Investigating the life history strategy of an African savanna tree, *Sclerocarya birrea* subsp. *caffra* (marula)

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Declaration

I declare that this thesis is my own, unaided work, unless otherwise noted within the text. It is being submitted for the Degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any other degree or examination in any other university.

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Abstract

Lack of understanding of the life history attributes and responses of savanna woody plants to disturbances, as well as the observation of unstable population structures in a keystone, savanna tree, *Sclerocarya birrea* subsp. *caffra* (marula), prompted this study. This study employed a combination of empirical, experimental and model formulation techniques, aimed at achieving its ultimate purpose of understanding the life history strategy of marula in the face of disturbance. Four main population structures were identified for marula in the low altitude savannas of South Africa: 1) adult dominated, 2) juvenile dominated, 3) with a "missing size class" and 4) stable (negative J-shaped). Spatial variability in structure indicated different drivers affecting different populations. High annual mortality rates of up to 4.6% in adult trees, no recruitment out of the fire trap and little regeneration were observed in the Kruger National Park (KNP) between 2001 and 2010, and consequently even greater instability in the structure of these populations already observed earlier in the decade.

Growth rates of saplings between 2 and 8 m in height and 2 and 30 cm in stem diameter in the field were monitored between 2007 and 2010. Annual growth rates of up to 11 mm in diameter and up to 22 cm in height were observed. Annual relative growth rates ranged between 1.9 and 4.8% across sites. Growth rates were positively linked with rainfall and plant size. Growth rates, biomass allocation patterns, as well as storage and defence allocation in 3 to 28 month old marula seedlings were assessed under glasshouse conditions. Relative growth rates were highest directly after germination (20%), but did not exceed 5% thereafter. Allocation to roots (already 65% of the overall biomass at 3 months of age and >80% when older) was high regardless of soil type or provenance. Provenance affected height gain, and plants germinating from seeds collected at higher rainfall sites had faster height growth rates than those from seeds collected at lower rainfall sites. Allocation to storage in the form of root starch peaked at 35%, while allocation to defence in the form of phenolics in the leaves peaked at 18%, being relatively higher than other species. No trade-off between growth and defence allocation was observed. However, in the second growing season, growth at the start coincided with a 50% decrease in starch reserves in the roots. Reproductive maturity was found to occur after 46 years and escape from the fire trap after 12 years in a disturbance free environment. Marula trees appear to be able to live for up to 300 years of age.

High temporal variability in fruit production was observed, marginally linked to rainfall. Only 2% of seeds persisted for more than one year, and hence marula relied mostly on the current season's fruit crop for input of new germinants. Fruit production was highly synchronous across trees at a site. Very high levels of seed predation were observed. Marula seeds can remain dormant for at least 10 years when stored in the laboratory. Germination takes place after 3 mm of rainfall every four days for two weeks and is enhanced by acid digestion and high temperatures. Germination percentages are relatively low (<50% of the endocarps).

Marula seedlings appear highly adapted to fire, with high allocation to below-ground biomass and starch storage, as well as very thick bark from very small stem diameters, including a well developed resprouting response from very young. Marula stems were able to resist fire from 3.4 cm in stem diameter, and were completely resistant above 7 cm. Stem diameter growth was prioritised above stem height growth, indicating that in marula, diameter gain is more important than height gain in escaping the fire trap. Topkilled marula saplings are able to regain their prefire height within one season. However, rainfall patterns may have an overriding effect on these growth patterns. Adult trees appear to be made vulnerable to fire through bark stripping, toppling and pollarding and the subsequent invasion of the soft wood by borers.

On nutrient-poor granite soils, marula has a resistant strategy to herbivory, however on nutrient-rich basalt soils, marula overcompensates for herbivory even at very low levels. This may explain why marulas are more vulnerable on basalt soils in the KNP, having already been extirpated from the northern arid basaltic plains. Marula seedlings are extremely drought resistant through fast root penetration rates and high root: shoot ratios.

A simple demographic model was developed which predicted that marula populations are unlikely to survive given the current elephant impact in the KNP and if the fire interval is less than once every seven years. Even though marula is highly resilient to damage from herbivory or fire alone, the combination of frequent fire and heavy utilisation is proving fatal for marula populations in the KNP and elsewhere.

In terms of other savanna tree species, marula is an outlier in its life history strategy, being extremely well adapted to the effects of fire with very thick bark, extensive resprouting ability and fast growth rates, combined with very high allocation to root mass, and levels of storage and chemical defence, as well as having very drought tolerant seedlings. Its main weakness as an adult, appears to be its soft wood, which is susceptible to wood borer attack. The perplexing lack of recruitment at some sites in spite of the extraordinary ability of marula seedlings to resprout from an early age, withstand extensive drought, have fast root penetration rates, extremely high root reserve storage and resistance to fire at small stem diameters, combined with high levels of fruit production and low water requirements for germination, is probably due to a combination of the lack of a dense persistent seed bank, high inter-annual variability in fruit production, low germination percentages, high seed and /or seedling predation rates and possibly dispersal of seeds away from suitable habitats. Overall, the unstable population structures observed in the low altitude savannas of South Africa, specifically in the KNP, do not bode well for the future persistence of marula as a dominant canopy tree species.

Keywords: elephant, fire, growth, mortality, recruitment, regeneration

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List of Abbreviations

ANOVA: Analysis of variance
ANCOVA: Analysis of covariance
a.s.l.: above sea level
AGR: Absolute growth rate
AIC: Akaike information criterion
ALB: Albasini population in the south-western KNP
CBH: Carbon balance hypothesis
CV: Coefficient of variation
DTT: Delagoa thorn thickets ecozone in the KNP
dbh: diameter at breast height
EBP: Experimental burn plot
GLM: General linear model
GPS: Global positioning system
GT: Gabbro thronveld ecozone in the KNP
HE: Hlangwine enclosure
HPLC: High performance liquid chromatography
JPNR: Jejane Private Nature Reserve
KNP: Kruger National Park
L12: *Colophospermum mopane/ Acacia nigrescens* savanna in the KNP
L17: *Sclerocarya birrea/ Acacia nigrescens* savanna in the KNP
L23: *Colophospermum mopane* shrubveld in the KNP
L5: Mixed *Combretum/Terminalia seric*ea woodland in the KNP
LAR: Leaf area ratio
LARMR: Leaf area root mass ratio
LSD: Least significant difference
MAR: mean annual rainfall
MBW: Mixed bushwillow woodlands ecozone in the KNP
MKS: Marula knobthorn savanna ecozone in the KNP
MLS: Mean leaf size
NE: N'washitsumbe enclosure in the KNP
OHE: outside the Hlangwine enclosure in the KNP
ONE: Outside the N'washitsumbe enclosure in the KNP
PCA: Principal component analysis
PHA: Phabeni population in the south-western KNP
RGR: Relative growth rate
SCD: Size class distribution
SE: Standard error
SLA: Specific leaf area
SSL: Specific stem length
subsp.: subspecies
STT: Sabie thorn thickets ecozone
TPC: Threshold of potential concern
TSH: Tshokwane population in the KNP
WRF: Wits Rural Facility

Glossary of important terms

Allometry: the study of the change in proportion of various parts of an organism as a consequence of growth.

Browsers: ungulates with a diet composed of more than 75% leaf or woody plant matter.

Compensation: Refers to the degree of tolerance exhibited by plants. If related damaged and undamaged plants have the same fitness, then that family has the ability to compensate fully for herbivory. If damaged plants have greater fitness than their undamaged relatives, then plants have overcompensated and if they have lower fitness, they have undercompensated for herbivory.

Constitutive defences: traits constantly produced in the plant which reduce herbivore damage

Cotyledon stage: first developmental stage after germination in which stored resources in the cotyledons provide nutrients to the plant before it is able to photosynthesise

Defence: Any trait that confers a fitness benefit to the plant in the presence of herbivores.

Disturbance: broadly defined as any discrete event in time that disrupts ecological processes and/or structures (ecosystem, community, or population) by influencing resource availability or changing the physical environment.

Grazers: ungulates with a diet composed of more than 75% grass biomass.

Induced defences: traits that are produced only after herbivore damage and then reduce further herbivore damage.

Leaf area ratio: leaf area/total plant mass, in m^2/g (LAR).

Leaf area root mass ratio: total leaf area/root mass, in m^2/g (LARMR).

Mean leaf size: total leaf area/total leaf number, in mm^2 (MLS).

Ontogeny: course of development of an organism.

Recruitment: the addition of new individuals into a population, following the young plant to adult (reproductively mature) transition. The key constraint on recruitment involves escaping the herbaceous layer, where woody plants are vulnerable to competition from grasses and damage by fire and herbivores.

Regeneration: population recruitment arising from seeds and seedling establishment or vegetative growth. The major mode of occupation of new space is by seedlings. Many savanna woody species are able to persist following damage, recovering lost biomass through resprouting.

Resistance: any plant trait that reduces the preference or performance of herbivores.

Resource allocation: partitioning of resources to different plant structures or metabolic functions.

Resprouting: The terms *coppicing/resprouting/sprouting* have been used interchangeably in the literature. For the purposes of this study, *resprouting* is used to denote shoots growing from the stem or stem base/root crown of a plant following damage.

Sapling: a juvenile, nonreproductive plant, structurally simple but having progressed beyond the seedling stage.

Savanna: The largest biome in southern Africa, accounting for 45% of southern African vegetation and 35% of the vegetation of South Africa and consisting of both trees and grasses. The major environmental delimiting factor is limited rainfall that prevents the woody component from dominating, while the effects of fire and grazing maintain grass layer presence.

Seedling: defined here as a young woody plant, less than 0.25 m in height and smaller than 1 cm in stem diameter, with a supple non-woody stem and frequently bearing cotyledons.

Seedling establishment: young seedlings have sufficiently expanded a photosynthetic surface, extended their root system and accumulated belowground resources, thus having a higher probability of surviving adverse conditions.

Size class distribution: a graphical representation of population structure: in this case of the number of woody plants represented by stem diameter size categories.

Specific stem length: stem length/stem mass, in mm/g (SSL).

Storage effect: During years with conditions favourable for seedling regeneration, a species can establish a cohort of juveniles and once established these individuals are able to persist in adverse conditions. Reproductive potential is thus "stored" between generations, allowing the population to recruit strongly when conditions are favourable.

Tolerance: the degree to which plant fitness is affected by herbivore damage, relative to fitness in the undamaged state.

Trees: Plants with a normal lifespan greater than 10 years, which can grow to at least 2.5 m height with adequate water, lights and nutrients and if not browsed, cut or burned; in which more than four fifths of the aboveground dry biomass consists of wood by the time reaches two thirds of its potential height.

Woodlands: Vegetation formations dominated by trees, but not to the extent that other growth forms are excluded or restricted to minor niches. Tree canopies are not continuous or overlapping.

Woody plants: collective term for trees and shrubs, all perennial and possessing woody secondary thickening.

PART A: BACKGROUND AND SETTING THE SCENE

Chapter 1: Introduction

Background, rationale, aims and objectives, study species description, study site description, thesis overview

1.1. Background

Sclerocarya birrea subsp. *caffra* (marula, family Anacardiaceae) is a widely studied savanna tree species and a large body of literature on many aspects of its biology, ecology and uses has already been published (reviewed in Shone 1979; Hall *et al.* 2002; Shackleton *et al.* 2002). It has been established that marula is a widely utilised species in terms of browsing by game in protected areas and utilisation by humans in communal areas for fruit, carving wood, shade and marula beer. It is a keystone species in many parts of its natural range and is usually a community dominant in many landscapes in the low altitude savannas of South Africa (lowveld) (Van Wyk 1974; Witkowski and O'Connor 1996; Jacobs 2001; Shackleton *et al.* 2002). It also forms an integral part of African cultural beliefs and practices (Wynberg *et al.* 2002). In addition, according to the National Forest Act of 1998 (Act 84 of 1998), the marula tree is protected in South Africa. Protected tree species may not be cut, disturbed, damaged or destroyed and their products may not be possessed, collected, removed, transported, exported, donated, purchased or sold except under license. In summary, the marula tree is an extremely important indigenous South African tree in terms of economic, ecological and cultural value.

Increasing interest in marula as a commercial fruit tree has motivated most of the reviews of marula research including those by Shone (1979), von Teichman (1982; 1983), Hall *et al.* (2002); Shackleton *et al.* (2002), and Wynberg *et al.* (2002). These reviews conclude that much is known about the fruit and its uses, as well as growing marulas under artificial conditions. However, the available information on natural marula populations is fragmentary, based on a collection of observations which have not been integrated (Shackleton *et al.* 2002). Thus a reliable interpretation of the population dynamics and life history strategy of this extremely important indigenous savanna tree, cannot yet be made. In addition, little is known about the regeneration ecology of this species in its natural habitat (Shackleton *et al.* 2002). Until the unpublished study by Neke (2005), no monitoring of seed germination under natural conditions has been reported (Hall *et al*. 2002). In addition, minimal references to the crown and stem dimensions of individuals of marula of known or estimated age, or to rates of growth appear in the existing literature (Hall *et al.* 2002).

This lack of understanding of marula biology is not surprising, as in general, savanna ecosystems and their components have been relatively understudied compared to tropical forests and temperate grasslands (Witkowski and Garner 2000; Neke 2005). Within this context, a major concern is the lack of information on the ecology and biology of most tree species and their response to disturbance (Maze 2001). Without this knowledge, sustainable management of savanna ecosystems becomes all but impossible.

In addition to the lack of knowledge on the ecology of marula, concern has also been raised over the impact of heavy elephant (*Loxodonta africana* subsp. *africana*) utilisation of marula, especially adult trees, in areas such as the Kruger National Park (KNP) and Associated Private Nature Reserves (APNR) on the western boundary of the KNP (Coetzee *et al.* 1979; Jacobs 2001; Gadd 2002; Henley 2007). Marula has been found to be actively selected for by elephants (Shannon *et al.* 2008), and hence on the receiving end of the highest levels of utilisation. Further concerns regarding marula population persistence in its natural habitat, relate to reported unstable populations, with no immature trees and little evidence of recruitment in certain areas (Walker *et al.* 1986; Lewis 1987; Jacobs and Biggs 2002a). However, other studies indicate that there are many young recruits in some areas, but these recruits may not be reaching the subsequent size classes due to factors such as fire and elephants (Jacobs and Biggs 2002b; Neke 2005; H. Eckhart pers. comm.). Some populations show evidence of a “missing size class” (2-8 m in height), represented by a bimodal population structure. It has been postulated that this is a consequence of too frequent fire preventing individuals in the fire trap from escaping, in combination with heavy utilisation of individuals up to 8 m in height by elephant (M. Hofmeyr pers. comm.; R. Grant pers. comm. Jacobs and Biggs 2002 a and b; Neke 2005). It has also been noted that some populations of marula are nearly locally extinct, such as in the *Colophospermum mopane* shrubveld region further north in the KNP (Jacobs 2001), and also near the Limpopo River in the northern areas of South Africa and southern Botswana (O’Connor *et al.* 2007). It has been speculated that this decline in marula density could be the result of an increase in elephant density, together with too frequent fire (Jacobs and Biggs 2002b). In the low altitude savannas of South Africa it appears that marula populations with stable structures are few and far between.

In order to address these concerns and the gaps in the current knowledge of the biology of marula, identification of explanatory mechanisms for how marula populations are able to sustain themselves in environments where disturbance is a frequent and intense factor, shaping community dynamics, is required. In addition detailed experimental evidence which may lead to an understanding of the life history strategy and underlying ecophysiology is also required. A combination of field research, experimental manipulations and modelling studies are therefore needed to address these gaps.

1.2. Aims and Objectives

The aim of this study was therefore to explore the possible explanatory mechanisms for 1) how marula populations are able to sustain themselves in environments prone to disturbance and 2) why marula has such variable and unstable population structures, by assessing the adaptive responses and vulnerability of marula to disturbance in its natural habitat as well as under artificial conditions. This study focused on ecological and ecophysiological mechanisms rather than genetics, and was geographically confined to the low altitude savannas of South Africa (Figure 1), but could represent the response of marula throughout its range (Figure 2).

The broad objectives of the study included the following:

1. To compare marula population structures and densities across various populations in the low altitude savannas of South Africa with different disturbance histories and site attributes.
2. To quantify the response of a number of marula populations in the KNP to the conditions prevailing over the last decade, including increasing elephant density and changes to the fire regime.
3. To determine above-ground relative growth rates of marula saplings and young reproductive trees (with special emphasis on the “missing size class” (trees from 2-8 m in height)) and relate these relative growth rates to variation in spatial and temporal contexts by comparing between sites with different ecological conditions and between years and seasons of measurement.

4. To measure intrinsic growth rates and allocation patterns (defence and storage) of marula seedlings and saplings grown in the glasshouse from seed.
5. To assess the variability in fruit production and subsequent seed fate in adult marulas across sites in the low altitude savannas with varying disturbance histories and site attributes.
6. To understand the early seedling water requirements, germination potential and early seedling root growth in marula.
7. To measure the overall above-ground relative growth rates, adaptive growth responses and allocation patterns (storage and defence) of marula seedlings exposed to various types and degrees of damage including defoliation and fire topkill.
8. To quantify the effect of various rainfall regimes and drought on the growth and allocation patterns of marula.
9. To integrate all these factors into a modelling framework to provide predictive capacity for the future dynamics of marula populations.

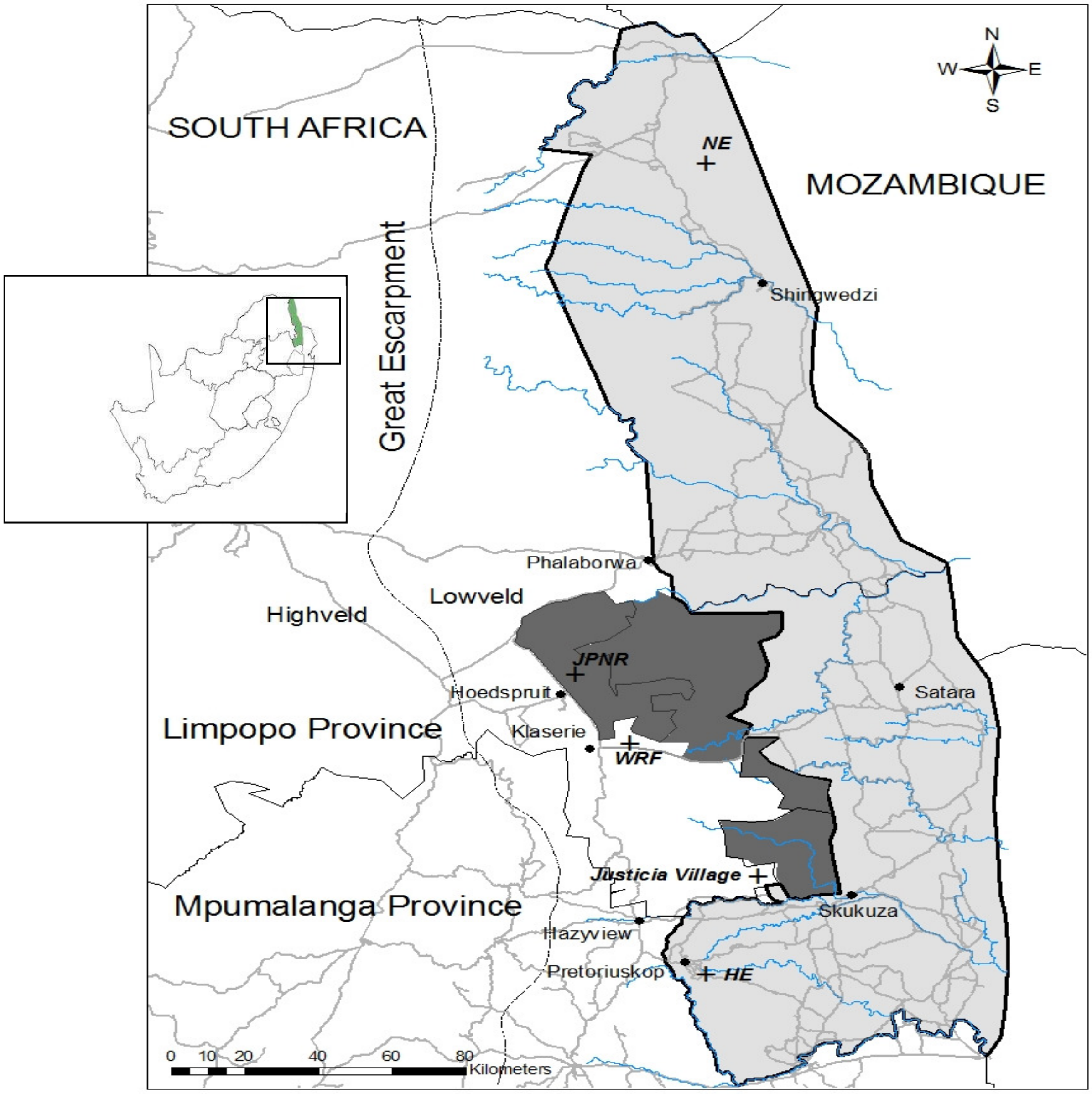


Figure 1: Location of the study area (South African low altitude savannas or lowveld) within South Africa and locality of the main study sites used in this study (marked with a cross). The Kruger National Park (KNP) is in light grey and the Associated Private Nature Reserves (APNR) is in dark grey along the western boundary of the KNP. NE=N'washitsumbe enclosure, HE=Hlangwine enclosure, WRF=Wits Rural Facility, JPNR=Jejane Private Nature Reserve.

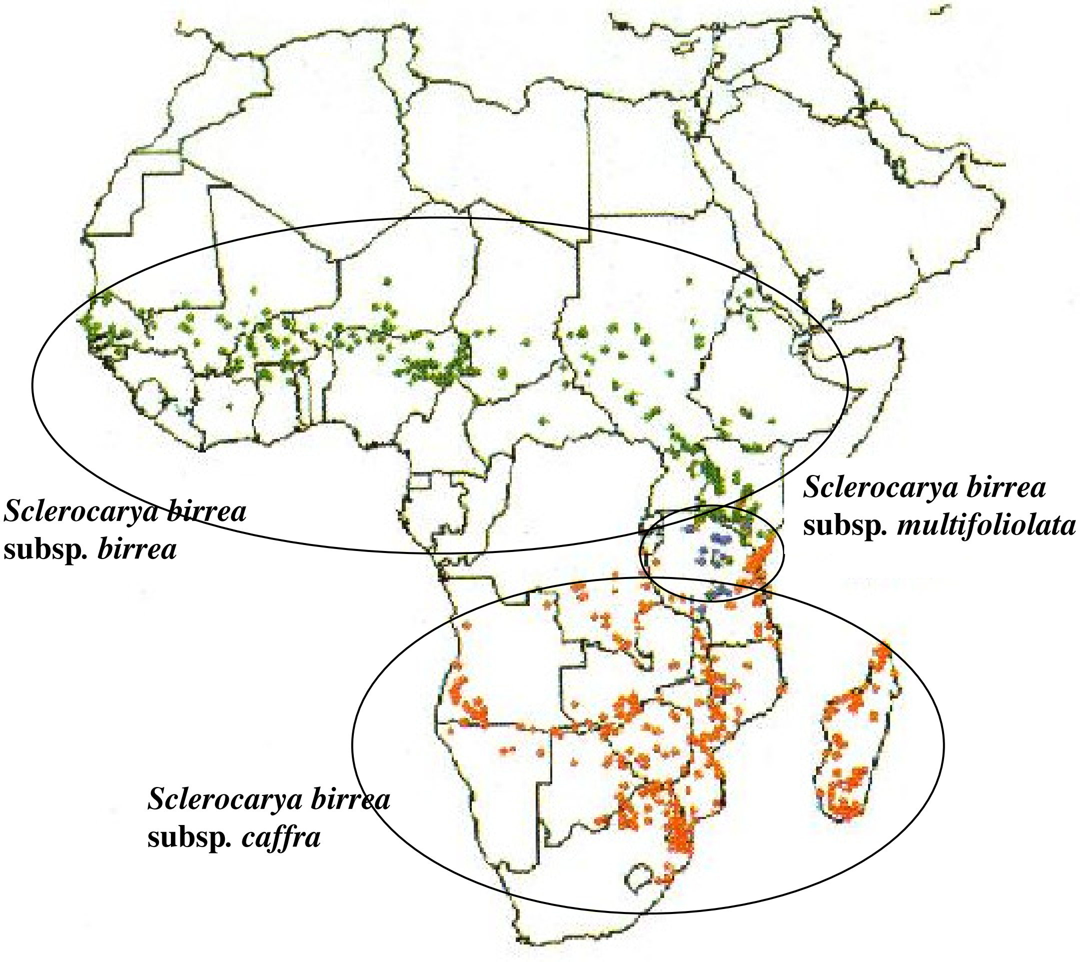


Figure 2: Distribution of the three subspecies of *Sclerocarya birrea* in Africa (including Madagascar) (modified from Hall *et al.* 2002).

1.3. Marula biology and ecology

Sclerocarya birrea (A. Richard) Hochst, an angiosperm, forms part of the mango family or Anacardiaceae (Palmer and Pitman 1972). *Sclerocarya* is a Greek name and literally means ‘hard’ ‘walnut’, referring to the hard kernel or stone (endocarp) inside the fruit. Anacardiaceae is a large family comprising over 60 genera and more than 500 species, of which 50 tree species, belonging to 10 genera, grow in South Africa and Namibia (Palmer and Pitman 1972). Well known examples include the mango, the cashew nut, and the pistachio nut (Palmer and Pitman 1972). There are three subspecies of *Sclerocarya birrea* in sub Saharan Africa (Figure 2). *Sclerocarya birrea* subsp. *caffra*, commonly known as marula or maroela, occurs across a very broad area within southern Africa, including the southern Democratic Republic of the Congo (DRC). The main factor limiting its range is its sensitivity to frost (Coates Palgrave 2002; Hall *et al.* 2002; Shackleton *et al.* 2002). Its altitudinal range is from coastal regions at sea level to 1 800 m (Hall *et al.* 2002). The mean annual rainfall (MAR) in its preferred habitat typically ranges between 400 to approximately 1000 mm (Shone 1979), which is seasonal in its distribution. It occurs mainly in the low altitude savannas of South Africa (lowveld), but extends through parts of the Northern Gauteng Province, KwaZulu Natal Province, Limpopo Province, and the Mpumalanga Province.

It is a keystone species in many parts of its range and is usually a community dominant (Shackleton *et al.* 2002). A keystone species has a disproportionate effect on the surrounding plant and animal species in a community. It is one of the most ecologically important trees in its natural environment, as it provides both food and shade (Hall *et al.* 2002). In addition, since marula is often a prominent element of the vegetation of a community, it has often been used as a descriptor species for the plant communities involved (Hall *et al.* 2002). Marula can be found in a diversity of vegetation types but typically in open, deciduous savanna and it shows a preference for well-drained soils (Shackleton *et al.* 2002). It may also be a constituent of denser woodland types such as forest, transition woodland, thicket, bushland, and shrubland and has also been recorded as a constituent of riparian vegetation and of forest patches (Acocks 1988, O’Connor 2010). In the low altitude savannas of South Africa, marula trees are mostly restricted to the crests and midslopes on both the granitic and basaltic substrates and in dolerite intrusions into both substrates (Coetzee *et al.* 1979; Jacobs 2001). However, preferred sites on the basalt plains are the more mesic convex upper parts where soils are leached and have relatively low clay content (Coetzee *et al.* 1979; Jacobs 2001). On the drier, basaltic clay soils, however, marula populations become less abundant, decreasing even further with an increase in clay content (Jacobs and Biggs 2002a). Marula tends to be sparsely distributed or absent from valley bottoms and is also poorly represented on well-defined ridges, although broad interfluves with extensive areas of sandy soils are a favourable habitat (Hall *et al.* 2002). The marula is frequently associated with species of the Combreteaceae usually in conjunction with mimosoid leguminous species.

Marula trees are deciduous and medium sized, reaching 18 m in height and 1 m in diameter (Palmer and Pitman 1972; Emanuel *et al.* 2005). The growth form is a single straight trunk, which branches high up into a few, bare branches that grow slightly upwards and horizontally to form a round spreading crown (Venter and Venter 1996; Jacobs 2001).

The marula tree can be identified by its lack of spines and imparipinnate compound leaves, which are composed of several leaflets as well as a terminal leaflet, arranged alternately and are clumped at the end of the branches (Palmer and Pitman 1972). The leaves remain on the tree from October to May (Hall *et al* 2002) in the lowveld of South Africa, but in wetter areas leaves may be retained for longer. The rounded crown and widely spreading branches do not supply water to stem flow to the same extent as the main branches of species with obconical canopies like *Acacia seyal* (Hall *et al.* 2002). This results in a lowered concentration of intercepted rainfall flowing to the region of the root crown.

The root systems of marula consist of shallow and extending roots thought to be sufficiently firmly anchored to commonly resist uprooting by elephants (Shone 1979). Marulas have been shown to tolerate soils with a very low fertility and exhibit a wide tolerance range for soil fertility (Hall *et al.* 2002). As for other species, a number of differences have been found between soils beneath the canopy of a marula tree and soils away from the canopy (Hall *et al.* 2002). These differences have been attributed to sequestration, in conjunction with architectural characteristics and the effects of root turnover (Soumare *et al.* 1994 in Hall *et al.* 2002). Soumare *et al.* (1994 in Hall *et al.* 2002) found that the soil was more acidic under the canopy than away from it. Organic matter was lower away from the trees than under their canopies. In addition, the cation exchange capacity and water holding capacity were higher under the tree canopies, probably due to the large quantity of roots present under the canopy. This higher water holding capacity is thought to allow for a rise in phosphorus diffusion, raising the level of available phosphorus. Marula forms a natural mycorrhizal association which seems important for the growth of healthy plants (Hall *et al.* 2002).

Marula is dioecious and therefore has separate male and female individuals, of which only the females produce fruit. Occasionally, however, bisexual flowers have been found on male individuals (Palmer and Pitman 1972). The male flowers are arranged in terminal spikes (approximately 5 to 17 cm long), whilst the female flowers are found singly or in clumps towards the ends of small branches (Palmer and Pitman 1972). The trees flower in spring from September to November (Van Wyk and Van Wyk 2007). The flowers are bright red when in bud but then open into small pinkish-white flowers; which are frequented by insects during the flowering season (Palmer and Pitman 1972; Hall *et al.* 2002). Morphological evidence and observations suggest that marula is an entomophilous species, that produces sticky pollen and secretes nectar, and that the honeybee is a major pollinator (Hall *et al.* 2002). The female trees produce fruit annually from December to March; however, fruit production appears to be episodic in certain populations (Shackleton 2002). The marula fruit is a highly specialised, large fleshy drupe or stone fruit (von Teichman *et al.* 1986), mostly produced on trees >13.6 cm in stem diameter (Shackleton *et al.* 2003). The fruit is fleshy, large, and almost spherical, approximately 5 cm long but varies both in size and shape (von Teichman *et al.* 1986; Emanuel *et al.* 2005; Van Wyk and Van Wyk 2007) (Figure 3a). The fruit is green and unripe in the canopy, however, ripens and turns yellow shortly after being dropped from the canopy, with the ripening time depending on the temperature and rainfall (Palmer and Pitman 1972; von Teichman 1982; Emanuel *et al.* 2005). The pulp is nutritious and high in vitamin C and therefore highly attractive to a variety of animals (Hall *et al.* 2002). The skin is tough and leathery and surrounds the fibrous juicy flesh which in turn encloses the endocarp, also known as the kernel or stone (Palmer and Pitman 1972; von Teichman *et al.* 1986) (Figure 3a). The endocarp is a hardened, lignified structure that encases one to four seeds (von Teichman *et al.* 1986) (Figure 3b). Pollination success affects the number of seeds per endocarp (Leakey *et al.* 2005). Since these seeds only have a thin, papery seed coat, the endocarp is important in seed protection (von Teichman *et al.* 1986). Each locule usually has one seed and is sealed by a lid also known as the operculum, which has to be ejected when germination commences (von Teichman *et al.* 1986) (Figure 3c). The endocarp does not inhibit water uptake; but does prevent germination through mechanical and chemical resistance (von Teichman *et al.* 1986). Marula seeds do not germinate readily when dropped from the canopy, but may remain quiescent for more than six months in a transient seed bank in the soil (Shone 1979; von Teichman *et al.* 1986), only germinating after sufficient rain the following growing season. The enforced quiescent period is not due to embryonic dormancy, but rather mechanical dormancy (von Teichman *et al*. 1986; Baskin and Baskin 2001). This dormancy can be broken through prior seed treatment, such as passing through the acidic digestive tract of mammals or manual removal of the opercula covering the seeds (von Teichman *et al*. 1986; Lewis 1987).

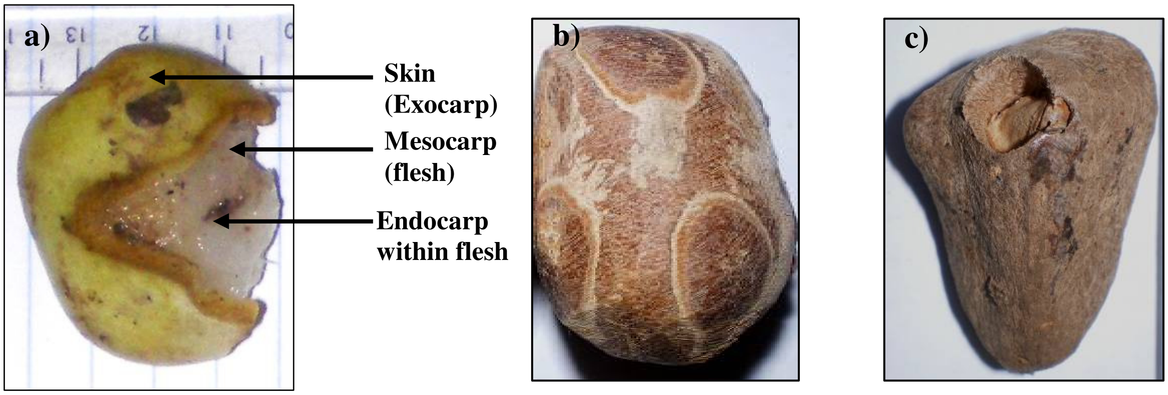


Figure 3 a) A ripe marula fruit with skin and flesh still attached; b) an apical view of a marula endocarp containing three seeds covered by opercula; c) a lateral view of a marula endocarp with one opercula removed revealing the seed inside.

Elephants appear to be the main dispersal agents of marula seeds under natural conditions; however, vervet monkeys (*Cercopithecus aethiops*) and chacma baboons (*Papio cynocephalus ursinus*) have also been observed eating the fermenting fruit and transporting them to new locations (Palmer and Pitman 1972). Remains of marula fruit have also been found in kudu dung (*Tragelaphus strepsiceros*) (Shone 1979; Estes 1991). Parrots, rodents and other small mammals, such as ground squirrels (*Xerus inauris*), also target the nutritious seeds (Palmer and Pitman 1972; Symes and Perrin 2003), and caching of uneaten endocarps is common (pers. obs). Finally, people also disperse the seed as the fruit are highly favoured (Shackleton *et al.* 2002), indeed there is speculation that humans may have been the primary dispersal agent before the arrival of Europeans in southern Africa, and may even have expanded the geographic range of the species over millennia (Cunningham 1997). Marula seeds remain viable for up to several years in the laboratory (von Teichman *et al.* 1986; pers. obs.), indicating the potential for a persistent soil seed bank. Little is known about natural germination percentages or variability across populations. Previous studies report that germination percentages of treated endocarps can vary between 2 and 100% (Hall *et al.* 2002).

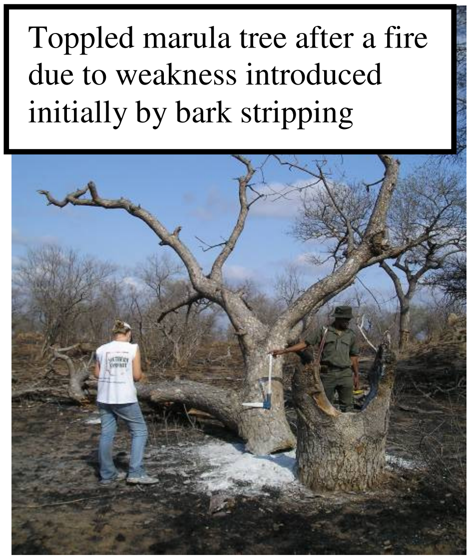
The importance of marula in the ecology of other plants and animals has been well documented. Since it often comprises 20% of the total woody biomass in areas where it is a community dominant, it produces a large area of shade which can be a key resource in semi-arid and arid areas. These areas often have higher soil moisture and nutrient levels than open environments and can provide suitable niches for organisms less tolerant of dry, hot conditions (Griffieon and O'Connor 1990). The crown of the tree is also an important habitat for small vertebrates and invertebrates as well as parasitic plants (Shackleton *et al.* 2002). Several Loranthaceous parasites have been recorded on marula and the woodroses (two species) have been described in detail by Dzeferos and Witkowski (1997) and Dzerefos *et al.* (1998). Marula was found to be the preferred host for this parasite (Dzerefos *et al.* 2003). The marula fruits are utilised by a wide array of different species and there has been no studies on what proportion of the diet or nutrient intake the marula contributes for these animal species (Shackleton *et al.* 2002). Elephants, kudu, giraffe (*Giraffa camelopardalis*), nyala (*Tragelaphus angasii*) and domestic cattle browse the leaves and even the bark (Palmer and Pitman 1972; Venter and Venter 1996). When the leaves are dry, they are eaten by impala (*Aepyceros melampus*) and grey duiker (*Sylvicapra grimmia*) (Palmer and Pitman 1972). Elephants have also been known to eat the bark and the roots of the tree. Shone (1979) also noted the tendency of *Panicum maximum*, a high quality fodder grass, to occur under the crown of marula trees, adding to the attraction of the tree. Humans also utilise the seeds, fruit, bark and leaves extensively; either as a food source, making beer, or for medicinal or carving purposes (Palmer and Pitman 1972; von Teichman *et al.* 1986). Humans in rural landscapes are heavily reliant on the tree from both a utilitarian and cultural perspective (Shackleton *et al.* 2002).

The leaves are also utilised by the larvae of at least eight species of butterfly and moth species including several emperor moth (Saturniidae) caterpillars (Picker *et al.* 2002), and the African lunar moth *Argema mimosa* (Palmer and Pitman 1972; Van den Berg 1990; Van Wyk and Van Wyk 2007). The larvae of the Ermine moth (family Yponomeutidae) encase the entire tree in silk and feed on the leaves (Picker *et al.* 2002). This effect is only temporary, however, as the trees regenerate their leaves once the old leaves have dropped. Certain species of mosquitoes have been found to be intimately linked with the tree as they breed in water that has collected in holes in the trunk (Van Wyk and Van Wyk 2007). The small marula fruitfly (*Pardalaspis cosyra*) is also a common predator of the marula fruit (Annecke 1982). Eggs are laid in an oviposition puncture on the fruit which leaves dark marks where rotting sets in. The larval maggots then feed off the rotting fruit (Pena *et al.* 2002).

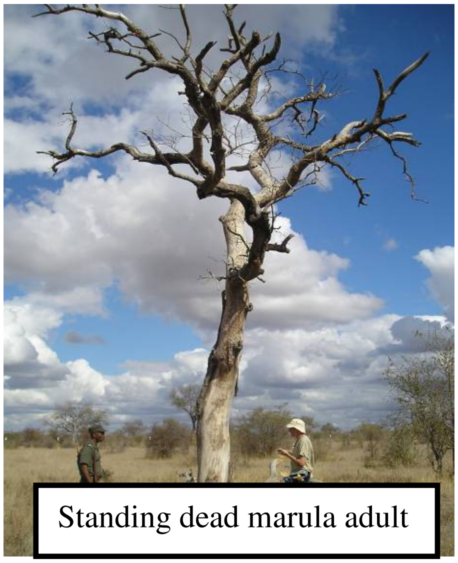
Marulas are resilient to most types of damage. They can resprout from the base or epicormically if toppled (ie. pushed over; the roots can either remain in the soil or the tree can be uprooted to varying degrees), or if the canopy is broken, or if the canopy is burnt by fire (Coetzee *et al.* 1979; Gadd 2002; Jacobs and Biggs 2002b). In addition, they can regrow stripped bark, recovering the underlying wood relatively quickly (Coetzee et *al.* 1979; Jacobs and Biggs 2002b). Marula trees have also been shown to be relatively drought resistant (Hall *et al.* 2002; O'Connor 2010) and seedlings can survive extended periods of summer drought (Botha 2006). Hence it is expected that marulas are able to sustain relatively high levels of damage before they die (Figure 4).

Mature marula tree in the KNP with new leaf flush after a fire in October 2008

Marula flowers



Toppled marula tree after a fire due to weakness introduced initially by bark stripping

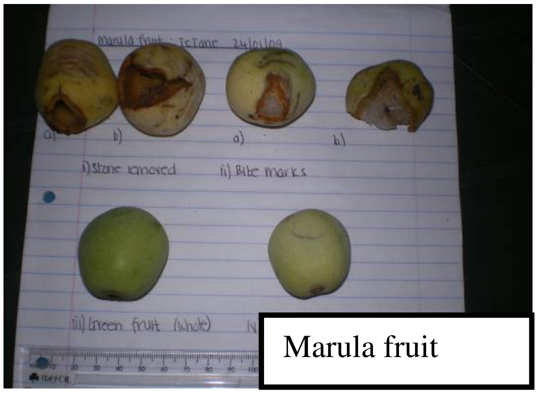


Standing dead marula adult

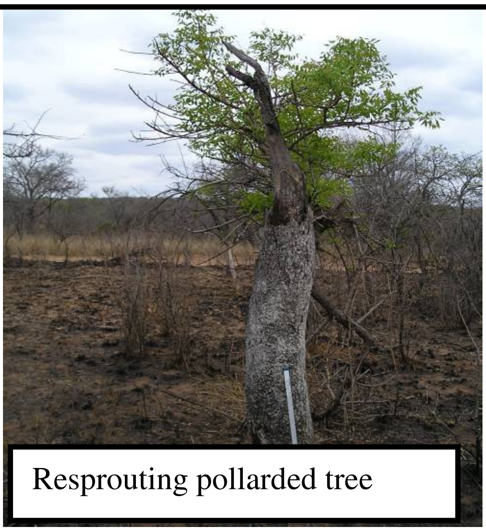
Ring barked adult marula

Freshly ring barked marula

Fruiting marula tree infested with caterpillars causing the leaves to turn brown



Marula fruit



Resprouting pollarded tree

Resprouting marula after a fire

Bark recovery in bark stripped experimental marula tree

Marula seedling roots grown under glasshouse conditions

Figure 4: Images of marula from a reproductive perspective as well as in terms of damage from herbivores and fire.

1.4. Savannas and the determinants of woody population dynamics

The primary factors which determine structure and function in ecosystems (ecological determinants) in southern Africa are climate, hydrology, soil type, herbivory and fire (Walker 1982; Sankaran *et al.* 2005). The savanna biome in southern Africa covers approximately 46% of the land surface making it the largest biome in southern Africa (Low and Rebelo 1996). A mixture of a herbaceous ground layer and a distinct upper layer of woody plants characterises this biome. The most important factor delimiting the boundaries of savannas is the lack of sufficient rainfall which inhibits the upper woody layer from dominating. Tied with fires and herbivory, the overall combination keeps the herbaceous layer dominant (Low and Rebelo 1996). This combination of herbaceous and woody components and their relative abundance is highly dynamic and varies according to soil nutrients, rainfall, herbivory and human activity (Scholes 1997). Rainfall and soil nutrients further determine the modifying impact of fire and herbivory on the savanna state (Huntley and Walker 1982).

Woody plant dynamics are therefore thought to be mostly influenced by the interactive effects of fire, competition from the grass layer, rainfall, soil texture and nutrients and herbivores (Scholes and Walker 1993; Scholes 1997). The interactive effects of these factors will depend on the woody species, the plant life history stages affected, as well as the intensity, frequency and season that the modifying factors (i.e. disturbance) may occur (Noble and Slatyer 1980). Woody plant dynamics are thought to be mostly influenced by the interactive effects of these factors while still in the seedling phase (Higgins *et al.* 2000). Other authors, however, believe seeds and seedlings to be less important in savanna dynamics than established trees (Hoffmann 1996).

1.4.1. Soil nutrients and topography

In South African savannas, soil nutrients and topography play a crucial role in woody vegetation dynamics (Scholes 1997). These savannas can be divided into infertile and fertile, based largely on soil type (Venter *et al.* 2003). Nutrient-rich soils support fine leaved woody species such as *Acacia* and other Mimosaceae species, which are deciduous with many thorns and preferred by herbivores. Grasses are nutritious and dense. On nutrient-poor soils, broad leaved woody plants such as species in the Combreteaceae and Caesalpinaceae dominate, which are also deciduous but without thorns. Grasses are unpalatable and sparse. Mopane veld is an exception to this broad categorisation as broad leaved mopane dominates in the hot and dry, but nutrient-rich northern basalt regions of the KNP (Venter *et al.* 2003). Soil nutrients are also influenced by the catenal sequence where nutrient-rich soils occur in valleys and low lands due to movement of fine clay particles downward and nutrient-poor soils occur on the toplands, which have a high sand content and are well drained, but where the nutrients have been leached out (Scholes and Walker 1993).

1.4.2. Rainfall

South African low altitude savannas are broadly divided into semi-arid (<700 mm per annum) and mesic savannas (>700 mm per annum) (Shackleton 2002; Venter *et al.* 2003). Rainfall modifies the effects of the soil type and under a high rainfall regime, more plant biomass growth is possible which increases the intensity and frequency of fires.

1.4.3. Fire

Our understanding of the actual mechanisms whereby fires influence the structure and functional dynamics of savanna ecosystems is not complete (Maze 2001). Injury by fire affects savanna trees by either 1) killing them outright; 2) killing the aboveground parts only (topkill), after which regrowth occurs from the stem base or root crown; 3) scorching the aboveground parts and resprouting occurs from the stem canopy (epicormic sprouting) (Bellingham and Sparrow 2000; Higgins *et al*. 2000). Low intensity and less frequent fires in savannas leads to an increase in woody plant density and biomass, whereas high intensity and frequent fires lead to a reduction in woody biomass and density (Scholes and Walker 1993). Frequent fires affect species composition by selecting for woody species with a high potential for vegetative reproduction (Gignoux *et al.* 1997; Hoffmann 1998). Most savanna tree species go through a prolonged period of dieback of shoots at the juvenile stage of their development (Luoga *et al*. 2004) and frequent fires maintain this process until the shoots reach a height, which allows them to escape from the flame height (Gignoux *et al.* 1997). Since seedlings suffer from repeated shoot dieback, saplings are assumed to be the first stable stage during the growth of trees in savannas and could therefore be used to predict their productivity (Zida *et al.* 2007). Trees in savanna ecosystems have developed resistance strategies in response to fire, including fire resistant bark, resprouting ability, seed dormancy and fire stimulated germination and the rate at which seedlings reach critical stages in the life history (Noble and Slatyer 1980). Gignoux *et al.* (1997) suggest that differences in fire resistance between species may have important effects on the structure and dynamics of savanna ecosystems.

The climate and fuel load conditions make the whole range of marula potentially subject to fires (Hall *et al.* 2002). In areas where fuel loads reach 4 tons/ha, fires increasingly tend to suppress woody plant regeneration and eliminate many smaller established individuals (Trollope *et al.* 1998a). Suppression of marulas by fire, along with other woody species, is inferred in the vegetation assessments of Lamprey *et al.* (1967), Coetzee *et al.* (1979), Jacobs (2001) and O'Regan (2005). Onochie (1964) conducted fire related woody vegetation monitoring on marula and found that populations had actually increased under all fire treatments that were applied, except the annual late burning regime which tended to eliminate it. These observations and others indicate a well-developed fire tolerance in marula. Some reports have found evidence for fire protection rather than fire tolerance (Hall *et al*. 2002), where marulas will grow in areas protected from fires such as bush clumps and termitaria. In spite of this, the evidence for fire tolerance in marula has been established and it is attributed to the thick bark, strong capacity to replace small branches lost when fires are particularly severe (Hall *et al.* 2002). The main effect of fire on woody vegetation of the KNP was topkill of stems and branches, forcing the plant to utilise stored reserves and coppice from the collar region of the stem (Trollope *et al.* 1998b; Jacobs and Biggs 2001). The effect of frequency of burning on woody species has been found to be significant and the physiological state rather than the time of year determined the reaction of trees to fire (Trollope *et al.* 1998b).

A fire experiment was started in 1954 with the aim of providing a scientific basis for fire management policies within the KNP (O'Regan 2005, Higgins *et al.* 2007). The experimental design was a pseudo-randomised block design in which 12 fire treatments were replicated four times each, within each of four major vegetation types defined in the KNP by van der Schijff (1958). Each plot was approximately 6.5 ha in size. For marulas in the experimental burn plots in the KNP (see O'Regan 2005 for a full explanation of their layout and design), no density differences were recorded in any of the fire treatments to which it was exposed to between 1954 and 1998, and it appeared from the data that the highest densities were recorded in the October triennial burn (Enslin *et al.* 2000). Field observations of marula in the KNP have, however, indicated that there has been a significant decline in their density as a result of the interaction of fire and elephants: elephants are killing the adult trees through bark stripping and toppling, while fire is preventing the seedlings from becoming established (Viljoen 1988; Trollope *et al.* 1998a; Jacobs and Biggs 2002 a and b).

Jacobs and Biggs (2001) further investigated the effect of various management fires on marula in the KNP. The effect of fire season, frequency and intensity was investigated. Trees taller than 2-3 m (2.75 m specifically for marula) were generally resistant to fire in the KNP (Trollope 1982; Jacobs and Biggs 2001). Marula plants up to 1.5 m in height were particularly susceptible to fire and were affected by the interaction of frequency and season (Jacobs and Biggs 2001). Generally the structure and not the density of marula seedlings was affected by fire. Fire intensity augmented the change in the structure of marula seedlings/saplings to a multi-stemmed morphology (especially in August treatments). Adult trees did not respond to fire. The triennial August burns appeared to have the greatest impact on marula seedlings probably due to the extremely high fire intensities resulting in increased topkill. The annual August treatments seemed to attract herbivory, resulting in this treatment having similar height and basal diameter responses to the triennial August burns. The February burns and biennial August burns were found to have the least impact on the structure of marula seedlings indicating favourable conditions for reaching a fire escape height.

1.4.4. Herbivory

Heavy browsing by mammals can inhibit the development of woody vegetation (Luoga *et al.* 2002, 2004) and the browsing damage incurred by a given browser, depends on its intensity and frequency as well as the developmental stage of the plant (Drexhage and Colin 2003). The outcome of herbivore impact on a particular woody species depends on the nature of the damage, the ability of the plant to recover, its demography and role that it plays in a plant community (Coetzee *et al.* 1979).

The most detrimental impact on woody vegetation has come from elephants in parts of Africa where they are confined to areas such as National Parks. In these areas, there is mounting evidence that woody vegetation regeneration is being suppressed and mature trees ring barked or felled (Caughley 1976). According to Shone (1979), animals such as elephants do little damage to mature marula trees. Small individuals within the browsing line are, however, significantly affected by small browsers. Goat browsing is sometimes sufficiently severe to kill young marula trees in rural areas (Shone 1979) and the detrimental impacts of impala on marula seedlings have received some documentation (Haig 1999). During an assessment by Coetzee *et al.* (1979), concerns regarding the high elephant damage to marula trees along the main roads in the KNP were raised contradicting Shone (1979). Elephants have been found to have a greater effect on size distribution of a woody species than on its density and the greatest decline is usually noted in the 1 m tall size class (Hall *et al.* 2002). Caughley (1976) has suggested that mopane seedlings establish with little interference from elephants and grow to a height of 1 m before being browsed by herbivores. Elephants have changed the structure of mopane stands from a symmetrical size class distribution, to a bimodal distribution of scrub mopane, with a large proportion of individuals of less than 2 m and a second group of mature trees taller than 8 m (Caughley 1976). The extent to which elephants affect the savanna woody component depends on factors such as tree density and age structure, fire, soil type and other herbivore species (Trollope *et al.* 1998a) and this further complicates an understanding of this complex problem.

Marula trees tend to show a bimodal population structure throughout the majority of the KNP, as there are large numbers of trees less than two meters in height and a large numbers of trees greater than eight meters in height, and very few individuals between two and eight meters (Jacobs and Biggs 2002b). It is well-known that elephants favour marulas for their fruit, and these mega-herbivores (Owen-Smith 1992) have been known to push down the trees, either exposing their roots or physically damaging the trunks of the trees (Jacobs 2001). This means that these individuals are removed from the larger size classes and reintroduced into the lower size classes as coppicing trees, and hence are often below the fire line. Therefore, although these individuals are able to coppice and re-grow, fire keeps burning these trees back. In addition to this, fire also prevents the seedlings from becoming established into the population.

Marula trees are dioecious and a possible preference of elephants for fruiting females (Hemborg and Bond 2006), has a great potential to affect the ratio of male to female trees in a population, and so affect fruit production and therefore the future recruitment dynamics of this species.

Scholes *et al.* (2003) stated that while the dominant drivers of vegetation change in the KNP are fire and large herbivores, both interact strongly with rainfall variability, particularly with the occurrence of prolonged drought. Jacobs and Biggs (2002b) stated that previous research has shown that the feeding behaviour of elephants varies according to the size classes of woody plants, whereby elephants feed on trees ≤2 m (marula seedlings) but in fact favour trees >2 m (mature marula trees) for both feeding and display. In particular, elephants favour marula trees for their fruit (Gadd 2002) and are known to push down trees, either exposing their roots or physically damaging and snapping the trunks of the trees, whereafter these individuals coppice (resprout) or die (Jacobs and Biggs 2002b). Toppled trees are significantly more likely to be killed by fire than undamaged standing trees. The bark of marula is also highly favoured by elephants, however it has been shown to regrow very quickly (Coetzee *et al.*1979). The interactive effect that fire and bark stripping may have on adult trees has received some attention (Moncrieff *et al.* 2008), indicating that bark stripping may be important in making adult trees vulnerable to fire.

1.4.5. Allometry and growth form

Woody plants adapt to the effects of the various disturbances in savannas through a variety of mechanisms including but not limited to: growth rates, resprouting ability, bark thickness, growth form, bark regrowth and quick recovery from damage, plant architecture and allometry, as well as chemical and physical defences.

Plant growth form determines the life history of a woody plant as it influences light interception, height gain, stability against wind, defence against herbivores and fire and timing of reproduction (Koyama and Hotta 1990; Archibald and Bond 2003). The final architecture of a plant is a trade-off between conflicting requirements of the various plant functions and translates into a physical trade-off between height gain and diameter growth at the sapling stage (Archibald and Bond 2003). Fire and herbivory will strongly influence growth form in savannas, whereas light interception would be important in forests. To escape fires, woody plants must grow tall enough to escape the fire trap (3 m in height (Trollope 1984)). Therefore plants growing in an environment with frequent fires would be expected to develop a pole like growth form due to rapid height gain. Plants growing in an environment with high levels of herbivory would instead be expected to invest in defensive structures such as spines and highly branched shoots developing a box-like cage structure. These theories assume that height gain is the only defence against fire, whereas increases in diameter may also instil fire resistance due to thicker bark (Wilson and Witkowski 2003). Neke (2005) found that marula had the lowest height gain per diameter increment out of 11 common savanna woody species studied. Marula saplings appeared to invest mostly into stem diameter when below 3 m in height, contrary to the strategy of many other species, which invested in height gain up to 3 m and then once out of the fire trap invested in diameter growth.

Enquist *et al.* (1999) found that for tropical dry forest species there was essentially a trade-off in growth rate with allocation to tissue density: species that allocate less biomass to their stems (light woods), increase in basal diameter faster than species that allocate more (dense woods). Marula has a very low wood density (590 kg/m^2) and as expected has rapid basal diameter growth in relation to height gain (Neke 2005).

1.5. Study sites

1.5.1. Greater study area

This study was restricted geographically to the north–eastern low altitude savannas (lowveld) of South Africa (Figure 1). This low lying area is bounded by the footslopes of the Drakensberg Great Escarpment to the west and the Mozambique coastal plain to the east (Venter *et al.* 2003). It consists mainly of plains with low to moderate relief and forms part of the Eastern Plateau Slope (Kruger 1983). It is on average 300 m above sea level and slopes gently towards the east (Venter *et al.* 2003). In South Africa the area traverses both the Mpumlanga and Limpopo Provinces.

Historically this area was never densely populated due to diseases such as malaria and tsetse fly, erratic rainfall and high temperatures, and poor soils (Pollard *et al.* 2003). During the colonial period the area was heavily utilised for hunting and combined with the rinderpest epidemic in the late 1800's, much of the formerly abundant game had been eradicated from the area. Establishment of the KNP on the eastern border of South Africa began in the early 1900s to protect the remaining game. With the establishment of the Apartheid regime in the 1940s, homelands were created to control land tenure and blacks were forced into centralised settlements on farms approximately 2000 ha in size. Establishment of tropical fruit plantations, sugar cane plantations and cattle and game farms occurred in the surrounding areas. Today the human populations reach 300 people per km^2 (Pollard *et al.* 2003) in the former homelands and the landscape consists of a patchwork of various land uses including agriculture, plantation forestry, mining, settlements and protected areas such as game farms, game and nature reserves and the KNP (Coetzer *et al.* 2010).

Geologically the area is dominated by ancient granitoid rocks of Swazian and Randian age which are grouped together as basement complex primarily composed of gneiss, granite or migmatite (Venter 1990). The Timbavati Gabbro consisting of intrusive rocks intrudes into the basement complex as irregular and discontinuous sills and dykes (Venter 1990). The strike of the lithology is normally north-south dividing the area into roughly north to south bands of different geology. A thin strip of sedimentary rocks separates the granitic and basaltic rock formations.

Climatically the area falls into two main zones, the lowveld bushveld zone (rainfall of 500-700 mm/annum and potential evaporation of 6 mm /day in October) and the northern arid bushveld zone (300-500 mm/annum rainfall and potential evaporation of 6 mm/day in October) (Venter *et al.* 2003). The climate is tropical to subtropical and drought is endemic (Pollard *et al.* 2003). It is characterised by high mean temperatures in summer and mild frost free winters with average temperatures of 17.3-24.4°C, minimum temperatures ranging between 9.5-20.4°C, and maximum temperatures in the range of 26.1-32.4°C. Rainfall is seasonal and concentrated between October and April and summertime humidity is high. Mean monthly relative humidity at midday in summer ranges from 50 to 53% and in winter from 37-42% (Venter *et al.* 2003). Powerful convective thunderstorms deliver most of the rainfall due to the combination of moisture and heat, creating an unstable atmosphere in summers (Venter *et al.* 2003). The mean storm size is approximately 0.9±0.5 mm. Typically the rainfall intensity is high, leading to high erosion rates of unprotected soil (Venter *et al.* 2003). There is a spatial trend in temperature from cooler in the south to warmer in the north. Rainfall decreases sharply from more than 1200 mm/annum along the escarpment to less than 500 mm along the western boundary of the KNP.

Six major rivers transect the lowveld most originating near the escarpment and flowing east towards Mozambique and into the Indian Ocean. Pollard *et al.* (2003) provide detailed maps of the topography, temperature, rainfall and geology across the lowveld. Typical savanna soil catenal sequences are evident throughout the region. Hence, upland soils on slope crests are shallow, coarse and dystrophic, whilst bottomland soils are deeper, finer textured and more eutrophic (Witkowski and O'Connor 1996; Shackleton 2002).

1.5.2. Specific study sites

1.5.2.1. Kruger National Park (KNP)

The KNP encompasses an area of 1 948 528 ha and forms part of the Lowveld region of South Africa, traversing both the Mpumulanga and Limpopo Provinces. The park varies in altitude between approximately 200 m a.s.l. on the eastern plains and up to about 900 m a.s.l at Pretoriuskop (van Wyk 1994). The climate is subtropical with hot summers and warm dry winters (average temperature range: 17.3 - 24.4 °C). The average annual rainfall ranges from approximately 740 mm at Pretoriuskop in the southwest to about 440 mm per annum in the northeast. Most of this rain falls between November and March. Over the past century, the pattern of rainfall has been characterized by extended wet and dry periods with cycles of about 10 years (Jacobs and Biggs 2002a). Seven perennial rivers (the Crocodile, Sabie, Olifants, Letaba, Shingwedzi, Luvhuvhu and Limpopo) run from west to east through the park (van Wilgen *et al.* 2000). The topography of the KNP is generally flat with gently undulating plains. Geologically the KNP is divided into two almost equal sections i.e.: predominantly granitic formations in the western half and the basalts in the eastern half. The two major formations are divided by a narrow belt of shale and sandstone. Soil profiles follow a similar trend, decreasing in depth from the south (1-3 m) to the north (0.5–1 m) of the park, especially for coarse-grained soils derived from granitic material (Venter and Gertenbach 1986). Gertenbach (1983) described 35 landscapes within the KNP, which are areas with distinct geomorphology, climate, soils and vegetation pattern together with the associated fauna. The nutrient-poor granite derived substrates tend to be dominated by deciduous, broad-leaved species with no thorns from the families Combreteaceae (especially *Combretum* and *Terminalia* spp.) and Caesalpiniaceae. The more nutrient-rich clay soils are dominated by species from particularly the Mimosaceae (especially *Acacia* spp.) that are deciduous, with fine compound leaves and thorns (Venter and Gertenbach 1986). The flora within the park comprises 1 983 species; including over 400 tree and shrub species, and over 220 grasses (van Wilgen *et al*. 2000). The fauna within the park is also diverse, including 147 species of mammals and 492 species of birds. Elephants occur at densities of up to 2 elephant/ km^2 (Shannon *et al.* 2008) and continue to increase.

Marula is distributed in the southern and central regions of the KNP, is rare between the Olifants and Shingwedzi Rivers and becomes common again in the north near Punda Maria and Pafuri (C. Helm, pers. obs.).

The fire management policy in the KNP has changed repeatedly over the years to meet different management objectives. From 1948-1956 a fire suppression policy was implemented (van Wilgen *et al.* 2000), until the observed effects led to concern over bush encroachment and deterioration of grazing and fuel accumulation (Trollope *et al.* 1998a). Accumulation of fuel load, led to a series of large fires in 1954, leading to approximately one quarter of the KNP being burnt in uncontrollable wildfires (van der Schijff 1958). This then led to the implementation of a triennial burning policy from 1956 to 1992 (van Wyk 1975), with the KNP divided into blocks, and controlled prescribed block burning being practiced during late winter. Management then re-evaluated the fire management policy, and in 1992 this was changed to a *laissez faire* burning policy where only lightning induced fires were allowed to burn. However, in practice the system was being dominated by fires ignited unintentionally or intentionally by humans (van Wilgen *et al.* 2003). In 2002 this policy was again altered to combine point ignitions with unplanned and lightning fires, aiming to burn an annual target area determined by rainfall and fuel conditions (van Wilgen *et al*. 2008). Herbivory by grazers has an important influence on fires through the consumption of grass fuels and browsing (particularly by elephant) has a strong impact on tree mortality subsequent to fires (van Wilgen *et al.* 2000).

Specific areas within the KNP were chosen for various parts of this study. Since marula population surveys had already been conducted in the central and southern regions of the KNP, these trees were reassessed to obtain a longer term perspective. Additional surveys were conducted in the south western parts of the park and near Pretoriuskop. Two enclosures within the KNP were chosen to assess the effects of herbivore exclusion on marula growth, fruit production and seed fate. These were the 254 ha N'washitsumbe enclosure (NE) (also known as the roan enclosure), north of Shingwedzi in the northern KNP and the 220 ha Hlangwine enclosure (HE) east of Pretoriuskop in the southern KNP. The N'washitsumbe enclosure was established in 1968 (Levick and Rogers 2008), while the Hlangwine enclosure was established in 1972 (Goodall 2006), both to exclude all animals larger than hares apart from rare antelope grazers such as roan (*Hippotragus equinus*) and sable (*Hippotragus niger*). The northern enclosure occurrs on basalt soils while the southern one occurrs on granitic soils. The dominant vegetation types where the study took place in the southern KNP include the Granite Lowveld, Pretoriuskop Sour Bushveld and Gabbro Grassy Bushveld in the Lowveld Bioregion (Mucina and Rutherford 2006).

1.5.2.2. Wits Rural Facility (WRF)

WRF is situated within the Bushbuckridge district, in the southernmost section of South Africa's Limpopo Province (Figure 1). It is 350 ha in size near Klaserie on the western boundary of the KNP. The region is characterised by medium rainfall (ca. 680 mm per annum), concentrated in the summer season (October to May) and is classified as semi-arid. Rainfall is extremely variable and intra-seasonal drought is common. Frost is rare and if it does occur will be confined to valleys and low lying areas. The topography of the study region is gently undulating. Soils are underlain by granitic gneiss with local intrusions of gabbro which give rise to sandy, well drained soils. The vegetation of the area is classified as Mixed Lowveld Bushveld (Low and Rebelo 1996) or Granite Lowveld (Mucina and Rutherford 2006). Combreteaceae species dominate the woody vegetation with marula and *Dichrostachys cinerea* also making significant contributions. WRF is a protected area mainly utilised for the purposes of research and environmental education. Small and medium antelope are present which result in a low to moderate browsing and grazing pressure. No large herbivores such as elephant or rhino are present and little harvesting of natural products takes place. Fire is used as a management tool but with low frequency, with particular areas having a fire return interval of between 3 and 8 years (W. Twine pers. comm.).

1.5.2.3. Jejane Private Nature Reserve (JPNR)

JPNR is situated in the 4 500 ha Mohlabetsi Conservancy (western boundary of the KNP), 5 km from Hoedspruit and consists of the farms Ptn 1 Vienna 207KT (Vienna Game Farm) and Ptn 1 Antwerpen 60 KU (J. Thomson 2004, pers. comm.). The property is approximately 2 070 ha in size. The underlying geology of this reserve is gneiss and granite (Peel *et al.* 1993). It lies between 450 and 500 m a.s.l. (Peel *et al.* 1993) and has a very low mean annual rainfall of 450 mm/annum (South African Weather Bureau). Jejane is predominantly *Acacia nigrescens–Sclerocarya birrea* savanna (Peel *et al.* 1993). The reserve has had no elephant activity, apart from passing vagrants, for at least 150 years. No major fire has been recorded at JPNR for 15 years.

1.5.2.4. Justicia Village

The communal land adjacent to Justicia Village approximately 15 km west of the KNP is heavily grazed by goats and cattle with low fire frequency due to the low abundance of grass. The dominant vegetation type is the Granite Lowveld in the Lowveld Bioregion (Mucina and Rutherford 2006). Heavy human utilisation of the woody vegetation for fire wood is evident and agricultural plots for growing vegetables are continually being added (pers. obs.). The mean annual rainfall is 550 mm. Fire is frequent but of low intensity due to the heavily grazed grass layer (Moncrieff *et al.* 2008).

1.6. Structure of the thesis

This thesis has been divided into four parts and 12 chapters (see Figure 5, which describes how the parts relate to each other). Each chapter represents one stand alone paper (unless otherwise specified), and hence some repetition is unfortunately inevitable. An attempt has been made to minimise this repetition by not including detailed study site and species descriptions in every chapter and by referring to other chapters where similar methodology applies, wherever possible. Parts of certain chapters have already been published/ in press or are currently under review. This is indicated at the beginning of the chapter concerned. It must also be noted that the experimental design for particular sections in some chapters may appear rather haphazard. This is because during the course of many experiments/trials a number of “side issues” were noted and the decision was made to report on these results in conjunction with the main experimental results. Hence for example, the growth rates of seedlings were assessed in a number of glasshouse growth trials that were originally established to determine responses to herbivory, storage, etc.

Part A: General introduction and background including species characteristics and population structure at various sites in the lowveld of South Africa and observed rates of change in some populations. Against this backdrop the aims of the thesis are strategically placed to address the issues raised.

Chapter 1 is a general introduction with thesis layout.

Chapter 2 describes the population structure of various marula populations in the lowveld of South Africa under different management regimes, rainfall regimes, disturbance regimes (fire and elephant) and with different historical contexts.

Chapter 3 quantifies marula damage and mortality over time and provides the reason why concern is warranted for marula populations in protected areas with disturbances such as elephants and fire.

Part B: Life history characteristics of marula

Chapter 4 describes the inherent growth rates of marula saplings between 2 and 8 m in height at three sites where the trees are protected from fire and herbivory.

Chapter 5 describes the inherent allocation physiology of marula in terms of growth, defence, and storage in marula seedlings of different ages (3- 28 months) grown in the glasshouse.

Chapter 6 describes the reproduction potential and seed fate in marula at five sites in the lowveld.

Chapter 7 summarises the early seedling requirements and survival for marula seedlings grown in the glasshouse.

Part C: Adaptive responses of marula to disturbances.

Chapter 8 describes the responses of marula seedlings to four levels of defoliation.

Chapter 9 describes the responses of marulas to fire and herbivory.

Chapter 10 describes the responses of marula seedlings to rainfall regime and variability.

Part D: Synthesis and discussion of all information previously known and obtained in this study.

Chapter 11 synthesises the information and uses it to parameterise a simple population model for marula to understand its current dynamics in the lowveld and to predict the future dynamics.

Chapter 12 is a general discussion of the overall findings as well as conclusions and recommendations.

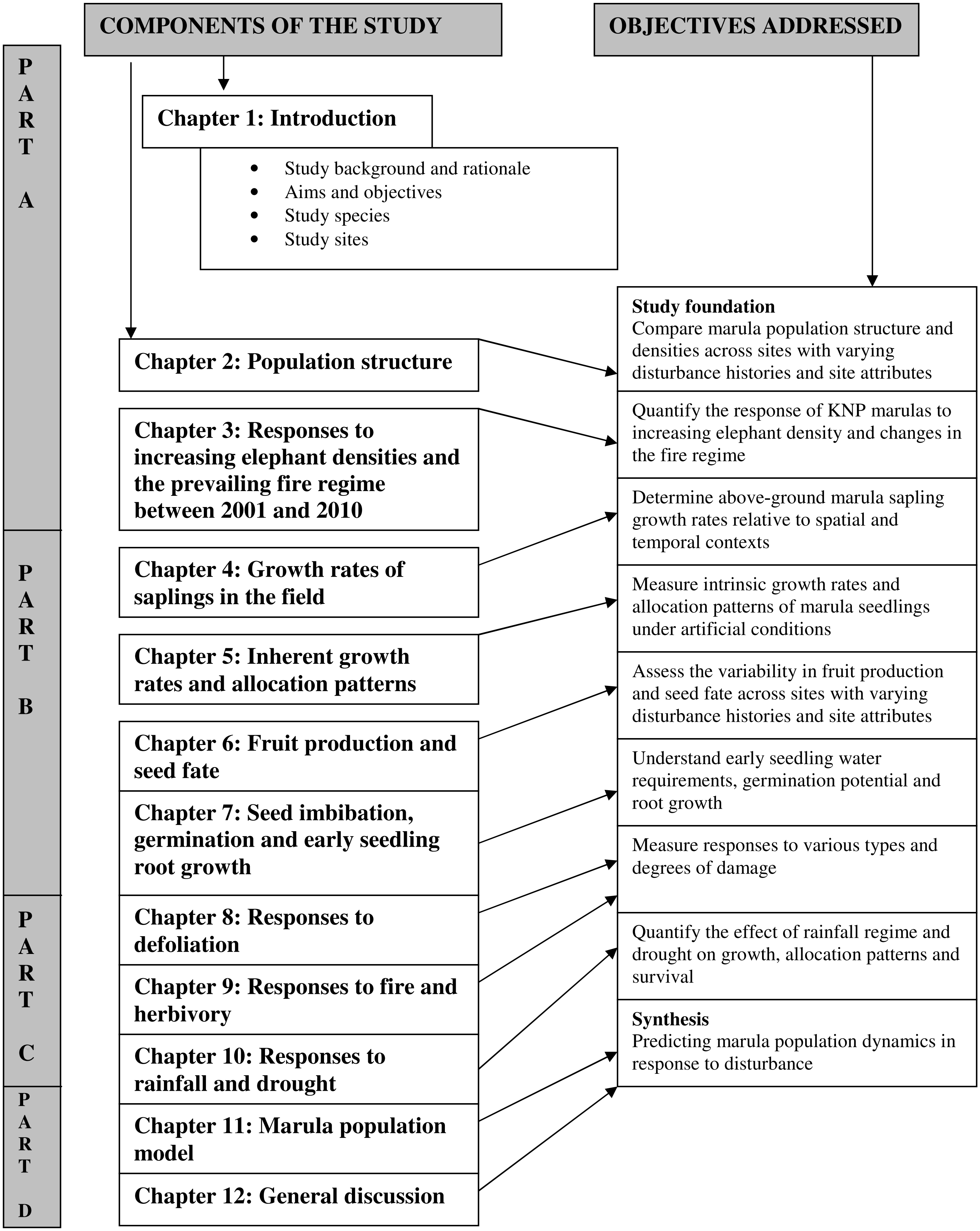


Figure 5: Thesis graphical overview.

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Chapter 2: Why is there such variation in *Sclerocarya birrea* subsp. *caffra* (marula) population size structure?

Description of the main population structures in the lowveld and possible explanations for how they came about

2.1. Abstract

Due to the availability of a large number of datasets on marula populations from different areas in the low altitude savannas of South Africa, comparisons between populations could be made. Four distinct groups of populations were identified: 1) adult dominated populations consisting of only adults and no juveniles or seedlings. These occurred in low rainfall protected sites with no elephant or fire, as well as at medium rainfall sites in the Kruger National Park (KNP) with both high elephant density and frequent fire. The size class distributions of this group were bell-shaped, indicating an unstable population which may have come about through circumstances that no longer occur in these areas, for example human settlement. High impala utilisation of seedlings or rodent seed predation appear to be the main cause of the lack of recruitment for these populations, since fire is absent from the no elephant site; 2) stable population structures in populations protected from elephants, as well as at high rainfall sites with elephants, indicating that without elephants and in suitable habitats, marulas are able to attain a stable size structure and instability is not inherent; 3) juvenile dominated populations with almost no adults. Adults are relegated to a few refugia in these populations, where elephants are unable to reach them. These populations occur in the drier northern granitic and central KNP gabbroic soils. This indicates that rainfall and soil type have interacting effects with elephant impact on the population structure. No individuals were found on the dry northern basaltic soils, indicating that these populations have already been extirpated; 4) juvenile dominated populations consisting of mostly juveniles and few adults, occurring on the central and southern basalt plains of the KNP, as well as the central and southern mixed bushwillow woodlands on granite of the KNP. These populations have "missing size classes" varying between 5-40 cm in diameter and 2-8 m in height. All these sites have fire and elephants. This unstable structure was most likely caused by fire suppression of juveniles and elephant utilisation of individuals in the "missing size classes" to varying degrees in different areas. The wide "missing size class" in some populations indicates that elephant pressure on fire escaped saplings is of more concern than fire suppression of the juveniles.

2.2. Introduction

Many years of field research are required to gain an understanding of the population dynamics of long-lived woody plants, and thus insights into their long-term dynamics are difficult to obtain. Size class distributions (SCD) are the result of growth, recruitment and mortality (Kirkpatrick 1984), acting over an extended period of time and have frequently been used as indicators for future demographic patterns in woody plants (Bullock *et al.* 1996; Condit *et al.* 1998). Woody plant size is considered to be of greater importance than age in determining population structure, as vital rates are size-dependent (Harper 1977).

Although data on SCDs of a population are easily collected, they are ‘snapshot’ representations of population composition at one point in time (Condit *et al.* 1998; Lykke 1998; Neke 2005). Condit *et al.* (1998) point out that SCDs do not correlate with the growth rate of larger individuals nor with their survival, and attempts to accurately estimate rates of change or to predict future changes are confounded by assumptions on the behaviour and identity of the disturbance agents (Wiegand *et al.* 2000). In addition growth and mortality rates affect SCDs and thus further complicate their interpretation. For example, fast-growing species with high survival rates will have fewer juveniles than slow-growing species with low survival rates (Neke 2005). Wilson and Witkowski (2003) and Bond and Midgley (2001) also warn that SCDs can be misinterpreted as small individuals may not be true seedlings, but rather resprouting individuals having experienced different disturbance events, which could be older than larger individuals and with different growth dynamics. Thus, it has been suggested that static information on SCDs of woody plants is not a good predictor of future population trends (Condit *et al.* 1998; Lykke 1998). Instead, demographic information on a species is much more important for the prediction of future dynamics. However, obtaining long-term data on such information is impractical in many situations where management decisions need to be made immediately (Lykke 1998). Since long-term data on vegetation changes in savannas are generally lacking, Condit *et al.* (1998) and Lykke (1998) have made suggestions for utilising a new method for the assessment of SCDs. This method is based on indications that the growth of the smallest size class is found to be a strong predictor of a SCD (Condit *et al.* 1998) and therefore uses the analysis of SCDs to obtain information on the rejuvenation status of trees. Growth in the smallest size class should correlate with the SCD because, in this size class, increases in growth should always flatten the distribution (Condit *et al.* 1998).

When describing SCDs one of the most important factors considered is the skewness of the distribution. Skewness specifies the degree of asymmetry. The SCD can have an L-shape (positive skewness) or J-shape (negative skewness), or it can be symmetrical (zero skewness) (Hara 1988). Inverted J-shaped SCDs are typical of strongly recruiting populations with decreasing numbers of larger plants. When episodic recruitment is involved the shape of the distribution will depend on the interval between recruitment events (Harper 1977; Lykke 1998). If there are long delays between recruitment events, the population may consist of mature tree stands without any recruits, resulting in a non-inverted J distribution (Owen-Smith 2007). If recruitment is highly variable, due to factors such as frequent fires or intense herbivore utilisation on recruits, the tree population may not reach a stable size distribution (Owen-Smith 2007).

Both height structure and basal stem diameter structure are of importance when examining SCD's. The vertical structure in the landscape indicates the feeding heights available for browsers as well as the proportion of the population in the fire trap. Heights will also provide information on shading effects on shorter individuals in denser woody vegetation. However, heights are often modified by factors such as damage by elephant, herbivory, lightning, or fire and therefore do not necessarily represent the actual sizes of trees as most biomass is below-ground (Rutherford 1983). Basal diameter is a much more accurate measurement of the actual size of a tree as it is not modified by damage. The age structure of the population can therefore be much more accurately interpreted from basal diameter SCD's. However examining a combination of both height and stem diameter will provide the highest level of information.

SCDs of marula have been determined and assessed by a variety of recent studies including work by M. Hofmeyr (unpublished data.), Walker *et al.* (1986), Jacobs and Biggs (2002), Gadd (2002), Buchanan (2005) and Neke (2005). A poor representation of seedlings, saplings and immature trees in these populations has been highlighted by these assessments (Hall *et al.* 2002), and the phenomenon of a "missing size class" (trees 2-8 m in height) has been identified by a number of authors (Jacobs and Biggs 2002; Neke 2005). In Nylsvley Provincial Nature Reserve, Walker *et al.* (1986) offered episodic recruitment success at intervals possibly exceeding 10 years as the explanation for the unstable marula population structure observed there. Recent attempts at explaining the "missing size class" have drawn on hypotheses that: (1) elephants (Jacobs and Biggs 2002) or small browsers such as impala (Haig 1999) are preventing seedling recruitment; (2) excessive fire is preventing seedlings from establishing into the upper canopy; (3) the fire/herbivore trap is resulting in a recruitment bottleneck (Midgley and Bond 2001); or (4) that episodic recruitment in response to rainfall and other favourable conditions could simply be a life history strategy of marula. Additional explanations could be based on competition from the present cohort of individuals in a population, preventing the recruitment of the next cohort until space opens up. However, there is no evidence of even aged stands such as those present in *Acacia tortilis* in East Africa, where the rinderpest pandemic killed most of the browsers in the 1880s and other diseases killed impalas in the 1960s, were used in explanation (Prins and van der Jeugd 1993).

The opportunistic spread of marula individuals into disturbed sites has also been reported (Hall *et al.* 2002). In the Kruger National Park (KNP), the vegetation changed between 1950 and 1970, with a shift from *Acacia nigrescens / Combretum imberbe* dominance to marula dominance on the basalt soils (van Wyk 1974). Overgrazing by large wild ungulates, more intense fires and where *Acacia nigrescens* mortality was concerned, woodborers, were all put forward as explanations for this shift in dominance (van Wyk 1974). Marula trees did not increase in number but rather managed to survive the disturbance whereas the other species did not.

Marula populations have been studied in a variety of habitats in the low altitude savannas of South Africa and many data sets (published and unpublished) are available from which to glean population structure data. This chapter aims to describe the observed marula population structure and density at up to 22 sites that vary in terms of disturbance history (elephant densities, browser densities and fire frequencies), soil type and climate. It is hypothesised that populations exposed to little disturbance will have stable SCDs, while those exposed to frequent fire and intensive herbivory, will have unstable non-recruiting SCDs.

This study therefore had the following objectives:

a) Assess and compare the density of juveniles and adults for each population
b) Assess and compare the height and stem diameter size distributions for each population
c) Analyse the population trends in terms of stability, dominance and evenness for each population.
d) Identify common trends which could allow for grouping of populations based on size class distribution.

2.3. Methods

2.3.1. Population descriptions

A number of marula populations have been studied by previous researchers in the north-eastern low altitude savannas of South Africa and some of these datasets were acquired (Table 1; Figure 1). Some datasets overlapped in terms of vegetation types in the KNP (specifically M. Hofmeyr (2002 unpublished) with Jacobs and Biggs (2002)), but these studies were conducted at different times and by different researchers and hence the populations were analysed separately.

In addition to these data sets, further transects were surveyed: 1) at Wits Rural Facility (WRF) in August 2008 (8 transects), 2) in the KNP within (4 transects) and outside (6 transects) the Hlangwine enclosure (HE) in January 2009, near Albasini (ALB) in October 2008 (3 transects), on the Phabeni road (PHA) in July 2008 (3 transects), and 3) at Jejane Private Nature Reserve (JPNR) near Hoedspruit in January 2008 (8 transects) (Table 1; Figure 1). These additional transects were surveyed as part of the greater study presented in this thesis and were included here as they made useful contributions to the population structure data already available. In addition they covered areas in the KNP which had not been previously studied by other researchers. JPNR was included in particular, as it was a reserve with no elephants and no fire but with large numbers of browsers.

Based on the available data, 22 reproductively isolated populations were defined in this study for comparison (Table 1; Figure 1). However, since only density data was available from Gadd (2002), population structure data could only be derived for 19 of these populations. These populations cannot be directly compared to data already published by Jacobs and Biggs (2002), as the raw data was subdivided using different criteria to those used in the original study. Impala densities were difficult to obtain for the different populations but estimates based on Jacobs and Biggs (2002) are provided in Table 1. According to KNP databases, impala densities were 0.03/ha, 0.05/ha and 0.08/ha in the entire northern, central and southern regions respectively in 2000; while in 2010 the densities were 0.03/ha, 0.12/ha, 0.12/ha (J. Botha, Scientific Services, KNP, unpublished survey data).

2.3.2. Sampling and measurement

For the field sites surveyed by this study, transects of 40 m wide for individuals ≥1m in height and 2 m wide for individuals <1m in height, were surveyed perpendicular to access roads. These were of variable lengths due to variations in site conditions such as roads. Stem basal diameter 30 cm from the ground or above the basal swelling and height were measured for each tree encountered. This overall methodology was chosen as it matched the methods used by M. Hofmeyr (unpublished data) in 2002. The methodologies used by the various researchers varied within and between populations (Table 2). All these studies represent sufficiently large samples of the marula populations at the various sites and provided representative numbers of both adults and juveniles.

Diameter above the basal swelling was recorded for all trees except for the M. Hofmeyr and Jacobs and Biggs (2002) trees, which measured the diameter at breast height. Diameter at breast height (1.3 m) was converted to basal diameter using the allometric regression equation in Appendix 1.

Table 1: Population descriptions of 22 marula populations in the low altitude savannas of South Africa for which datasets could be obtained and which were analysed in the current study. See Figure 1 for further location information. Impala densities taken from Jacobs and Biggs (2002). * Very high densities due to provision of artificial water points (see Parker and Witkowski 1999). ** Broad estimate.

Population	**Location**	**Researcher**	**Year surveyed**	**Area surveyed (ha)**	**Geology**	**Fire frequency**	**Elephants**	**Disturbance rating**	**Browsers**	**Impala densities/ha (year)**	**Long-term average rainfall (mm)**
Sites outside the Kruger National Park											
Wits Rural Facility (WRF)	-24.48 S; 31.13 E	This study	2008	32	Granite with dolerite intrusions	Low	No	Low	Few	0.11 (2002)	680
Jejane Private Nature Reserve (JPNR)	-24.25 S; 30.98 E	This study	2009	8.84	Granitic gneiss	None	No	Medium	Many	3 (2007)	450
Private reserves (PR): Kapama, Tshukudu, Thornybush	-24.43; 31.02; -24.48; 31.13 -24.26; 30.96	Gadd (2002)	1997	45	Granitic gneiss	Low	Yes	High	Many	*	506
Sites inside the Kruger National Park											
Hlangwine enclosure (HE)	-25.21 S; 31.29 E	This study	2009	10	Granite	Low	No	Low	None	0 (2008)	737
Outside Hlangwine enclosure (OHE)	-25.19 S; 31.30 E	This study	2009	19.86	Granite	High	Yes	High	Many	2**	737
Sabie thorn thickets west (STT west)	-25.02 S; 31.40	Hofmeyr	2002	25	Granite	High	Yes	High	Many	2**	550
Sabie thorn thickets east (STT east)	-24.99 S; 31.80 E	Hofmeyr	2002	20	Granite	High	Yes	High	Many	1.7 (1997)	603
Gabbro thornveld north (GT north)	-24.41 S; 31.61 E	Hofmeyr	2002	15	Gabbro	Low	Yes	High	Many	2.15 (1997)	572

Population	**Location**	**Researcher**	**Year surveyed**	**Area surveyed (ha)**	**Geology**	**Fire frequency**	**Elephants**	**Disturbance rating**	**Browsers**	**Impala densities/ha (year)**	**Long-term average rainfall (mm)**
Gabbro thornveld south (GT south)	-25.00 S; 31.85 E	Hofmeyr	2002	10	Gabbro	Low	Yes	High	Many	1.7 (1997)	603
Mixed bushwillow woodlands (MBW)	-24.45 S; 31.53 E	Hofmeyr	2002	25	Granite	High	Yes	High	Many	2.15 (1997)	572
Marula knobthorn savanna (MKS)	-24.43 S; 31.78 E	Hofmeyr	2002	40	Basalt	High	Yes	High	Many	1.7 (1997)	544
Marula knobthorn savanna north (L17 north)	-24.43 S; 31.78 E	Jacobs and Biggs (2002)	2000	240	Basalt	High	Yes	High	Many	1.7 (1997)	544
Marula knobthorn savanna south (L17 south)	-25.22 S; 31.95 E	Jacobs and Biggs (2002)	2000	160	Basalt	High	Yes	High	Many	1.7 (1997)	620
Delagoa thorn thickets (DT)	-24.61 S; 3177 E	Hofmeyr	2002	15	Basalt	Medium	Yes	High	Many	2.15 (1997)	544
Mixed bushwillow woodlands north (L5 north)	-24.66 S; 31.63 E	Jacobs and Biggs (2002)	2000	260	Granite and gneiss	Medium	Yes	High	Many	2.15 (1997)	572
Mixed bushwillow woodlands south (L5 south)	-25.17 S; 31.63 E	Jacobs and Biggs (2002)	2000	140	Granite and gneiss	Medium	Yes	High	Many	2.15 (1997)	562

Population	**Location**	**Researcher**	**Year surveyed**	**Area surveyed (ha)**	**Geology**	**Fire frequency**	**Elephants**	**Disturbance rating**	**Browsers**	**Impala densities/ha (year)**	**Long-term average rainfall (mm)**
Mopane knobthorn savanna (L12)	-23.03 S; 31.04 E	Jacobs and Biggs (2002)	2000	400	Granite	High	Yes	High	Many	1.2 (1997)	515
Mopane shrubveld north (L23 north)	-22.56 S; 31.28 E	Jacobs and Biggs (2002)	2000	200	Granite	High	Yes	High	Many	1.2 (1997)	515
Mopane shrubveld south (L23 south)	-23.49 S 31.51 E	Jacobs and Biggs (2002)	2000	200	Basalt	High	Yes	High	Many	1.2 (1997)	504
N'washitsumbe enclosure (NE)	-22.78 S; 31.26 E	Jacobs and Biggs (2002)	2000	17.4	Basalt	Medium	No	Low	None	0 (2008)	515
Albasini (ALB)	-25.03 S; 31.28 E	This study	2008	12	Gabbro	High	Yes	High	Many	1.5**	550
Phabeni (PHA)	-25.03 S; 31.38 E	This study	2008	8.2	Gabbro	High	Yes	High	Many	1.5**	550

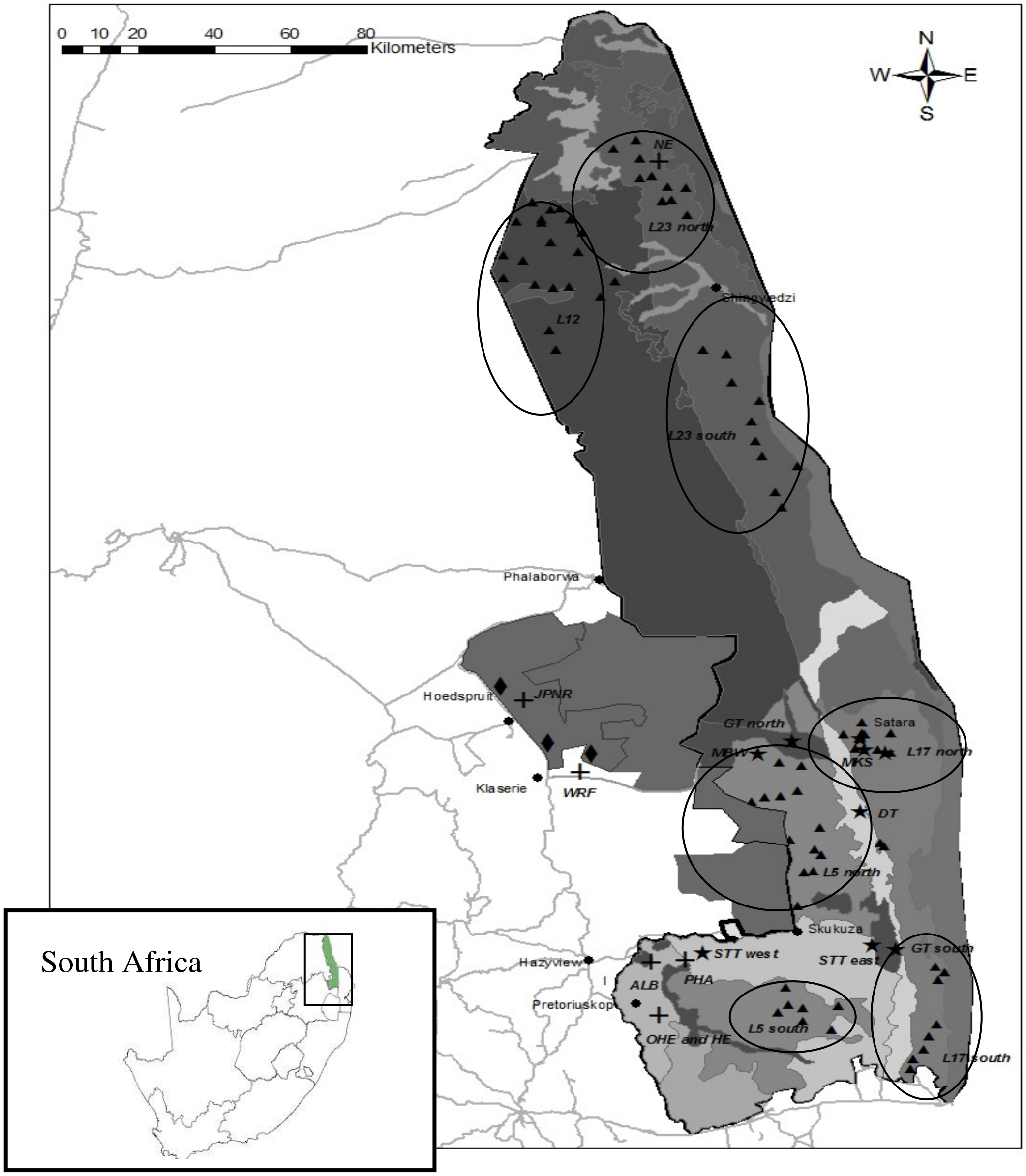


Figure 1: Map of 22 study populations assessed in the low altitude savannas of South Africa between 1996 and 2008. The western boundary of the Kruger National Park (KNP; black border) has been opened to private nature reserves (uniform grey). The shaded sections in the KNP indicate the different ecozones in the park (Grant and Thomas 2006). The black triangles represent all the transects surveyed by Jacobs and Biggs (2002) in 2000 and are grouped using circles, the black stars represent the sites surveyed by Hofmeyr (unpublished data) in 2002, the diamonds the private reserves surveyed by Gadd (2002) in 1996, and the crosses additional sites surveyed in this study. Abbreviations can be found in Table 1.

Table 2: Methodology used by the various studies to survey the marula populations in the low altitude savannas of South Africa.

Study	**Sites surveyed**	**Sampling unit**	**Sampling size**
Hofmeyr (2002)	5 ecozones in KNP	3-8 transects per ecozone	>1 m plants: 1250 m long, 40 m wide <1 m plants: 1250 m long, 2 m wide
Jacobs and Biggs (2002)	4 landscapes in KNP	20 road transects per landscape and 16 seedling transects per road transect	>2m plants: 2000 m long, 100 m wide <2m plants: 100 m long, 5 m wide
Jacobs and Biggs (2002)	N'washitsumbe enclosure (NE) in KNP	1 road transect and 48 seedling transects	1500 m long for >2 m plants 100 m long, 5 m wide <2 m plants
Gadd (2002)	3 private reserves near Hoedspruit	30 road transects per reserve	1000 m long, 5m wide

2.3.3. Data and statistical analysis

Both height and diameter SCDs were analysed and presented in terms of tree density (trees/ha). Toppled trees (n=135; 3%) were excluded from all SCD analyses. R freeware (V) and Statistica V.6 were used for statistical analyses.

2.3.3.1. Population density and structure

Density estimates were made from individual trees and not the number of stems, as some trees were multistemmed (responding to disturbance). Stems >14 cm in basal diameter were considered reproductively mature adults while stems <14 cm were classified as juveniles (Shackleton *et al.* (2005). Percentages and densities of mature and juvenile plants were reported for each population. ANOVA followed by Fisher's LSD was used to compare the density of juvenile and mature plants between populations. Paired t-tests were used to compare juvenile and mature tree densities within each population.

A general linear model was used to determine the most parsimonious model (using AIC) to explain the variation in overall tree density, reproductively mature tree density and juvenile tree density using the presence of elephant (yes or no), soil type (granite vs basalt) and mean annual rainfall as predictor variables.

2.3.3.2. Size class distributions

Basal diameter size classes were delimited as follows: <0.5, 0.5-2, 2-5, 5-9, 9-14, 14-20, 20-30, 30-40, 40-50, 50-60, 60-70, >70cm. These size classes were chosen to include more individuals with increasing size as advised by Condit *et al.* (2008), as well as to be biologically meaningful with certain class cut-offs based on life history stages such as fire resistance and reproduction. Height size classes were delimited as follows: <0.25, 0.25-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-8, 8-10, 10-12, 12-15, >15 m. In determining the number of woody plants represented by each size class, account was taken of the two-width plot design with the number of woody plants ≤1 m in height adjusted according to the area sampled for larger plants. Diameter SCDs were compared using the Kolmogorov-Smirnov distribution test. Diameter SCD slopes, to be used as indicators of population structure were calculated using the methods of Condit *et al*. (1998), Lykke (1998), and Obiri *et al.* (2002). A least squares linear regression using the corrected abundance per size class ($\ln(N_i+1)$) as the dependent variable and the size class midpoint ($\ln(d_i)$) as the independent variable were performed for all sites to obtain the SCD slope. As size classes varied in width, the number of trees in each size class was divided by the width of the size class to obtain the corrected abundance (N_i). Following Everard *et al.* (1995), negative slopes indicate ongoing recruitment (or growth suppression), with more individuals in smaller size classes than in larger size-classes; flat slopes indicate equal numbers of individuals in small and large size-classes showing either little recruitment or a relatively high number of large plants, perhaps from previous recruitment events; and positive slopes indicate little recent recruitment but possibly episodic recruitment in the past (or perhaps accelerated growth across intermediate size classes). Steepness of the slope was used to further describe recruitment trends. Steep negative slopes indicate better recruitment than shallow slopes.

Diameter and height SCDs were also compared between sites using Principal component analysis (PCA). Backeus *et al.* (2006) found that the use of PCA for comparing SCDs of different species or sites was an easy and rapid way to obtain an overview of the size distribution at different sites. A PCA was performed with number of individuals in basal diameter classes as ‘‘items’’ and populations as ‘‘samples”.

2.3.3.3. Population trends

Population stability at the various sites was measured by means of a) the permutation index (P) introduced by Wiegand *et al.* (2000), b) Simpson’s index of dominance as applied to size class evenness (see Wiegand *et al.* 2000; Botha *et al.* 2002) and c) the standard deviation around the mean quotient between successive size classes, also expressed graphically (Walker *et al.* 1986; Shackleton 1993; Botha *et al.* 2002; Shackleon *et al.* 2005), termed the quotient index (Q). These analyses were conducted on the diameter SCDs only. The Permutation Index (P) is used to measure the degree of deviation from the monotonic decline expected in an undisturbed population. Size-classes are ranked from smallest (most frequent) to largest (least frequent). A monotonically declining population will have P=0 and a population with a discontinuous SCD will have a P>0. The higher the P, the less stable the population. Simpson’s Index of Dominance describes the probability that any two trees drawn at random from the same community are of the same size class, by measuring the occurrence of dominant (more frequent) size classes (Wiegand *et al.* 2000, Botha *et al.* 2002). The Simpson Index measures the evenness of occupation of size classes, ignoring the order in which the size classes are arranged (Wiegand *et al.* 2000). Values above 0.1 reveal that the size frequency is steeper than would be expected from an exponentially declining population, while values below 0.1 show that the size classes are more evenly distributed, i.e. showing a higher rate of attrition between successive size classes than expected for a regularly recruiting population with constant growth between size classes. The combination of the Simpson’s and Permutation indices is considered effective in describing size frequency distributions that deviate from ‘ideal’ regularly-recruiting populations and which are shaped by rare recruitment events and irregular growth (Neke 2005). Population constancy (Harper 1977) was examined by calculating quotients between successive size-classes and displaying the results graphically. Quotients are a measure of growth from one size class to the next. Constant quotients between successive size-classes indicate a stable population, while fluctuating quotient values indicate an unstable population (Shackleton 1993; Botha *et al.* 2004).

2.4. Results

2.4.1. Population density and structure

Population densities ranged from 0 (L23 north and south) to 88.88 trees/ha (HE), with a mean±SE of 20.11±1.96 trees/ha (Figure 2). Mean adult and juveniles densities were 4.15±0.53 and 15.93±1.93 trees/ha respectively. Adult tree densities were significantly different from juvenile tree densities at: JPNR, OHE, PHA, ALB, STT east and west, L5 north and south, L17 north and south and L12. Some populations had no juveniles (PHA, STT east and west, JPNR, ALB) and no trees were found at L23 north or south. There seems to be a general trend of decreasing adult density with decreasing rainfall in the KNP sites (Figure 2). PR stands out from the other sites with elephants, as these sites only have low elephant densities, which were introduced less than a decade before the surveys were conducted.

Up to 40.2% of the variation in total tree densities could be explained through rainfall, elephant presence, soil type and their two-way interactions (Table 2). At lower rainfall elephants decreased the tree density much more than at higher rainfall (Figure 3). Soil type did not affect tree density when elephants were absent but in their presence, tree densities were lower on basalt than on granite. At lower rainfall, tree density on basalt was lower than on granite.

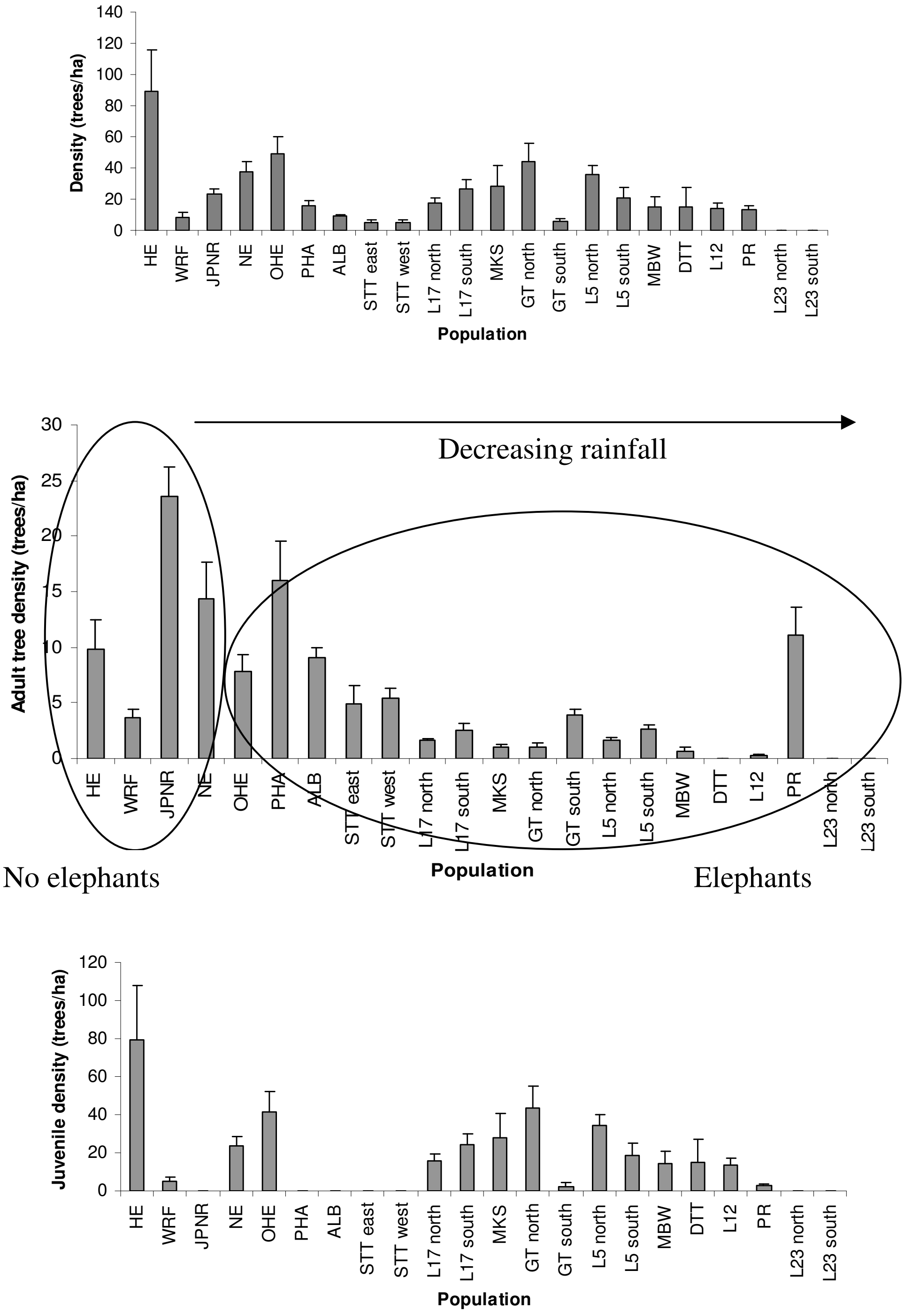


Figure 2: Range of marula densities (mean±SE) (adult/ reproductively mature (>14 cm in basal diameter), juvenile and in total) for 22 populations assessed in the low altitude savannas of South Africa. See Table 1 for abbreviations. Populations arranged from left to right in terms of elephant absence and presence and then with decreasing rainfall per group.

Table 3: Multiple regression results for adult, juvenile and total tree densities between twenty two populations in the low altitude savannas of South Africa. See Table 1 for abbreviations.

Response variable	**Model R^2 (%)**	**Model F**	**Model df**	**Model p**	**Predictors**	**Coefficient**	**SE**	**t**	**p**
Log(trees/ha+1)	40.20	15.78	6,141	**<0.0001**	Intercept	-4.13	2.58	-1.60	0.1100
					Rainfall	0.015	0.0048	3.14	**0.0021**
					Elephant	-3.47	0.81	-4.30	**<0.0001**
					Soil type (Soil)	3.64	1.13	3.23	**0.0015**
					Rainfall*Elephant	0.0029	0.0013	2.29	**0.0240**
					Elephant *Soil	0.84	0.31	2.67	**0.0085**
					Rainfall*Soil	-0.009	0.0020	-4.58	**<0.0001**
Log(reproductively mature trees/ha+1)	54.44	42.71	4,143	**<0.0001**	Intercept	5.90	0.69	8.54	**<0.0001**
					Rainfall	-0.0078	0.0011	-6.87	**<0.0001**
					Elephant	-3.76	0.43	-8.84	**<0.0001**
					Soil	0.14	0.053	2.66	**0.0087**
					Rainfall*Elephant	0.0056	0.0007	7.54	**<0.0001**
Log(juveniles/ha+1)	27.41	10.72	5,142	**<0.0001**	Intercept	-5.04	2.66	-1.90	**0.0600**
					Rainfall	0.018	0.005	3.84	**0.0002**
					Elephant	-2.006	0.74	-2.73	**0.007 2**
					Soil type	1.47	1.42	1.03	0.3000
					Elephant *Soil	1.21	0.39	3.09	**0.0024**
					Rainfall*Soil	-0.0068	0.0023	-2.86	**0.0049**

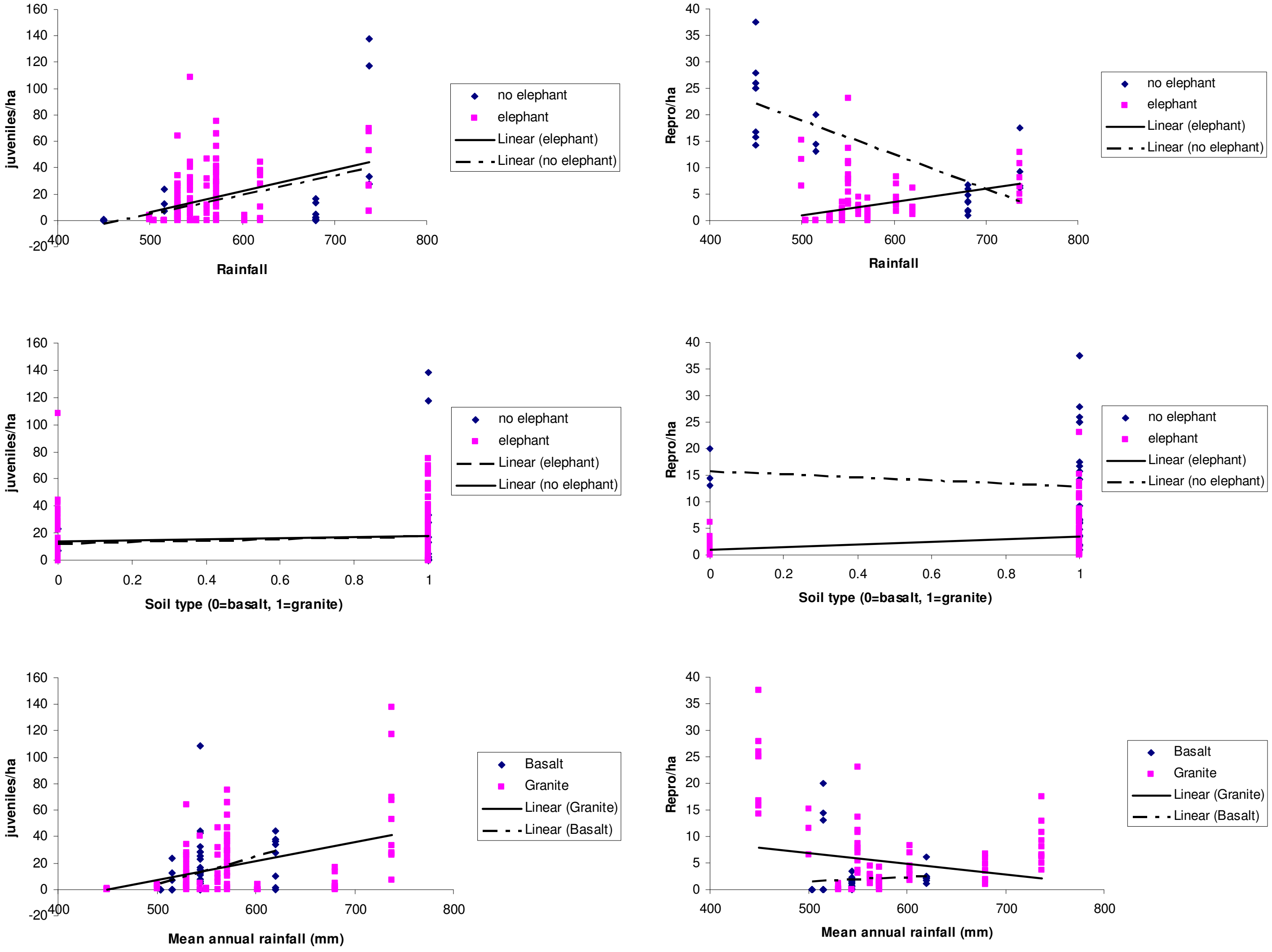


Figure 3: Marula juvenile and adult (reproductively mature (repro/ha)) tree densities as a function of soil type, elephant presence and mean annual rainfall for 22 sites in the low altitude savannas of South Africa.

2.4.2. Size class distributions

SCDs could be classified into 4 main groupings. Kolmogorov-Smirnov tests showed significant differences ($p<0.05$) between the distribution of the basal diameters of the populations in the four main groups (described below).

1) Adult dominated populations

Five populations including JPNR, PHA, ALB, STT east, STT west consisted almost entirely of adult trees and recruitment failure had occurred for a number of years (Figure 4). Kolmogorov-Smirnov tests showed no significant differences ($p>0.05$) between the distribution of the basal diameters of these populations. At JPNR most trees were between 30-40 cm in diameter and 10-12 m in height and the stem diameter SCD approximated a normal curve (Figure 4) as did that of the other four populations. At STT east and west most individuals were between 50 and 60 cm in diameter and 12-15 m in height. A younger population was evident at ALB with most individuals 30-40 cm in diameter and 8-10 m in height. At most of these sites there was a sudden drop off of individuals >50 cm in diameter. At GT south a similar situation exists but a recruitment event appears to have occurred resulting in the presence of some individuals between 0.5 and 2 cm in diameter. No significant differences in the distributions of GT south and the other populations in this group were found.

2) Healthy recruiting populations

The density distributions at HE, OHE, WRF and NE all showed rotated sigmoid curves with most trees concentrated in the 0.5-5 cm diameter and 0.25-1 m height class (Figure 4). These results indicate healthy recruiting populations. Although it appears that NE has a "missing size class" between 5 and 20 cm in diameter, this is most likely an artefact of the sampling design as many individuals in these size classes were observed on subsequent visits (pers. obs.). Kolmogorov-Smirnov tests showed no significant differences ($p>0.05$) between the distribution of the basal diameters of these populations, except between WRF and OHE and HE, most likely due to the much greater marula densities at the Pretoriuskop sites (HE and OHE). Importantly there were no structural differences inside and outside the enclosure (HE and OHE), nor between the two enclosures in the KNP (NE and HE).

However, Jacobs and Biggs (2002) found significant differences between inside and outside the roan enclosure (NE and ONE), with the population outside the enclosure (L23) almost extirpated in 2001.

3) Juvenile dominated populations

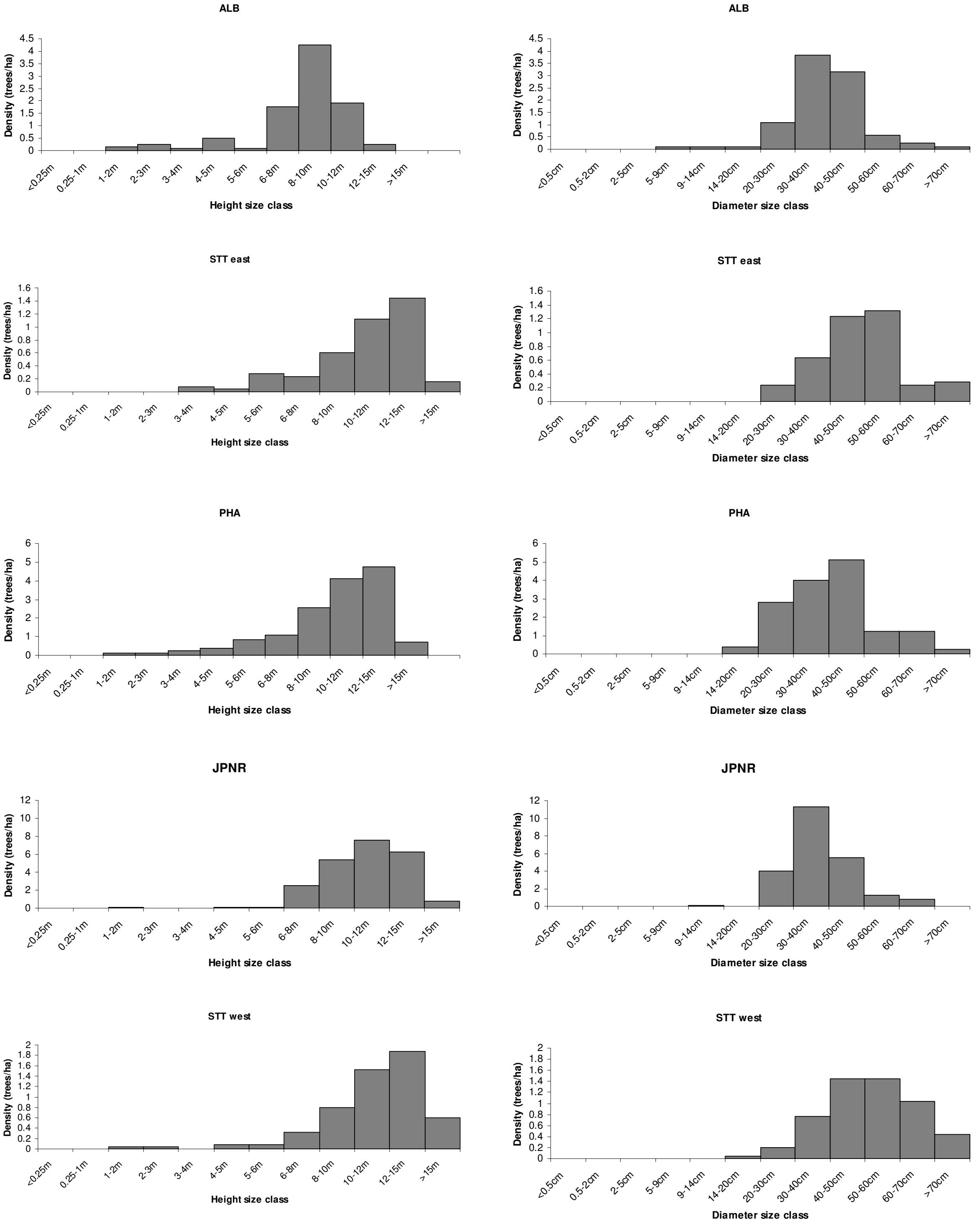
The density distribution at DT could be represented by a normal curve but with most individuals between 5 and 9 cm in diameter and 0.25-1 m in height and no adults (Figure 4). The density distribution of L12 was similar but with most individuals between 0.5-2 cm in diameter and a very low adult density of <0.5 trees/ha. The Kolmogorov-Smirnov test showed no significant difference ($p>0.05$) between the distribution of the basal diameters of these populations. These populations consist almost entirely of juveniles and hence recruitment would rely entirely on the escape of the small individuals from the fire and browsing traps, which does not appear to have occurred for many years.

4) Populations with a "missing size class"

These are separated into two subgroups, but a Kolmogorov-Smirnov test showed no significant difference ($p>0.05$) between the distribution of the basal diameters of these populations between subgroups a) and b) (see below).

a) The density distributions of MKS, MBW and GT north could be represented by rotated sigmoid curves but with very low adult densities (≤1 tree/ha) and the presence of a "missing size class" between 14 and 20 cm in diameter and 2-6 m in height. The Kolmogorov-Smirnov test showed no significant difference ($p>0.05$) between the distribution of the basal diameters of these populations.

b) The remaining populations (L5 south and north, L17 south and north) consisted mostly of juveniles with adult densities between 1 and 2.6 trees/ha. The ubiquitous presence of a "missing size class" between 5 and 9 cm in diameter also sets this group of populations aside from 4a. The Kolmogorov-Smirnov test showed no significant difference ($p>0.05$) between the distribution of the basal diameters of these populations.



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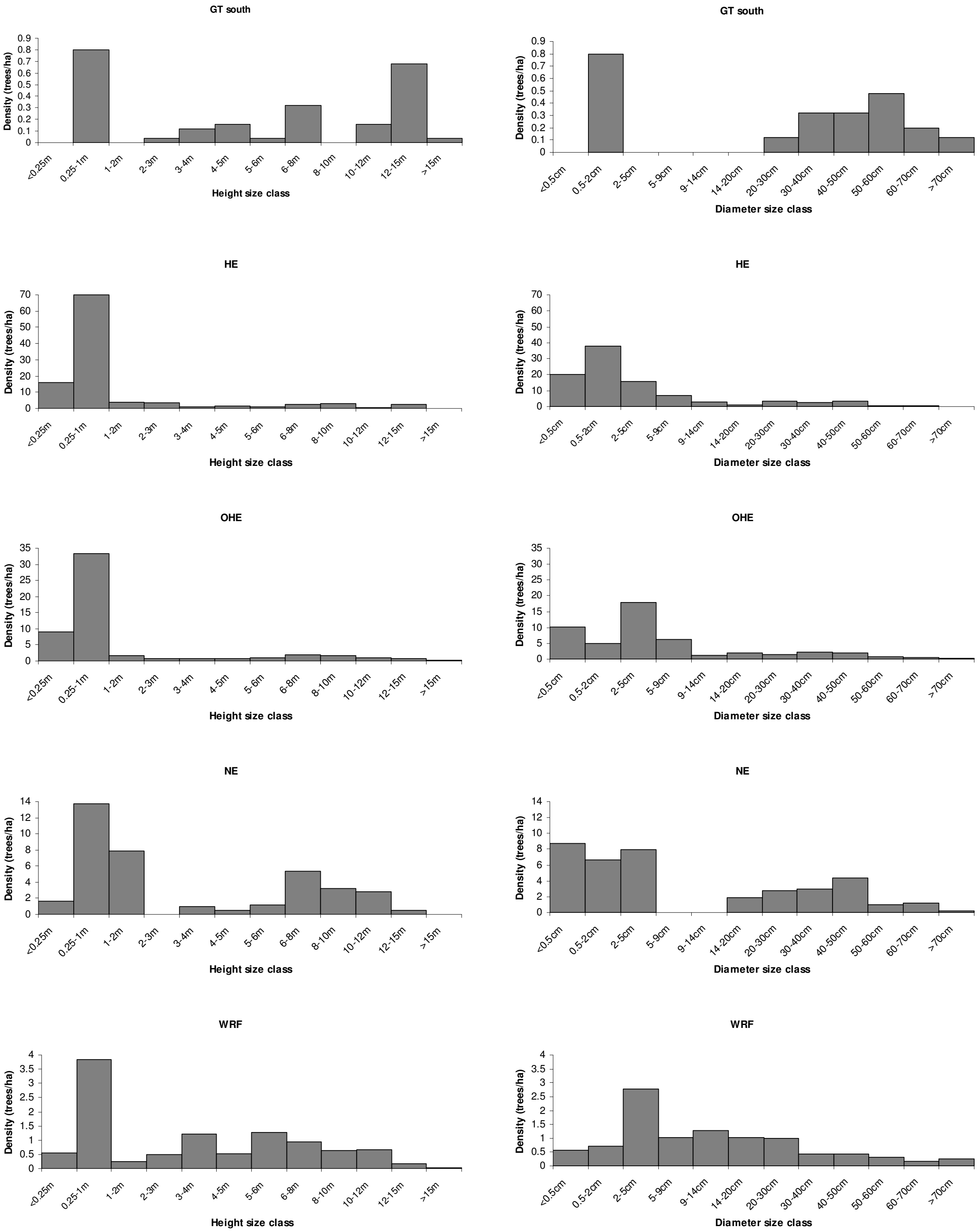

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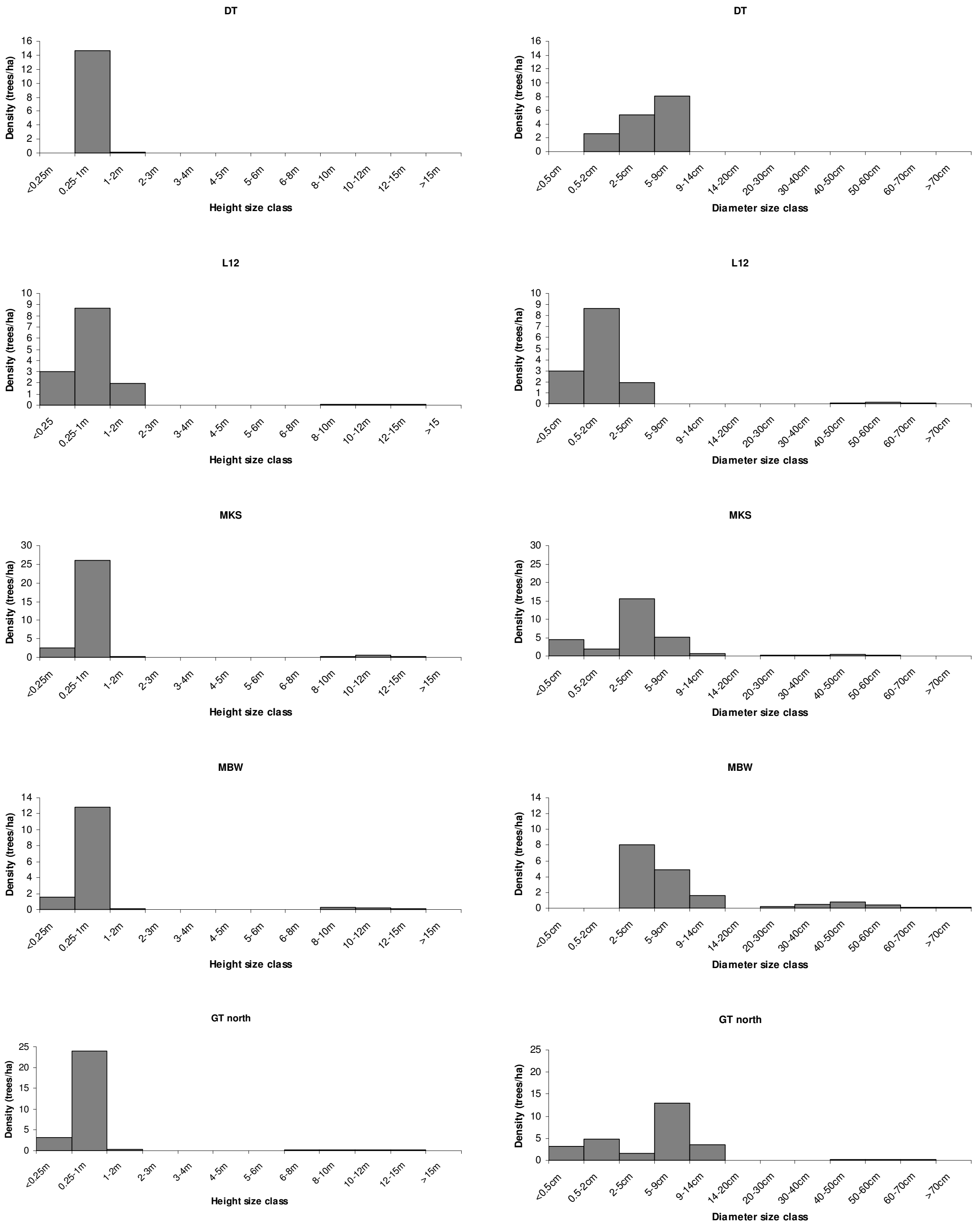

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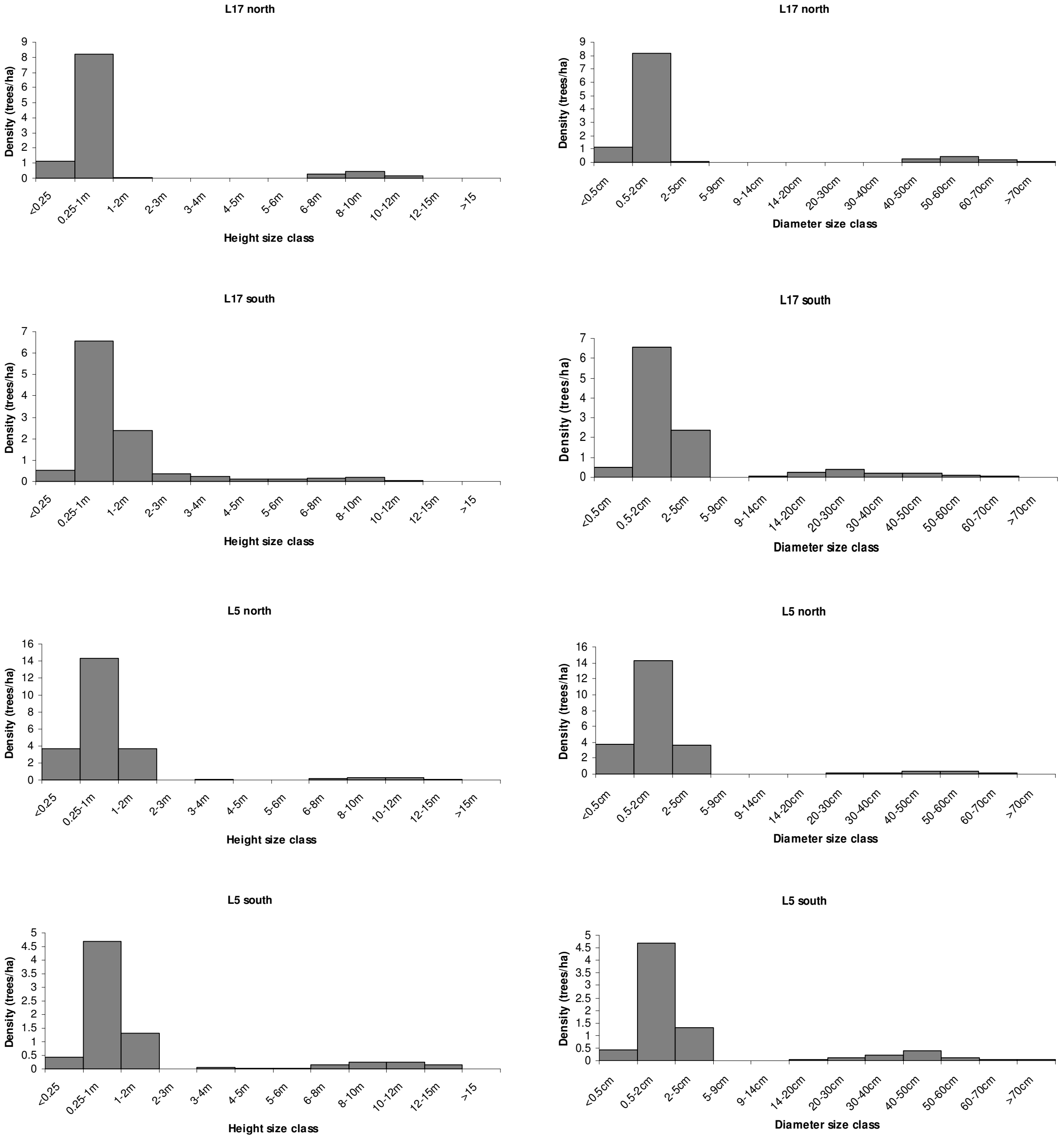


Figure 4: Height and diameter size class distributions (trees/ha) of marula at nineteen sites in the low altitude savannas of South Africa. Note the different y axis scales due to large variations in density across sites.

The prinicipal component analysis (PCA) using stem diameter indicated that axis 1 could explain 74% of the diameter variance and 84% of the diameter environment relation (Figure 5). Axis 1 represents the sequence of populations with HE on the far right and the group of JPNR, ALB, PHA and STT east and west (almost no juveniles) on the left hand side. The sites on the left hand side of the diagram have more individuals in the larger size classes, while those on the right have more individuals in the smaller size classes. HE is strongly associated with individuals between 0.5 and 2 cm in diameter while OHE is associated with individuals 14-20 cm in diameter. Density is indicated on the y axis. The large separation of HE and OHE indicates the large difference in density between these two populations. Axis 1 of the PCA using heights explained 95% of the height variability and 96.1% of the height environment relation (Figure 5). The sites on the left side of the diagram have more individuals in the taller size classes.

2.4.3. Population trends

SCD slopes were negative for HE, NE, OHE, L17 north and south, MKS, GT north, L5 north and south and L12 indicating more individuals in smaller size classes than larger size classes (Table 3). L12 and L5 north had the steepest slopes. Positive slopes were found for PHA and STT east and west, indicating that little recruitment was taking place.

Permutation indices ranged from 8 (DT) to 70 (PHA) (Table 3). Permutation indices >40 (WRF, PHA, ALB, STT east and west, L17 north, GT south, L5 north and south, MBW and L12) indicate that recruitment and mortality are highly episodic events.

The SCDs of all populations were steeper than would be expected from an exponentially declining distribution (Simpson's Index of Dominance range: 0.13-0.64) (Table 3). WRF and NE had relatively even distributions while L5 north and south, L12, and L17 north had populations strongly dominated by small individuals (high indices).

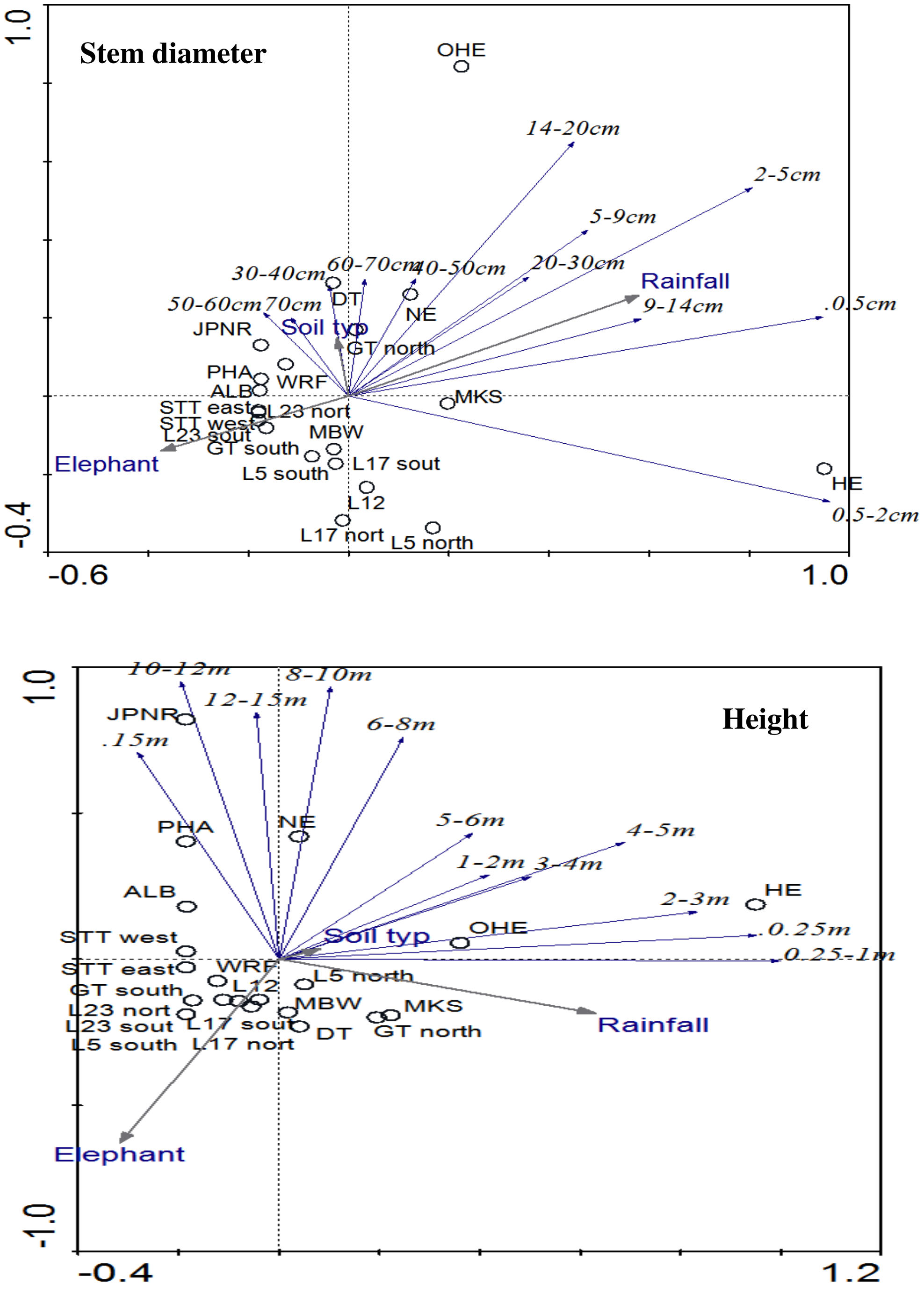
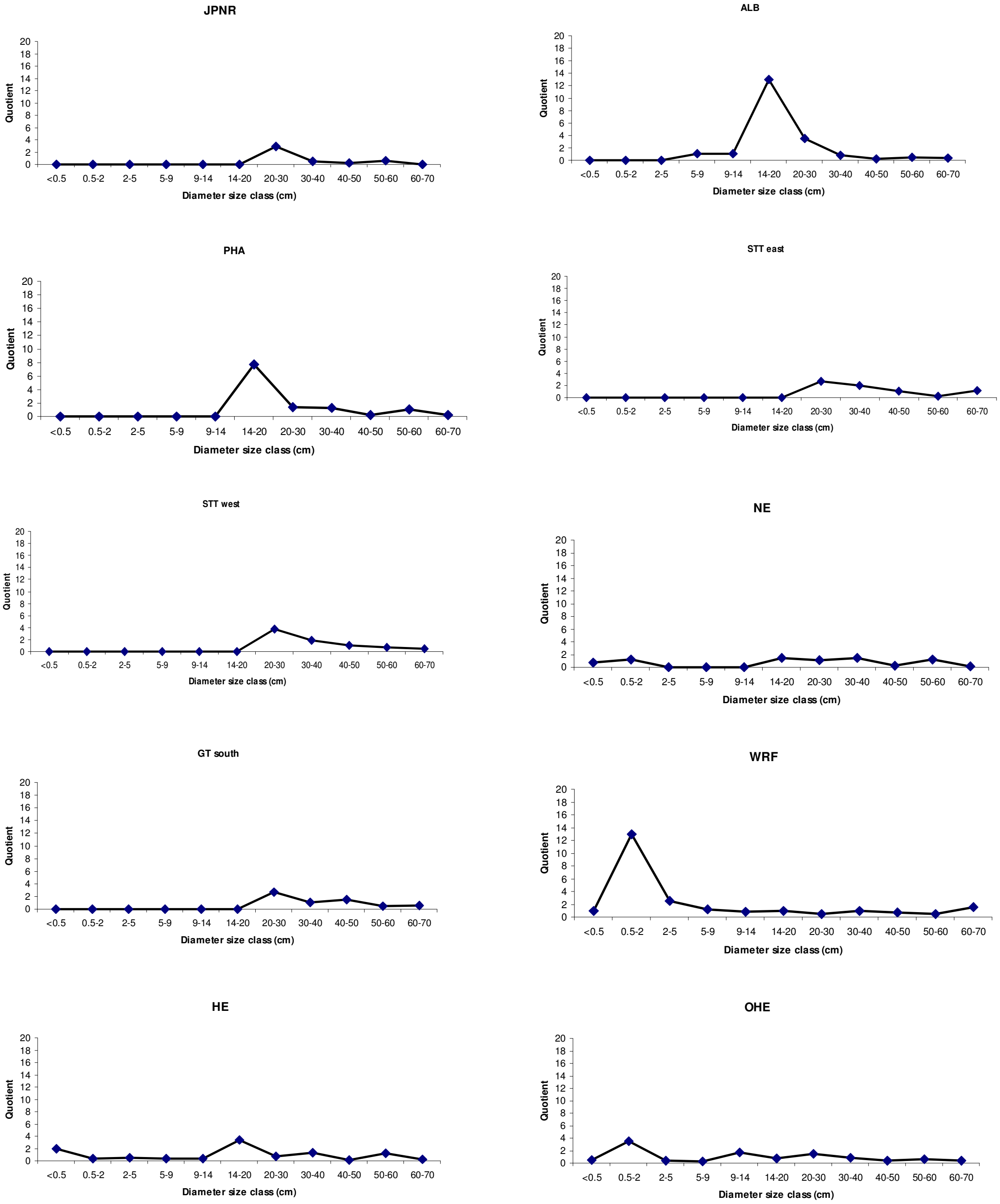


Figure 5: Principal component analysis (PCA) plot of tree density (trees/ha) with stem diameter and height size classes as explanatory variables and sites as samples and supplementary environmental variables. Site abbreviations can be found in Table 1.

Quotients calculated between successive size classes indicated that none of the populations were evenly distributed (Table 3; Figure 6). The mean Q for L17 north (2.98) and very high standard deviation (6.18) indicate that this population is highly unstable due to high irregularity of movement from one size class to the next. WRF, ALB and L17 south and L5 south also show very unstable populations due to high mean Q. HE and OHE show relatively stable populations with little fluctuation.

Table 4: Percentage juveniles, slope of the regression of diameter size class midpoint and number of trees per size class, permutation index and Simpson's index of dominance for marula at nineteen sites for which diameter distribution data was available in the low altitude savannas of South Africa. Different gray scales represent the four groups identified from the SCD's in Figure 4, with the grey scale lightest for the adult dominated group (Group 1) and darkest for the "missing size class" group (Group 4).

SCD groups/ sites	**Juveniles (%)**	**Slope**	**R^2**	**F**	**p**	**Permutation index**	**Simpson's index of dominance**	**Mean Quotient±SD**
PHA	0.0	0.2	0.31	4.54	**0.06**	70	0.23	1.97±2.84
JPNR	0.51	0.2	0.22	2.76	0.13	12	0.33	0.70±1.09
ALB	1.8	0.2	0.21	2.71	0.13	66	0.30	2.54±4.36
STT east	0.0	0.35	0.26	3.57	0.088	52	0.24	1.17±1.02
STT west	0.0	0.5	0.37	5.74	**0.038**	61	0.20	1.31±1.38
GT south	33.9	-0.76	0.13	1.45	0.26	47	0.19	0.56±0.86
HE	88.9	-1.1	0.94	165.61	**<0.0001**	12	0.24	0.96±0.98
WRF	57.7	-0.1	0.07	0.75	0.406	48	0.13	2.16±3.64
NE	61.8	-0.8	0.63	17.14	**0.002**	32	0.16	0.85±0.58
OHE	84.1	-1.0	0.92	110.92	**<0.0001**	19	0.20	0.98±0.99
DT	100.0	-0.4	0.23	3.02	0.11	8	0.39	1.22±1.59
L12	98.1	-1.4	0.68	21.27	**0.001**	39	0.46	2.61±4.32
MKS	96.6	-1.2	0.88	70.03	**<0.0001**	20	0.29	1.45±1.75
GT north	97.8	-1.0	0.79	39.49	**<0.0001**	29	0.30	1.32±2.32
MBW	95.8	-0.4	0.19	2.33	0.16	34	0.27	1.39±1.76
L5 north	95.4	-1.3	0.71	23.92	**0.0006**	34	0.44	1.43±139
L5 south	87.6	-1.0	0.58	13.71	**0.004**	36	0.44	2.67±3.67
L17 north	90.7	-1.0	0.48	9.21	**0.013**	47	0.64	2.98±6.18
L17 south	90.3	-1.1	0.67	21.07	**0.001**	28	0.44	2.41±3.91



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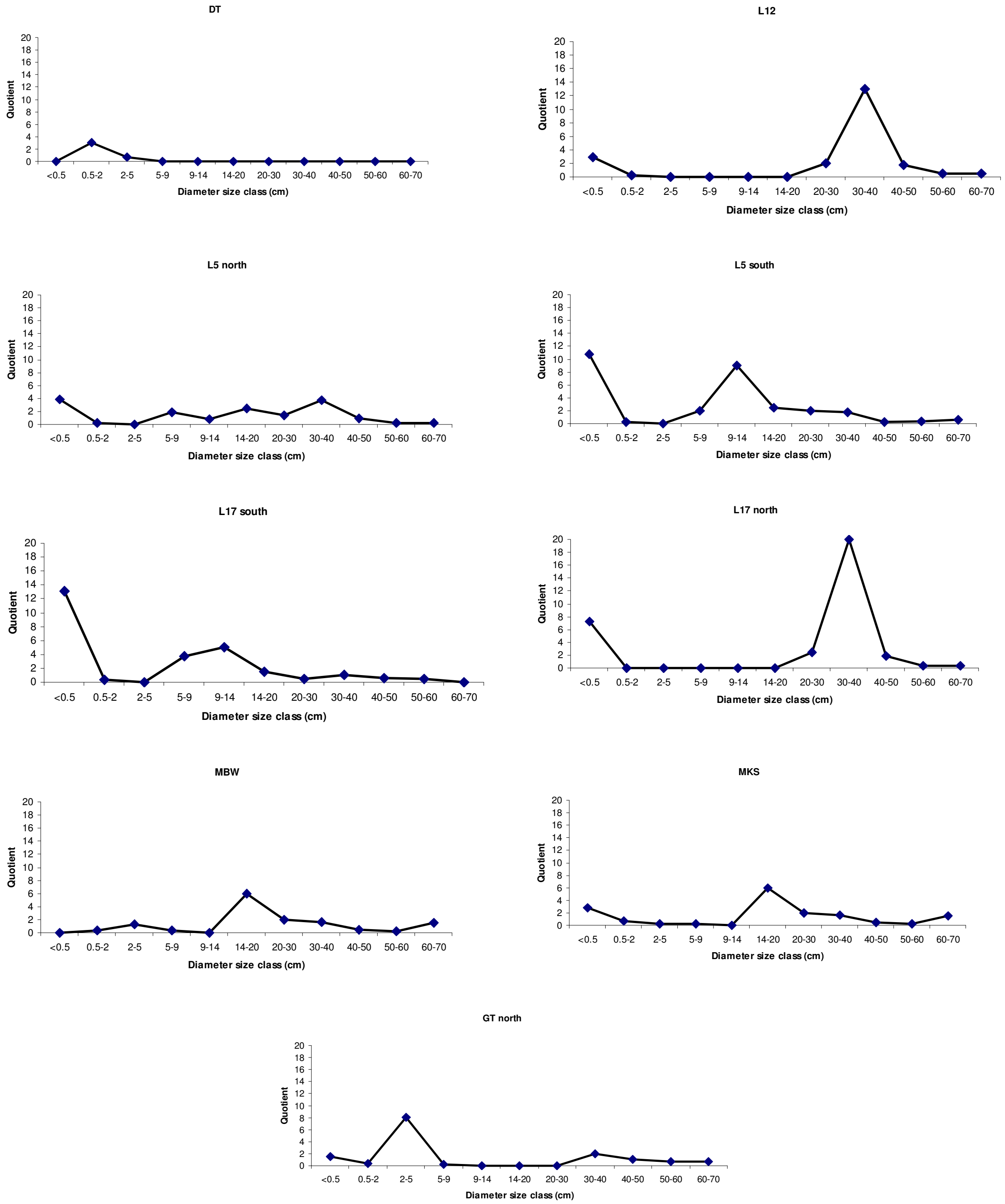

Figure 6: Quotient distributions for marula at nineteen sites in the low altitude savannas of South Africa.

2.5. Discussion

It is noteworthy that the marula SCDs differed so greatly between populations. Distributions did not conform to typical standard curves referred to in the literature, such as the reverse J-shaped curve, which is often cited as being indicative of a strongly recruiting population. However, four statistically and biologically distinct main groups of frequency distribution curves could be identified: 1) Adult dominated populations at JPNR, STT east and west, PHA, and ALB had normal curves, positive regression slopes and very high permutation indices, indicating that recruitment had not taken place in many years and is probably highly episodic. The sudden break off after 12 m or 15 m in height indicates either natural senescence at this height or an episodic recruitment event where the individuals <12 m at present were able to recruit. Other environmental influences such as soil type could also influence the size that trees could attain; 2) Healthy recruiting populations at OHE, HE, NE and WRF where there is a relatively stable size structure with many small individuals and few large individuals, but which are present relatively equally through the size classes and with either slightly negative or flat regression slopes; 3) Juvenile dominated populations at DT and L12 had almost no large individuals and negative regression slopes; 4) Populations with “missing size classes” at a) MBW, MKS, and GT north with many small and few large individuals distributed evenly through the higher size classes and with negative regression slopes; b) L5 north and south and L7 north and south have missing individuals in smaller size classes than in 4a, with steep negative regression slopes, high permutation indices and high levels of dominance of one size class.

The normal distribution curve representing the adult dominated populations (Group 1) is interpreted as showing a preponderance of individuals in intermediate diameter classes, which may be due to infrequent recruitment and selective felling of trees of larger sizes (Rao *et al.* 1990). According to Benton and Werner (1976), such a population is on the way to extinction if this trend continues (Rao *et al.* 1990). JPNR is the only site without elephants to have this distribution. There are reports that marulas were felled on a large scale in the lowveld in the 1950’s to make toilet seat covers (Shone 1979). The SCD at JPNR may reflect this history as individuals >50 cm in diameter are rare. The remaining populations in the adult dominated group coexist with elephants. Long-term recruitment failure has resulted in a cohort of individuals, the density of which is decreasing with time due to a combination of elephant utilisation and frequent fire (Helm *et al.* in press). Even-sized stands are undesirable since particular developmental stages are more vulnerable to certain environmental disturbances, stress or disease than others (Shackleton 1993). If most of the population is in one vulnerable stage there may be simultaneous loss of the whole cohort (Harper 1977). The lack of regeneration in these populations is cause for concern. Helm *et al.* (2009) noted a male biased sex ratio in these populations, indicating selection of females by elephants. If this trend continues, the recruitment bottleneck could become even more severe as new seed would be required for regeneration given the lack of juveniles in these populations. The lack of seedlings indicates that recruitment in these populations is highly episodic. Further evidence for this includes the sudden drop off of individuals >50 cm at ALB and PHA, indicating that the current high density of individuals between 30 and 50 cm in diameter was due to a massive recruitment event during particularly favourable years. Estimates of the age of standing marula trees would also be useful in determining when these individuals established. There is little reference to growth rates and ages of specific size classes in the literature (Hall *et al.* 2002). Age estimates will be investigated in Chapter 4.

The sudden drop off of individuals >60 cm in stem diameter, is, however, less evident at STT west. Severe bottlenecks at the seedling level exist at these populations and these could result from either severe rodent predation of the seeds or heavy herbivory on the seedlings. Since recruitment is continuous at other locations in the KNP with similar rainfall and soil type (eg. L5 north and south), rainfall *per se* does not appear to be the limiting factor. Gadd (2002) and Walker *et al* (1986) indicated that the high palatability of marula seedlings may result in high mortality when not protected by other vegetation, resulting in the dearth of individuals <30 cm in diameter in the private reserves. Walker *et al.* (1986) found a lack of individuals <50 cm in diameter and concluded that successful regeneration was highly episodic, probably every 10-20 years. Tchamba (1995) found in Cameroon, where marula (*Sclerocarya birrea* subsp. *birrea*) was a dominant species, weak regeneration and recruitment classes with mature trees making up 77% of the population. In addition to the risk of insufficient regeneration, elephant pressure may be exacerbated when trees grow in even-sized stands. This has been implicated in the die off of *Acacia xanthophloea* in Kenya (Young and Lindsay 1988) and *Acacia tortilis* in the Serengeti (Prins and van der Jeugd 1993).

Rotated sigmoid curves characteristic of “missing size class” populations (Group 4) indicate a slowing down of growth in one of the diameter classes or variable growth rates in subsequent classes. Unequal mortality rates between size classes could also result in this type of curve. The “missing size classes” observed in these populations have been reported to be the result of fire suppression of individuals vulnerable to fire and the removal of individuals which have recently been able to escape the fire trap by elephants (Jacobs and Biggs 2002). Indeed the individuals that are missing are between 14-20 cm in diameter for GT north, MKS and MBW but for L5 and L17 the missing individuals are between 5 and 20 cm. Evidence suggests that marula is able to escape the fire trap from 2.75-3 m in height (Jacobs and Biggs 2001), which is equivalent to approximately 7 cm in basal diameter (Appendix 1). Hence, the missing individuals at L5 and L17 could be due to fire suppression. However, the much larger “missing size class” gap at GT north, MKS and MBW may rather be due to browsing pressure on the larger individuals that have already escaped the fire trap.

The juvenile dominated populations (Group 3) are probably advanced cases of the “missing size class” populations (Group 4), where all the large individuals have been removed, leaving only individuals held in the fire and herbivore traps. The lack of individuals in L23 found by Jacobs and Biggs (2002), corresponding to the now virtually extirpated population outside NE, may be the ultimate consequence of the continued application of the drivers responsible for the SCDs in group 2 and 3. Adult tree loss is evident in the KNP at a rate of up to 7% per annum (Helm *et al.* 2009). A decrease in mature trees diminishes the sexual reproductive potential of a population through a lowering of seed production (Botha *et al.* 2004). The individuals within the fire trap, unable to multiply due to lack of seed input, will eventually die, unless allowed to escape, ultimately resulting in local population extinction.

The occurrence of the reverse J-shaped curve indicates predominance of lower diameter classes. Ecologically, this curve is explained by equal mortality rates among diameter classes across the entire range of diameters. When a reverse J-shaped curve plots as a straight line of negative slope on a semi-log scale, the distribution is called a negative exponential (Westphal *et al.* 2006). This curve type could probably be assigned to the healthy recruiting populations (Group 2), indicating relatively stable structures for these populations. However, most of the populations in this group had size structures defined by the various descriptive indices, as deviating from a monotonically declining distribution curve, with a steeper than expected attenuation in woody plant numbers with increasing stem diameter size. This could be a consequence of a high mortality of small plants and/or the suppression of growth into successive stem diameter size classes as a consequence of fire or as a result of damage to aboveground biomass. Indeed most of the individuals in these populations were between 2-5 cm in diameter and 0.5-1 m in height, indicating that growth suppression may be a factor.

The quotient plots also indicated that all marula populations were unstable with variable transitions from one class to the next. Quotient plots from other southern African studies have also shown that savanna tree species demonstrate unstable populations (Taylor and Walker 1984, Walker *et al.* 1986). Fluctuations in quotients between frequencies of trees in successive size classes is common in savannas where high levels of fire, megaherbivores and anthropogenic disturbances are experienced (Shackleton 1993; Botha *et al.* 2002). Stability of woody populations is therefore probably rarely attained in an environment typified by large climatic fluctuations, even under low fire, herbivore and harvesting pressures (Shackleton 1993). Unevenness in quotients may, however, also be an artefact of the class intervals chosen. Inappropriate class intervals could be a result of misinterpreting the shape of the growth curve (i.e. assuming a linear relation between age and size when in fact it is sigmoidal) (Walker *et al.* 1986). This will be further investigated in Chapter 4.

Marula has been found to have the most discontinuous and unstable population structure by a number of studies that have compared various species (Walker *et al.* 1986; Neke 2005). The population size frequency distributions of savanna woody plant species commonly deviate from the classic monotonically declining curves representing ‘healthy’, continuously recruiting natural forest populations, instead showing evidence of pulsed recruitment and sporadic growth in response to episodically favourable conditions (Neke 2005). Neke (2005), comparing 12 broad leaved tree species within the same region, found that marula had a relatively humped population distribution, with a higher representation of plants of intermediate size (20 to 52 cm) than those of the preceding size classes (4 to 20 cm in stem diameter). Interestingly, marula showed a lack of individuals sized 2 to 4 m in height. This however, could be due to the sampling design used in this study. Marula was defined as having large mature trees and poor seedling establishment. Neke (2005) also found a relatively high permutation index (69) for marula in protected areas, compared to other species which had indices ranging between 1 and 9.

Periodic tree regeneration has been proposed as the cause of irregular size distributions in *Acacia* trees (Prins and Van der Jeugd 1993; Wiegand *et al.* 2000). Variable tree growth is also suspected to cause irregular tree size frequency distributions (Ruess and Halter 1990; Grice *et al.* 1994; Rohner and Ward 1999). Possible factors causing irregular growth in marula could be a highly episodic rainfall regime, growth suppression by herbivores, borer infestation, or poor tree moisture status. This will be dealt with in Chapter 4 when tree growth is examined in detail. Neke (2005) did find that marula had a population structure that could be interpreted as being reflective of episodic recruitment. Evidence for episodic recruitment was also provided by this study for the adult dominated populations (Group 1). However, there was also some evidence from the current study, particularly in the KNP, that browsing or fire may have inhibited growth into larger diameter size classes resulting in irregular growth through the size classes.

2.6. Conclusions

Having identified four main groups for the population structures of marula, an understanding of the drivers behind these structures begin to emerge. Transitional states for marula population instability can be observed through an examination of these population structures. Figure 7 summarises all the possible factors affecting the SCD of marula. Some of these factors will be examined in later chapters as indicated. The different SCDs observed in this study indicate that marula is a highly dynamic species with multiple stages of vulnerability. Elucidation of the causes of these various SCDs requires teasing apart the effects of various factors influencing irregular growth from those influencing recruitment and mortality (Neke 2005).

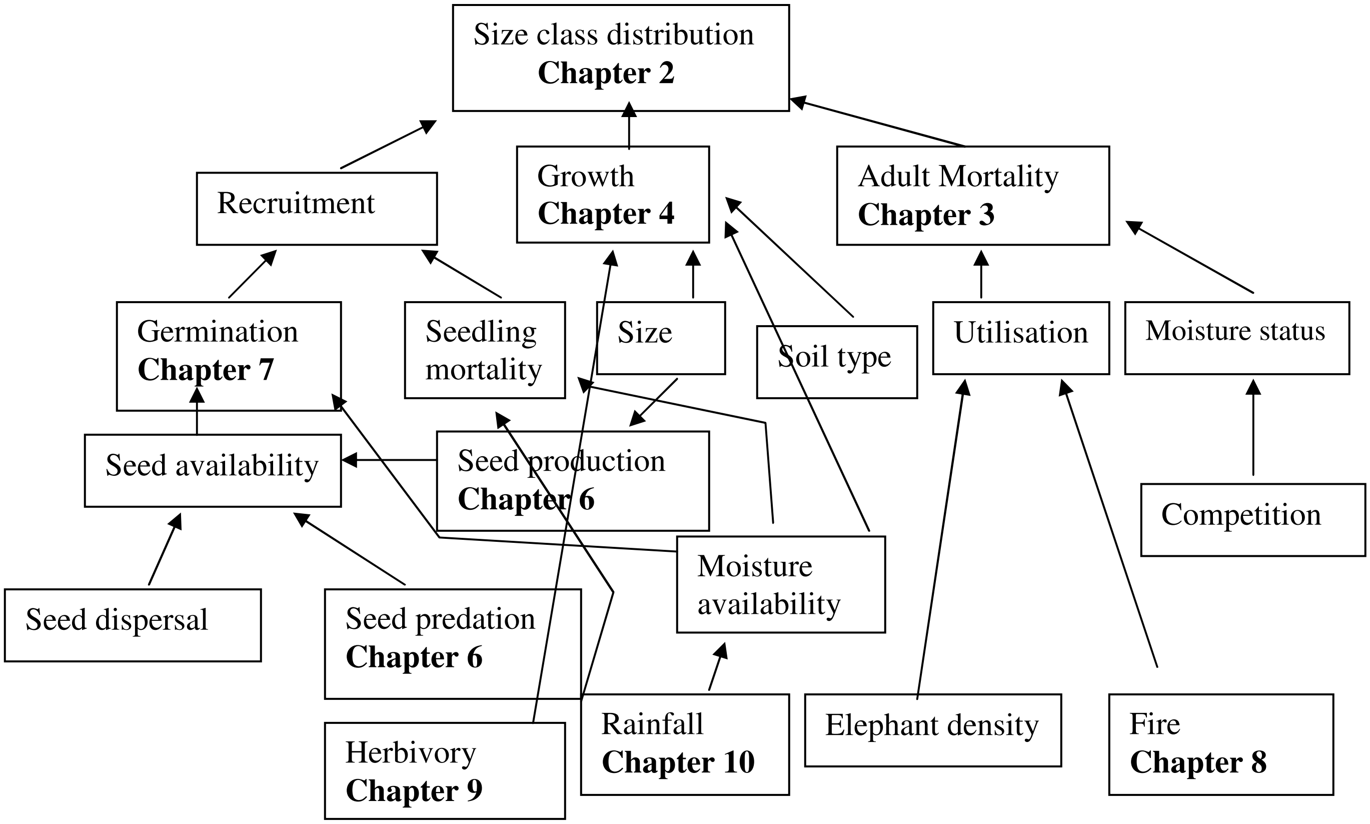

Figure 7: Graphical model of the factors influencing the population size class structure of *Sclerocarya birrea* subsp. *caffra* in the low altitude savannas of South Africa (Modified from Wiegand *et al*. (2000)).

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Chapter 3: Mortality, utilisation and recruitment in *Sclerocarya birrea* subsp. *caffra* (marula) in the Kruger National Park between 2001 and 2010

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3.1. Abstract

A resurvey of a number of marula populations (n=474 trees) in the Kruger National Park (KNP) originally documented in 2001, was conducted in 2008 to determine the response of these populations to the conditions prevalent during that time. These included an increasing elephant population and changes to the KNP fire policy. This was repeated in 2009 and 2010, with additional adult trees. The overall mortality rate was estimated to be 2.6% per annum between 2001 and 2008, with the mortality rate for individuals >5 m in height being 3.8% per annum. The highest level of mortality was found in the 5-8 m height class (7.3% per annum). In addition minimal recruitment into the seedling size class was observed (n=6). No growth of saplings <3 m in height to heights >3 m was observed. Almost half the individuals that were heavily damaged in 2001 had died by 2008. Utilisation levels on the surviving individuals throughout the sampled area had almost tripled between 2001 and 2008, with individuals between 3 and 8 m in height accumulating the most additional elephant utilisation. Annual mortality rates between 2008 and 2009 and 2009 and 2010 were 4.6% and 3.2% respectively. Toppling rates increased 0.75 fold in 2009 (to 3.1%) and decreased 3 fold in 2010 (to 1%), while pollarding rates remained consistent (range: 1.4 to 1.7%). Heavy elephant utilisation and tree mortality was concentrated in the Sabie thorn thickets ecozone on granites where marula density was highest. High spatial variability in mortality rates was observed. Marula populations at lower rainfall sites had higher annual morality rates than at higher rainfall sites. No relationship could be found between mortality rates and distance to the nearest perennial river. It was concluded that the sampled marula populations were not sustaining themselves under the conditions prevalent between 2001 and 2010. The spatial variability described in this study suggests that elephant utilisation is patchy in nature and hence this patchiness needs to be incorporated into any predictions for the future dynamics of these marula populations.

3.2. Introduction

Concern over the loss of mature trees in the Kruger National Park (KNP) due to the accumulation of damage by elephants (*Loxodonta africana* subsp. a*fricana*) and fire (Eckhardt *et al.* 2000), has prompted a host of studies on the keystone tree species in the park, including *Sclerocarya birrea* subsp. *caffra* (marula).

Jacobs and Biggs (2002a) reported that 7% of marula trees sampled within four landscapes in the KNP in 2000 were found dead, while Gadd (2002) found that 2% of the trees were dead in private reserves on the western boundary of the KNP in 1996. In addition, Shannon *et al.* (2008) found that 10% of trees >5 m in height (all species) sampled in the southern KNP were dead. However, such values tend to tell only part of the story about the mortality patterns in a population and the time period since death is unknown. For example, tree species with very hard resistant wood (high wood density), such as *Combretum imberbe* can remain standing dead for up to 80 years in semi-arid landscapes (Vogel and Fuls 2005). Hence, what is really needed is repeat sampling of marked individuals within a population to enable the actual annual mortality rate and other rates of change to be determined.

Understanding the effects of elephant impacts on a tree population requires investigation into the long-term responses of the population to these impacts. However, as elephant are only one of many possible influences on woody plant dynamics, our understanding of their actual effect is confounded by their interactions with other ecosystem drivers (Prins and van der Jeugd 1993; Ben-Shahar 1996, van de Koppel and Prins 1998; Whyte *et al.* 1998; Higgins *et al.* 2000; Zeng and Neelin 2000; Owen-Smith *et al.* 2006; Shannon *et al.* 2008). These drivers include fire, competition from the grass layer, rainfall, soil texture and nutrients, and other herbivores (Scholes and Walker 1993; Scholes 1997). In addition, there is little consensus over whether the dynamics of woody plants are mostly influenced by the interactive effects of these drivers while the tree is still in the seedling phase (Higgins *et al.* 2000) or whether this influence is greatest after the tree has established (Hoffmann 1996). Jacobs and Biggs (2001) suggested that the previous fire management policy (before 2002; see below) within the KNP was preventing the marula population from recruiting to adult size classes, and further evidence indicated that elephants were targeting individuals between 2 and 8 m in height causing mortality in this size class (Jacobs and Biggs 2002b). In contrast, Lewis (1987) and Haig (1999) attributed marula mortality to be mostly in the seedling phase due to browsing by impala (*Aepyceros melampus*), while Walker *et al.* (1986) suggested that recruitment of marula was highly episodic and hence limited by rainfall events.

Since marula is a keystone species in the KNP, a number of autecological studies have already been conducted including that of Jacobs (2001). In addition a large sample of marulas of all sizes was marked in 2001 (M. Hofmeyr, unpublished data). This provided the unique opportunity to determine whether similar processes as those described by Jacobs and Biggs (2002a) were operating between 2001 and 2010. This was achieved through two phases of investigation. The first phase, already published in 2009 (Helm *et al.* 2009; Appendix 3), involved a resurvey of the marked trees in 2008. This period was characterised by an increase in elephant density (0.8 to >2.0 elephants per km^2 between 1988 and 2008 (Shannon *et al.* 2008; Smit and Ferreira 2010)) and followed a number of changes to the KNP fire policy. From 1954 to 1992 the fire policy required the triennial burning of controlled block burns during late winter. In 1994 this was changed to a *laissez faire* burning policy where only lightning induced fires were allowed to burn. In 2002 this policy was again altered to combine point ignitions with unplanned and lightning fires, aiming to burn an annual target area determined by rainfall and fuel conditions (van Wilgen *et al*. 2008). Rainfall in the study area was approximately 140% and 150% of the long-term mean in 2004/2005 and 2005/2006 growing seasons respectively, while in 2006/2007 and 2007/2008 the rainfall was only 65% and 85% of the long-term mean respectively (N. Zambatis, unpublished data). Since almost 20% of the original sample of adult trees surveyed in 2001 had been lost by 2008, the second phase involved sampling additional adult trees in 2008 in areas not surveyed by the original survey and combining these with the remaining live individuals already assessed and then reassessing this sample in 2009 and again in 2010. Loss of large trees is of concern to KNP management, and an understanding of their mortality patterns over a wide spatial scale is essential. Rainfall during the 2008/2009 and 2009/2010 monitoring periods was 126% and 110% of the long-term mean respectively.

The overall aim of this study was to assess the response of a number of marula populations in the southern KNP to the conditions prevailing between 2001 and 2010. In doing so, the objectives were to: 1) determine adult mortality rates within and across populations in the KNP and relate these to temporal and spatial drivers; 2) investigate the relative levels of utilisation of the various size classes and compare these between years.

It was expected that 1) the increasing elephant population would result in the accumulation of higher levels of damage to individual marula trees and in the loss of targeted size classes and 2) the changes in the fire policy would allow opportunities for trees within the fire trap to reach a fire escape height of 2.75 m (Jacobs and Biggs 2001), and hence recruit to the taller size classes, and 3) Mortality rates would vary between life stages due to varying rainfall and fire patterns.

In addition it has been postulated that the current loss of large trees may be due to natural senescence of a cohort that established after the decimation of herbivore populations due to rinderpest and hunting at the beginning of the twentieth century (Prins and van der Jeugd 1993; Shannon *et al.* 2008). If this is the case then most of the senescence should be of the largest and hence oldest trees. This study aimed to determine whether there is evidence to support this hypothesis for marula.

3.3. Materials and Methods

3.3.1. Sampling

3.3.1.1. Stage 1: 2001 to 2008

In 2001 (July and August) and 2002 (March and August), 33 transects of approximately 1.25 km in length and 50 m wide (area = 62.5 ha) were laid out in the following ecozones: Delagoa thorn thickets (Ecca shales; 5 transects, DT), Marula knobthorn savanna (basalt; 9 transects, MKS), Mixed bushwillow woodlands (granite; 5 transects, MBW), Sabie thorn thickets (granite; 9 transects, STT), and Gabbro thornveld (gabbro; 5 transects GT), based on the classification of Grant and Thomas (2006) (M. Hofmeyr, unpublished data) (Figure 1). All trees <0.5 m in height were recorded within a 2 m belt transect nested in the middle of the larger transect. Each tree's location within each transect was recorded with a Global

Positioning System (GPS) but not physically marked. The height and circumference at 30 cm above the ground or above the basal swelling were recorded (the basal circumference was recorded for seedlings, young trees and multi-stemmed individuals) and damage scores of 0 to 4 (least to most damage), as coded by Jacobs and Biggs (2002b) were assigned to each tree encountered within each transect. The scoring was assigned as follows: 0= no damage; 1= <50% bark removed from the stem circumference and/or secondary branches broken; 2= <50% bark removed and secondary branches broken or >50% bark removed from the stem circumference or when one primary branch was broken; 3= >50% bark was removed from the stem circumference and one primary branch was broken or if more than one primary branch was broken; 4= ringbarked, or where the main stem was snapped (stem snapped or pollarded) but coppicing.

In the winter of 2008 (June to August) 31 of these transects were resurveyed by relocating each tree recorded in each transect in 2001/2002 using its GPS location. Two transects in the Delagoa thorn thickets were not resurveyed due to a lack of accurate GPS locations. This translated into the relocation and reassessment of 474 trees that were classified as alive in 2001/2002. For the analyses it was assumed that all trees found in 2002 had existed in 2001 and no significant changes in height or damage had taken place within that year. Any individuals <2 m in height not originally recorded in 2001/2002 within an approximated 10 m wide transect nested in what was thought to be the middle of the larger transect were also recorded. The number of individuals found in this transect was divided by five to make the area comparable to the original size sampled in 2001/2002 (i.e. 2 m wide), and the number obtained was assumed to be indicative of the number of new recruits since 2001. This approach was followed (only for the estimation of new recruits) since it was impossible to determine exactly where the nested transect had been placed in 2001/2002 due to a lack of transect markers. By increasing the size sampled five-fold it was assumed that the entire transect sampled in 2001/2002 was covered in 2008. The height and diameter at breast height and/or the basal diameter for each tree was recorded. A photograph of each tree was taken for future records and the presence of endocarps at the base of the tree (indicative of fruit production and hence a female tree) was noted.

Tree height was used to classify the marulas into size classes which were comparable to other studies (eg. Gadd 2002; Jacobs and Biggs 2002a) as well as to be biologically meaningful, as follows: 1 (0-1 m), 2 (1-3 m), 3 (3-5 m), 4 (5-8 m), 5 (8-12 m), 6 (12-15 m) and 7 (>15 m). Diameter size classes were also delineated as follows: 1 (<0.5 cm), 2 (0.5-5 cm), 3 (5-14 cm), 4 (14-30 cm), 5 (30-40 cm), 6 (40-50 cm), 7(50-60 cm), and 8 (>60 cm). A further size class of 0 was used to group dead (including missing mature) and toppled surviving individuals for certain analyses.

In an attempt to relocate each tree marked in 2001 in 2008, trees was further classified into one of three broad categories (surviving, missing, or dead) to indicate its fate since 2001 (Figure 2). The surviving category was further subdivided into four subcategories namely:
1) Standing trees = individuals >3 m in height that had not been toppled or stem snapped. Trees that were leaning over but where the canopy was higher than 3 m above the ground were also grouped into this category;
2) Pollarded = trees with main stem broken (removing the entire canopy above the snapped area) but which were coppicing;
3) Toppled trees = trees that had been pushed over and were lying on the ground but were coppicing. Toppled trees were not classified as dead as done by Jacobs and Biggs (2002a) as many examples of toppled trees surviving even intense fires were found during the surveys. In addition at least two trees classified as dead in 2001 due to toppling were found to be still alive in 2008. Trees that were bent at 90° but where the stem was not snapped clear off from the trunk, were also classified as toppled when the canopy was close enough to the ground to be affected by fire (<3 m);
4) Gullivers = individuals <3 m in height within the fire trap which were surviving due to basal coppicing from the rootstock after each fire event (following Bond and van Wilgen 1996).

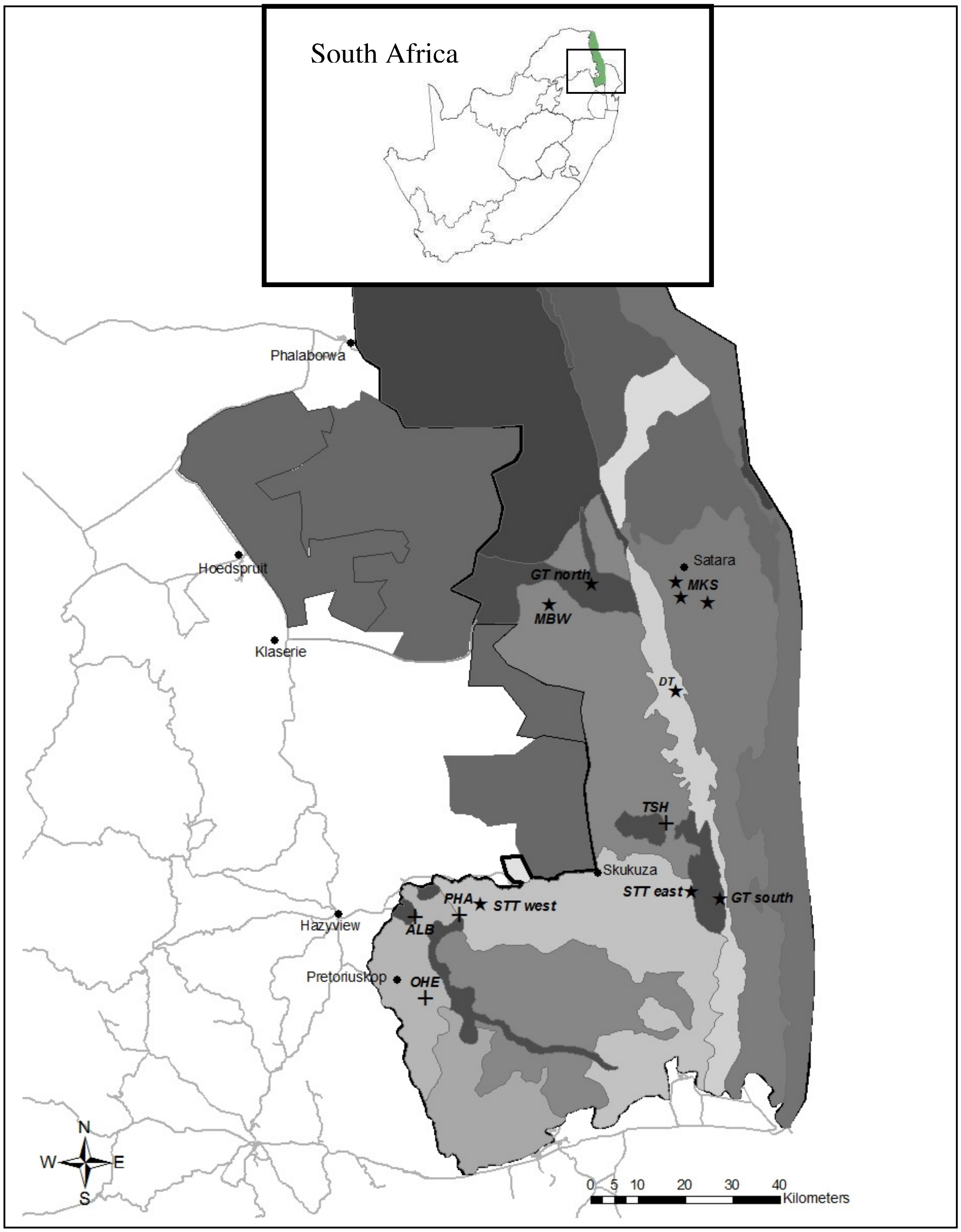


Figure 1: Map showing location of transects used to sample rates of change in marula populations between 2001 and 2010 in the southern Kruger National Park. Stars indicate the populations initially surveyed by M. Hofmeyr in 2001 and the crosses the additional populations initially surveyed in 2008.

The missing category consisted of all individuals that could not be relocated in 2008. This category was further subdivided into two subcategories: <3 m and >3 m in height in 2001. Individuals <3 m in height may have survived undetected at or below the ground as a rootstock (due to fire topkill) during the dormant season when the resurvey was conducted. For mortality analyses it was assumed that missing individuals >3 m in height were dead and were categorised as toppled dead, while individuals <3 m in height were assumed alive in 2008. This was thought reasonable as it is unlikely that the taller trees would not have been found during the resurveys in 2008.

Dead trees were further subdivided into:

1) Pollarded = main stem broken with no coppicing;

2) Standing = upright tree with bark coming off from the stem and no coppicing;

3) Toppled = pushed over and no coppicing.

The cause of mortality for toppled and stem snapped trees was assumed to be elephant damage, while for standing dead trees it was attributed to either ring-barking, natural senescence or boring insect activity (Jacobs and Biggs 2002b). Damage scores were reassigned to each surviving mature tree for direct comparison with the 2001 data. Surviving toppled individuals were excluded from damage score analyses.

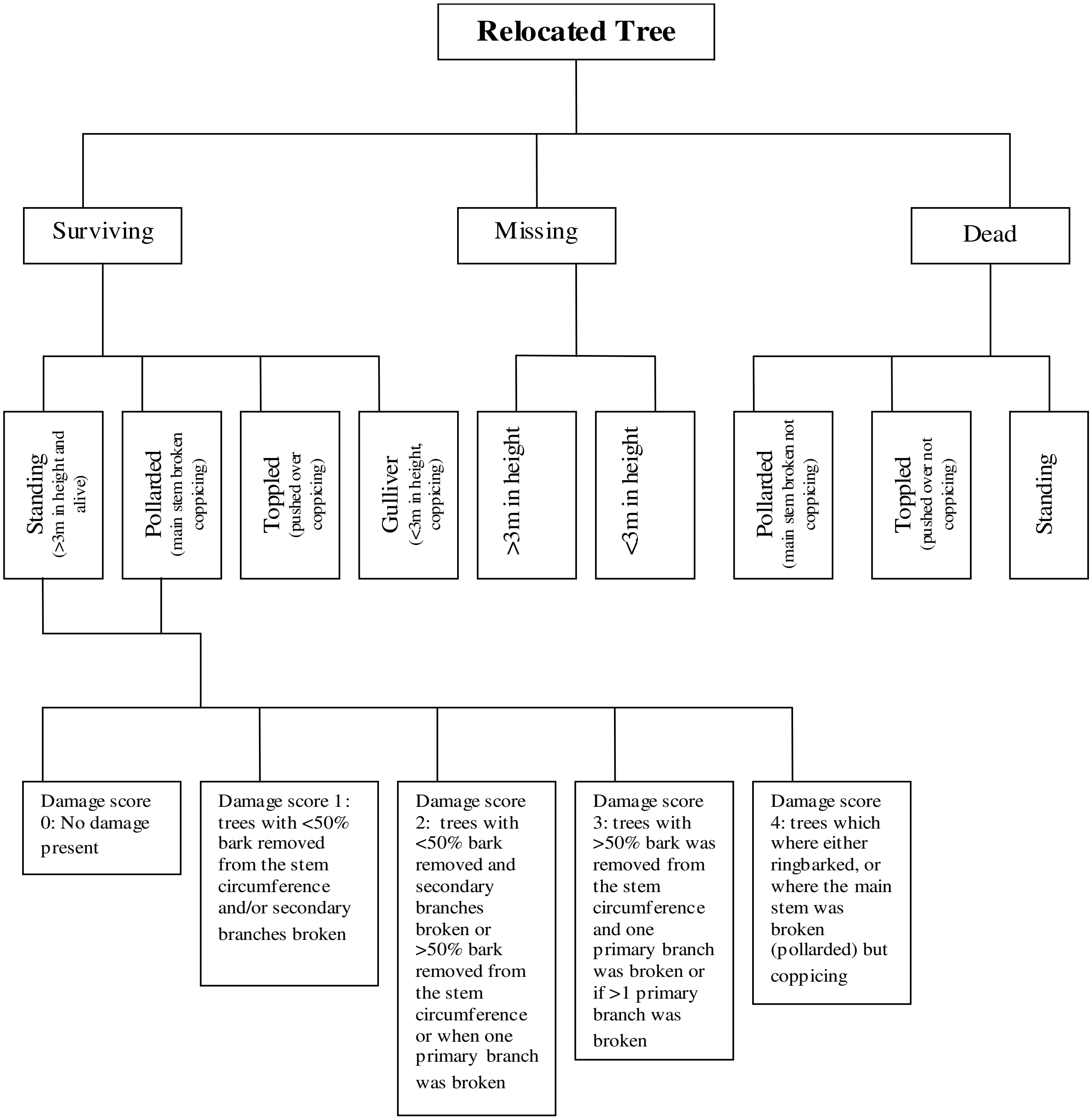


Figure 2: Dendrogram detailing the categorisation of each relocated tree to indicate the fate of the tree since it was first surveyed and the allocation of damage scores to surviving individuals.

3.3.1.2. Stage 2: i) 2008 to 2009 and ii) 2009-2010

In order to focus on the rates of change of large marula trees (>3 m in height), additional transects in the southern KNP were assessed in July, August and October 2008 in the Tshokwane (2 transects), and Pretoriuskop sections (9 transects), five of which fell into the Gabbro thornveld ecozone, three in the Sabie thorn thicket ecozone and three in the Pretoriuskop sour bushveld ecozone (Grant and Thomas 2006). Each transect was approximately 1 km in length and 40 m wide (40 ha). All additional trees were marked with a GPS and their height, basal diameter, % primary damage, % secondary damage and % bark stripping were recorded. Damage scores were assigned as described in Stage 1. This additional sample was added to a portion of the trees already surveyed in stage 1 (only those south of Skukuza) and all the transects were grouped into seven different populations: Tshokwane (TSH, 2 transects), Albasini (ALB, 3 transects), Pretoriuskop (OHE, 3 transects), Phabeni (PHA, 3 transects), Gabbro thornveld south (GT south, 2 transects), Sabie thorn thickets east (STT east, 4 transects) and Sabie thorn thickets west (STT west; 5 transects) (Figure 1). Most of these populations match those already described in Chapter 2. In July and August 2009 all trees >3 m in height (n=630) in these seven populations were relocated, categorised as surviving (toppled, pollarded or standing) or dead (toppled, pollarded, standing) and the surviving individuals were assessed for new damage (bark stripping, primary damage, secondary damage) (Figure 2). New damage scores were also assigned to each surviving tree for comparison with the 2008 data. For individuals that died the cause of death was determined as described above.

In July 2010, trees surveyed in 2009 that were still alive (n=600), were reassessed for new damage (% bark stripping, % primary damage, % secondary damage) and categorised as described above. Eight trees were not resurveyed due to logistical problems with aggressive rhinos. New damage scores were assigned to each surviving tree for comparison with the 2009 data.

3.3.2. Data analysis

All statistical analyses were conducted in Statistica v.6 (Statsoft, Inc. 2004) or R v.2.12 (R Development Core Team 2010) at a significance level of $p<0.05$.

A χ^2 test was then used to determine whether the number of individuals in each size class was associated with year of survey. A Wilcoxon paired test was used to assess differences in damage scores between surveys as well as differences in damage scores for each size class for those individuals that survived only.

Since elephant impacts on tree species are usually highest nearest to rivers (Smit and Ferreira 2010), transects were grouped according to relative distance to perennial water courses following the maps in Smit and Ferreira (2010). Mortality rates were then regressed against distance to water using linear regression. To further explore the spatial context, trees were also grouped by ecozone and the mortality rates compared using ANOVA and Fisher's least significant difference (LSD). This was only applied to the 2001-2008 data. In addition, trees were grouped into seven geographical populations as described above and the mortality rates across populations compared using ANOVA and Fisher's LSD. This was only applied to the 2008-2009 and 2009-2010 data as many of these populations were first surveyed in 2008.

To explore the effect of rainfall on mortality patterns, rainfall records for the various sections were obtained and transects were grouped into rainfall categories based on the rainfall during the monitoring periods. Contingency table χ^2 tests were used to determine whether mortality rates were associated with rainfall between years and between populations.

3.4. Results

3.4.1. Stage 1(trees of all size classes): 2001 to 2008

3.4.1.1. Fate

Of the 474 resurveyed trees, 13.7% (n=65) were conclusively dead in 2008 due to the presence of the dead tree, stump, bark or ash bed (fire) outline at the site where a tree was present in 2001 (Figure 3). Only 27.8% (n=22) of the missing individuals were >3 m in height in 2001 (Figure 3). This translates into an overall mortality rate across all size classes between 2001 and 2008 of 18.4% (2.6% per annum) (Table 1). Most of the dead individuals were between 8 and 12 m in height or 50-60 cm in basal diameter in 2001

(Figure 4). 63.1% (n=41) of the dead individuals had been toppled and 29.2% (n=19) had been pollarded (Figure 3). 69.5% of all the toppled individuals (n=41) had died while only 44.2% (n=19) of the pollarded individuals had died. Most of the remaining pollarded individuals were between 5-8 m in height and 30-40 cm in diameter, while the remaining toppled individuals were between 8-12 m in height and 30-40 cm in diameter (Figure 4). Of the mature individuals, those between 5 and 8 m in height (7.3%) and those between 30-40 cm (5.1%), had the highest annual mortality rate (Table 1). Up to 11.1% of individuals between 3 and 5 m in height were pollarded annually.

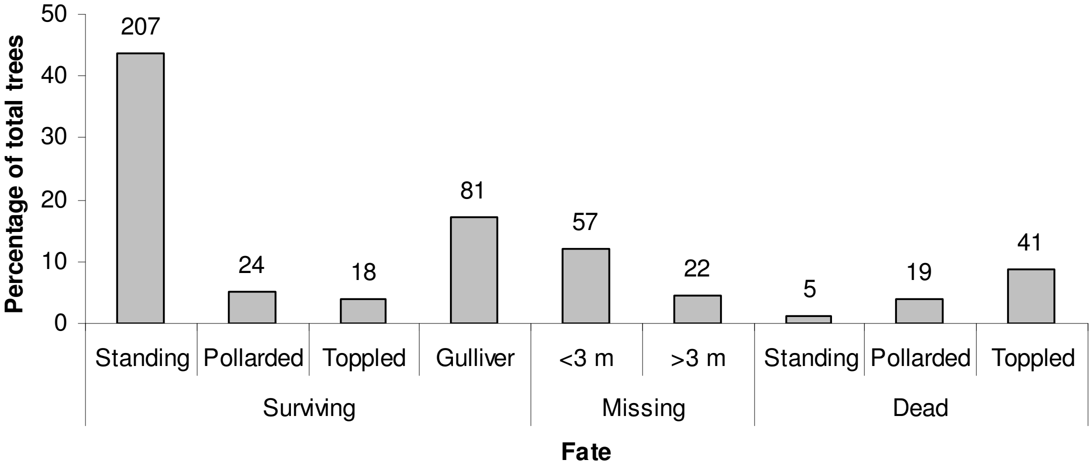


Figure 3: Fate of the resurveyed marula trees in the Kruger National Park between 2001 and 2008 (n=474). Numbers above the bars represent the number of trees in each class.

Table 1: Annual rates (%) of mortality, toppling and pollarding for each height and stem diameter size class of the resurveyed marulas in the Kruger National Park between 2001 and 2008 (n=474). Mature individuals are >5 m in height or >14 cm in diameter. Toppling and pollarding rates include both alive and dead individuals (n=99).

	Height size classes								**Mature total**	**Total**
	<1m	**1-3m**	**3-5m**	**5-8m**	**8-12m**	**12-15m**	**>15m**			
Mortality	0	1.8	2.0	7.3	3.9	2.7	2.4		3.8	2.6
Toppling	0	1.2	6.3	3.5	2.7	2	0.8		2.4	1.9
Pollarding	0	1.8	11.1	6.9	1.3	0.5	0.8		1.6	1.4
	Diameter size classes									
	<0.5cm	**0.5-5cm**	**5-14cm**	**14-30cm**	**30-40cm**	**40-50cm**	**50-60cm**	**>60cm**		
Mortality	0	0	0	4.8	5.1	3.8	4.3	2.1	3.7	2.6
Toppling	0	0	0	3.6	4.2	2.8	2.8	1.2	2.5	1.9
Pollarding	0	0	0.5	5.4	4.5	1.6	1.4	0.8	1.9	1.4

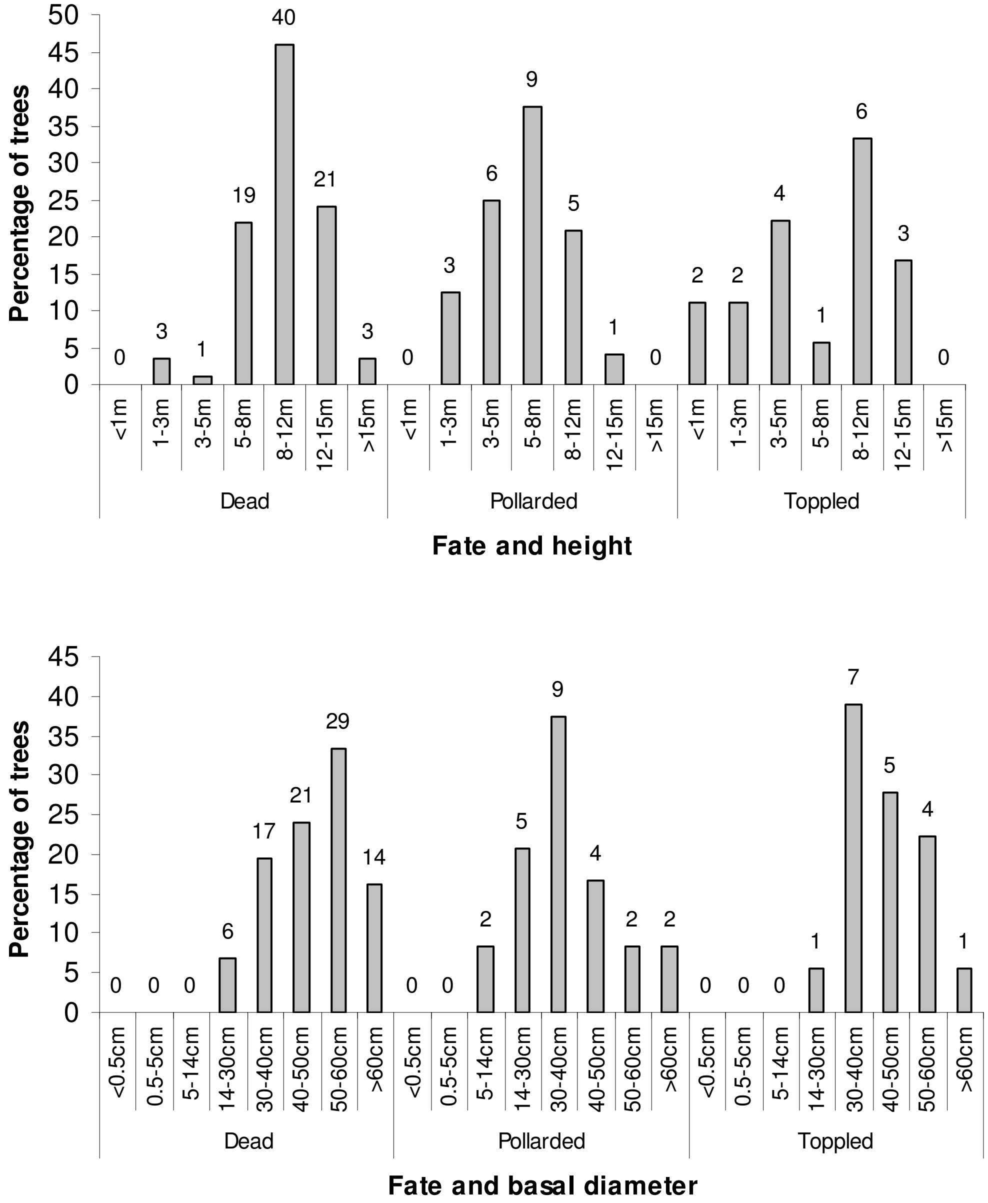


Figure 4: Percentages of dead, live pollarded and live toppled trees within each a) height and b) basal diameter size classes in 2008 for the resurveyed marulas in the Kruger National Park (n=474). Numbers above the bars represent the numbers of trees in each class.

The numbers of individuals in the different size classes were not significantly associated with year (height: χ^2_6=11.54, p=0.07; diameter: χ^2_6=9.92, p=0.13) (Figure 5). However, there was a substantial decrease in the number of individuals in some size classes, the greatest being in the 5-8 m height class (58% loss) and the 30-40 cm diameter class (50%). This resulted mainly from the removal of individuals from the 3 to 15 m height and 30-60 cm diameter size classes to class 0 due to toppling or complete removal by death (Figure 4).

A large proportion (48.3%) of the 2008 dead trees had no damage in 2001 (Figure 6). In addition, 58.6% (n=17) of the trees that had a score of 4 in 2001 were dead in 2008. The class containing the highest percentage of dead individuals (30.8%) in 2008, were those between 8-15 m in height with a damage score of 0 in 2001.

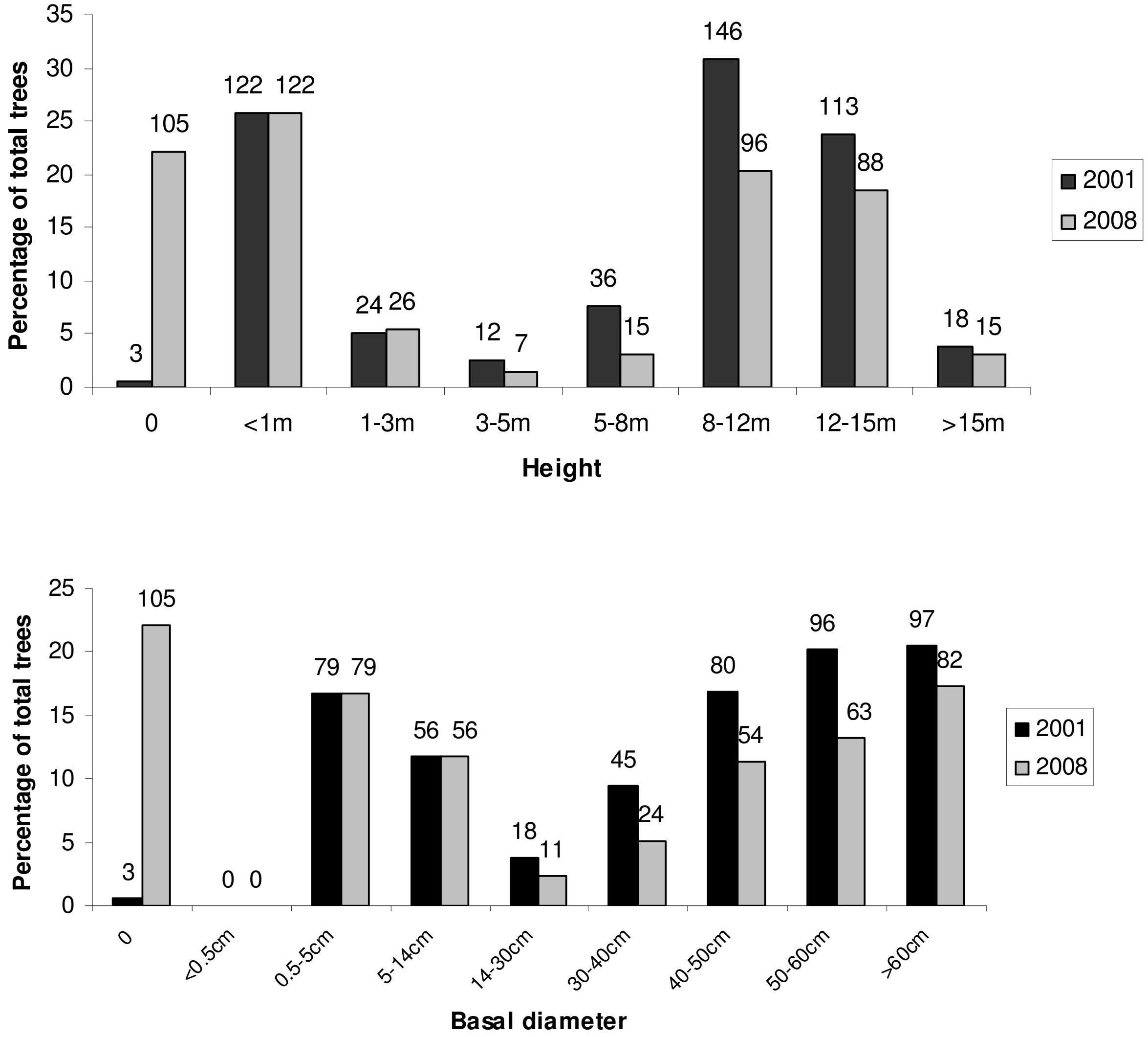


Figure 5: Comparison of the a) height and b) basal diameter distributions of the marked marula individuals in the Kruger National Park between 2001 and 2008. Size class 0 represents all toppled, dead and missing mature individuals (n=474). The values for 2008 exclude the new recruits. Numbers above the bars represent the number of trees in each height size class.

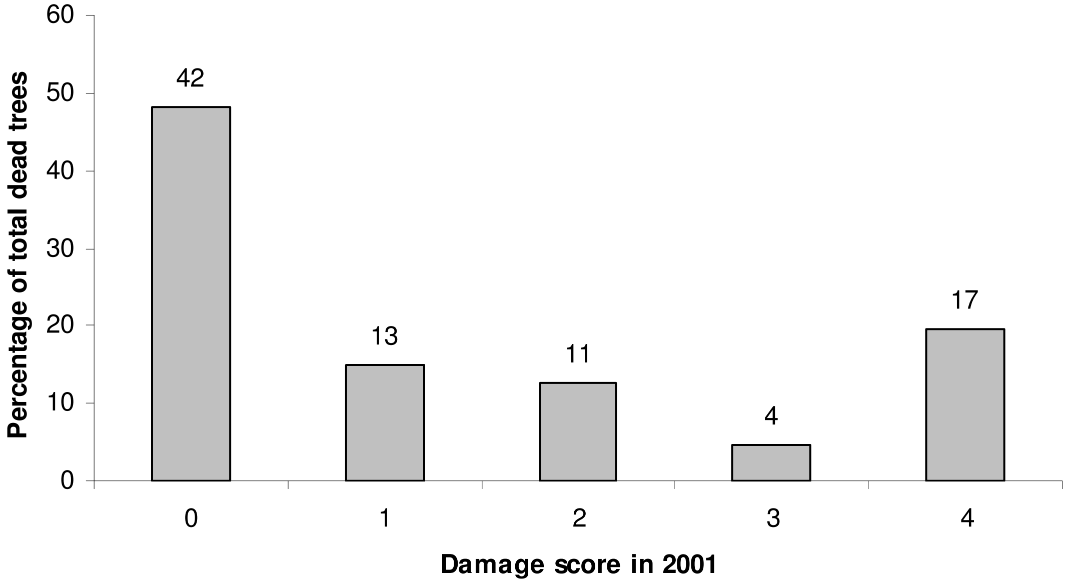


Figure 6: Damage scores in 2001 of all dead marula individuals (dead and missing >3 m) in the Kruger National Park in 2008 (n=87). Numbers above the bars represent the number of trees in each class.

3.4.1.2. Damage scores of the surviving individuals

Damage levels in the surviving mature individuals in 2008 (>5 m excluding toppled individuals) were significantly higher than for the same trees in 2001 ($p<<0.001$; n=222) (Figure 7). The greatest percentage increase was in damage score 2 which increased from 3.5% in 2001 to 39.0% in 2008. The changes in damage levels per size class indicated that the smaller size classes (5-8 m) had experienced most of the additional damage compared to the larger size classes (>15 m) (Figure 7). Individuals between 5 and 8 m in height with damage score 4 increased 4.5 fold, and those between 8 and 12 m increased 5 fold. The damage levels in each size class were significantly higher in 2008 than in 2001 ($p<0.005$). The greatest transition rates between damage scores was from score 0 to score 2 (Table 2).

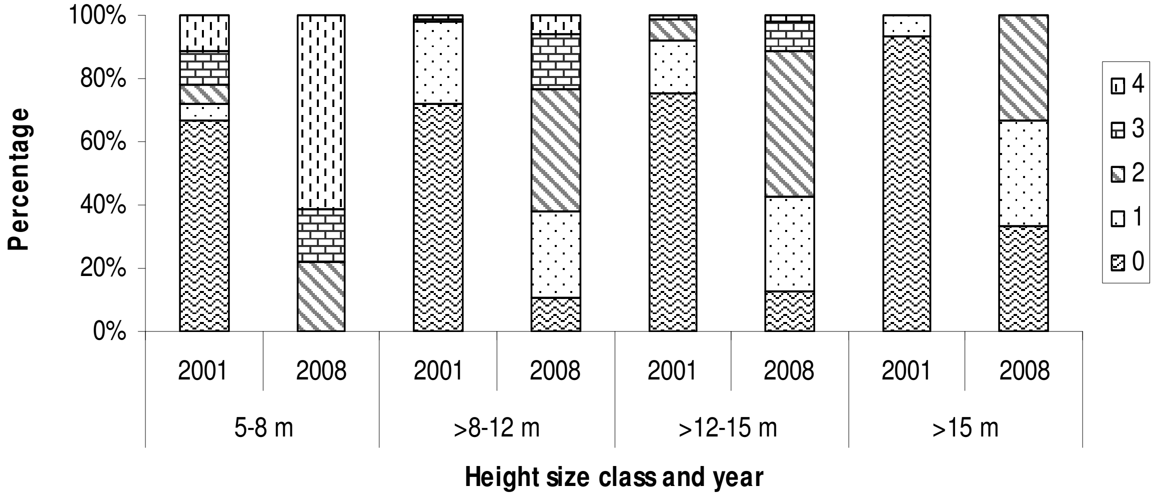


Figure 7: Comparison of the damage scores assigned to mature marula height size classes in 2001 and in 2008. For a description of the damage scores see Figure 2.

Table 2: Stage matrix and transition probabilities of damage scores for mature (>5 m in height) standing marulas (excluding toppled) in the Kruger National Park between 2001 and 2008 (n=222).

$$\begin{pmatrix} P_{0,0};\ P_{1,0}\ldots\ldots\ldots\ldots\ P_{4,0} \\ P_{0,1};\ P_{1,1}\ldots\ldots\ldots\ldots\ P_{4,1} \\ P_{0,2};\ P_{1,2}\ldots\ldots\ldots\ldots\ P_{4,2} \\ P_{0,3};\ P_{1,3}\ldots\ldots\ldots\ldots\ P_{4,3} \\ P_{0,4};\ P_{1,4}\ldots\ldots\ldots\ldots P_{4,4} \end{pmatrix}$$

$$\begin{pmatrix} 0.16 & 0.02 & 0.00 & 0.00 & 0.00 \\ 0.28 & 0.28 & 0.00 & 0.00 & 0.00 \\ 0.41 & 0.37 & 0.05 & 0.00 & 0.00 \\ 0.09 & 0.23 & 0.25 & 0.33 & 0.00 \\ 0.05 & 0.09 & 0.25 & 0.67 & 1.00 \end{pmatrix}$$

3.4.1.3. Recruitment rates

Of the surviving marulas (n=330), 24.5% (n=81) were gullivers (Figure 4). These gullivers showed an overall tendency to decrease in height since 2001 (60.5% had decreased in height), indicating that little recruitment to the higher size classes was occurring (Figure 8).

Twenty nine individuals <2 m in height which had not been identified in 2001 were found within the 10 m wide belt transects, translating into six new recruits since 2001. The new recruits occurred mainly in the GT (44.8%, n=13). However, most of these recruits (61.5%) occurred in the portion of the GT near Satara (GT north). No new recruits occurred in the STT.

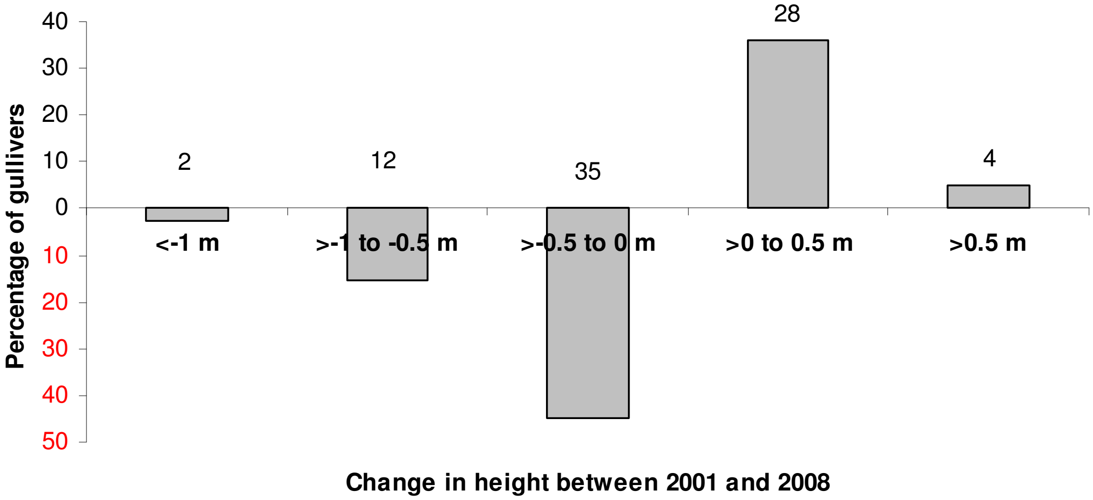


Figure 8: The percentage of gullivers resurveyed in the Kruger National Park in each height loss or gain category indicating the height lost or gained between 2001 and 2008 (n=81). Numbers above the bars represent the number of trees lost or gained. The bars below the x-axis represent the percentage of trees which showed height loss and the bars above the x-axis represent the percentage of trees which showed height gain.

3.4.2. Stage 2 (trees >3 m in height): 2008-2009 and 2009 to 2010

3.4.2.1. Fate

The sample of trees selected for stage 2 consisted of 521 standing, 55 toppled and 54 pollarded individuals. Between 2008 and 2009 the overall mortality rate was 4.6% (Table 3). Only the Pretoriuskop trees (OHE) had experienced a fire during this period in June 2009 which was very cool and only burnt the grass, hence not affecting the large tree sample. Between 2009 and 2010 the overall mortality rate dropped to 3.2% (Table 3), despite almost half of the trees assessed, occurring in areas that had been burnt in June 2010. Up to 26.4% of toppled individuals died between 2008 and 2009, while only 11% died between 2009 and 2010. Pollarded individuals died at a rate of 11-13% per year. Toppling was most common in individuals between 8 and 12 m in height and 40-50 cm in basal diameter, while pollarding was concentrated on individuals between 5 and 8 m in height and 30-40 cm in diameter (Figure 9). Most of the dead individuals in 2009 were between 1 and 3 m or 8 and 12 m in height and evenly distributed across the diameter size classes from 14-60 cm, while in 2010, most of the dead individuals were between 5 and 12 m in height and 30-40 cm in diameter (Figure 9). Most of the dead individuals had a damage score of 3 the previous year. Pollarding rates between 2008 and 2009 did not differ from those between 2009 and 2010 (χ^2_1=0.0012, p=0.97). However, toppling rates tended to be higher between 2008 and 2009 than between 2009 and 2010 (χ^2_1=3.33, p=0.068).

The height distribution of the sampled trees at the end of the 2008 monitoring season is presented in Figure 9. All the individuals in the 0.5-2 m height class had been pollarded. The number of individuals in the "missing size class" (2-8m in height) was also decreasing with each progressive year (Figure 10). The numbers of individuals in the different size classes were not significantly associated with year (height: χ^2_{14}=20.80, p=0.107) (Figure 10).

Table 3: Fate (number of trees dead, pollarded or toppled) relative to damage type and score the previous year for marula trees >3 m in height in the southern regions of the Kruger National Park between 2008 and 2010. Damage score 4 includes only ring-barked trees. Eight trees were not resurveyed in 2010 and these were assumed to be surviving and undamaged.

2008-2009						2009-2010					
2008 state	**2008 damage score**	**n**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**	**2009 state**	**2009 damage score**	**n**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**
Pollarded		55	7 (12.7)			**Pollarded**		56	6 (10.7)		
Toppled		54	14 (25.9)			**Toppled**		55	6 (10.9)		
Standing		521	8 (1.5)	8 (1.5)	16 (3.1)	**Standing**		489	7 (1.4)	8 (1.6)	5 (1.0)
	0	98	0 (0)	0 (0)	3 (3.1)		**0**	83	0 (0)	1 (1.2)	0 (0)
	1	128	1 (0.8)	3 (2.3)	2 (1.6)		**1**	123	1 (0.8)	0 (0)	1 (0.8)
	2	219	2 (0.9)	3 (1.4)	9 (4.1)		**2**	209	2 (1.0)	3 (1.4)	3 (1.4)
	3	74	3 (4.1)	2 (2.7)	2 (2.7)		**3**	74	4 (5.5)	4 (5.4)	1 (1.4)
	4	2	2 (100)	0 (0)	0 (0)		**4**	0	0 (0)	0 (0)	0 (0)
Total		629	29 (4.6)	8 (1.5)	16 (3.1)	**Total**		600	19 (3.2)	8 (1.7)	5 (1.0)

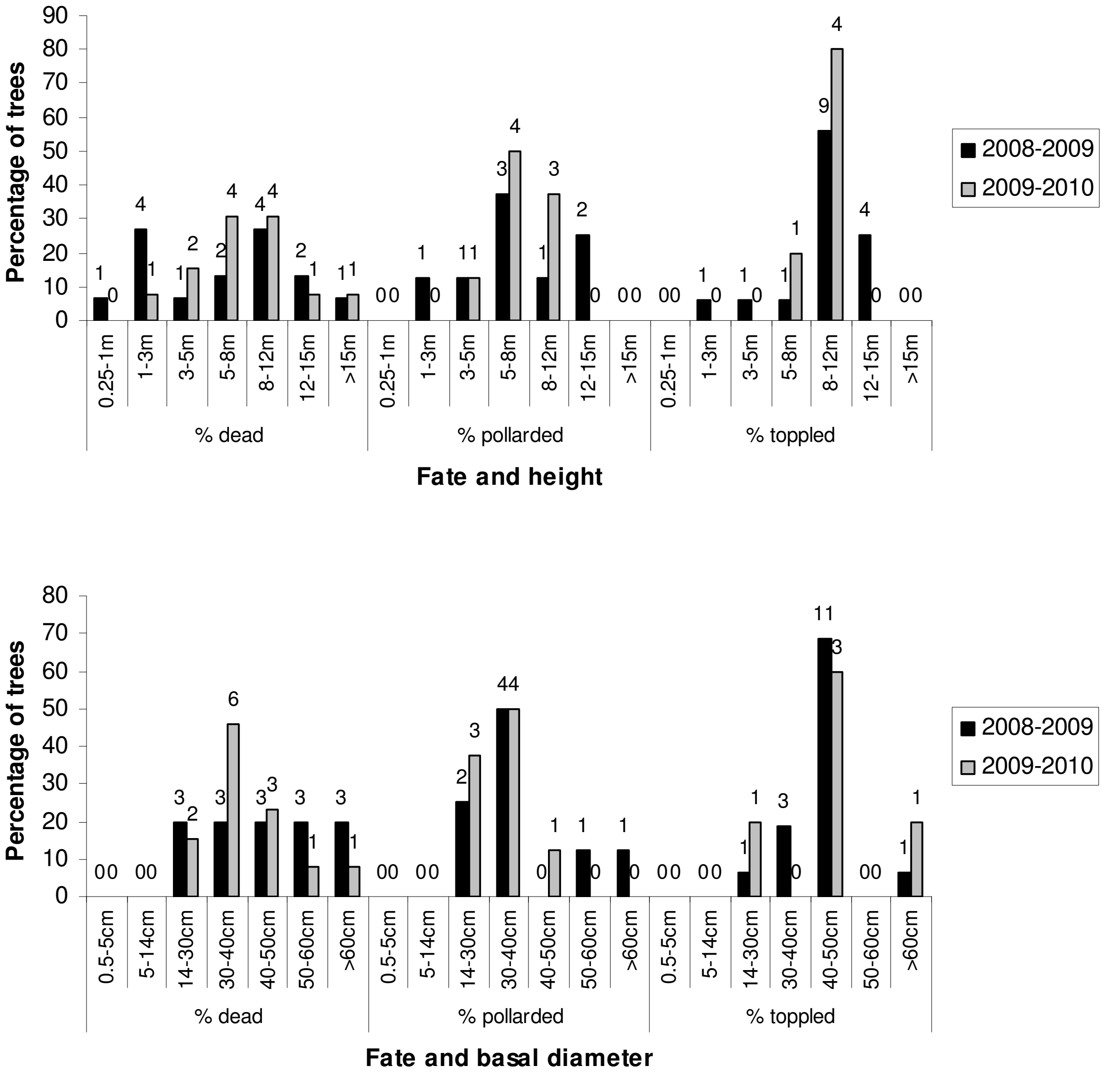


Figure 9: Percentages of dead, live pollarded and live toppled trees within each a) height and b) basal diameter size class for the two monitoring periods between 2008 and 2010 in the Kruger National Park. Numbers above the bars represent the numbers of trees in each class. Only the individuals that were standing at the beginning of each monitoring period were reassessed the following period (n_{2008}=576, n_{2009}=545).

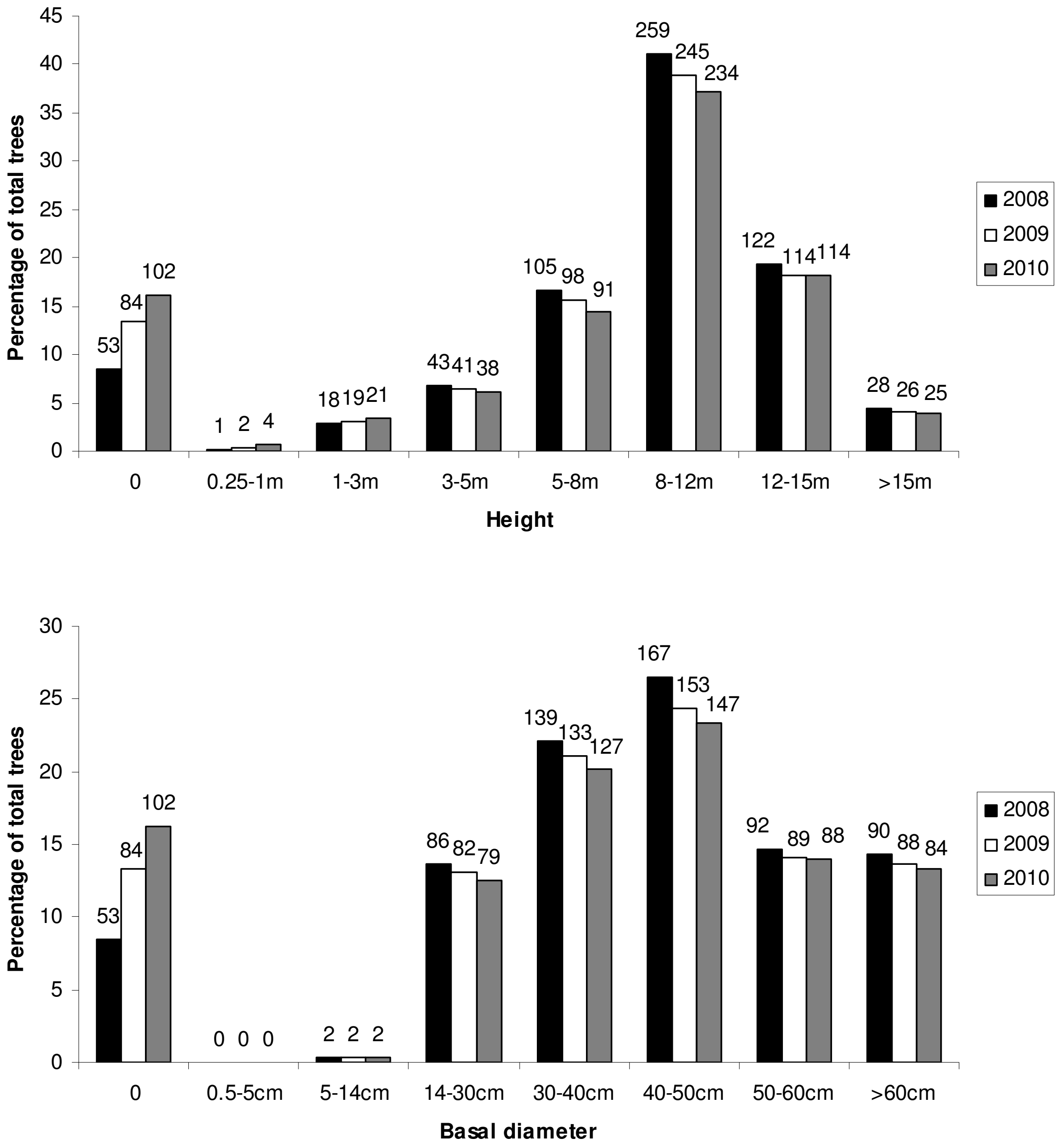


Figure 10: Comparison of the a) height and b) basal diameter distributions of the marked mature marula individuals (>3 m in height) in the Kruger National Park between 2008 and 2010 (n=629). Size class 0 represents all toppled and dead individuals. Numbers above the bars represent the number of trees in each size class.

3.4.2.2. Damage analysis of surviving individuals

The relative proportions of the damage scores for the standing population did not change between the two monitoring periods (χ^2_3=0.08, p=0.99). In 2009 14.2% of the standing population had a damage score of 3 while in 2010, this increased marginally to 15.2% (Table 3).

3.4.3. Overall summary 2001-2010

Given the data presented above, mean annual rates of loss can be determined for the various size classes (Table 4). In addition, given a certain type of utilisation, mean annual mortality rates can further be estimated (Figure 11). Such details are important for estimating transition rates between size classes in demographic models (see Chapter 11). Since heights can easily be modified by elephant utilisation, it is more biologically meaningful to make use of diameter size classes when modelling demographic parameters.

Table 4: Mean annual rates (%) of mortality, toppling and pollarding for each diameter size class for mature marulas (>3 m in height) in the Kruger National Park between 2001 and 2010.

	Diameter size classes					**Total**
	14-30cm	**30-40cm**	**40-50cm**	**50-60cm**	**>60cm**	
Mortality	3.6	3.9	2.5	2.9	2.2	3.5
Toppling	2	2.1	3.8	0.9	1.2	2.0
Pollarding	3.8	3.5	0.8	0.8	0.6	1.5

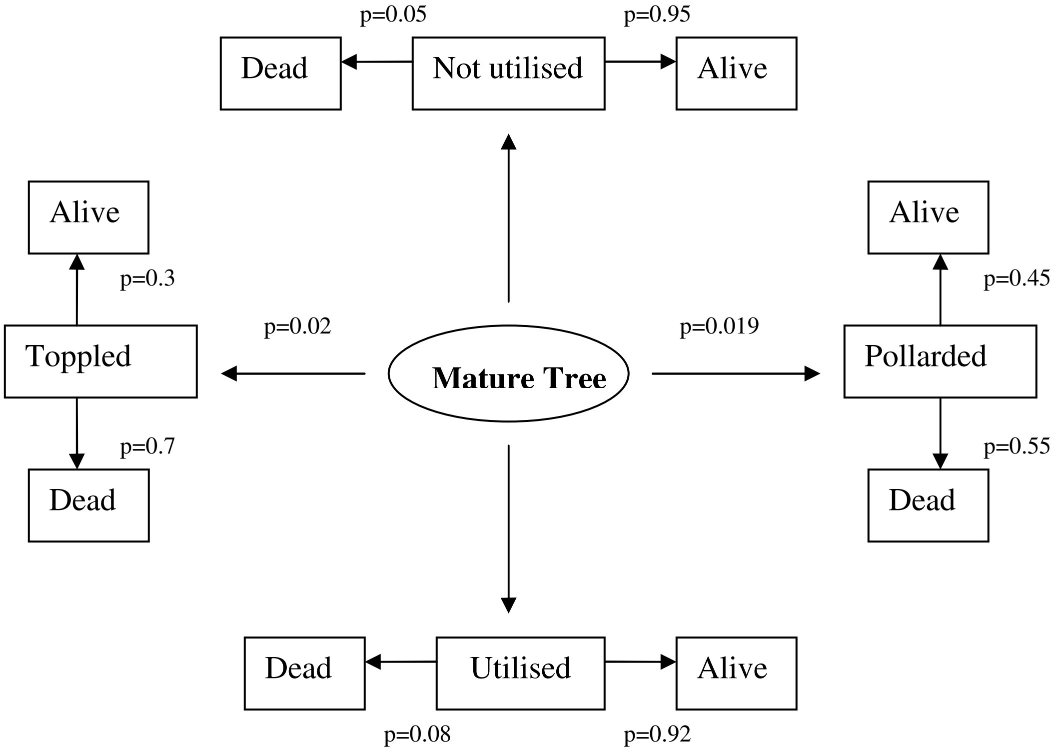


Figure 11: Conceptual diagram indicating the probability of survival of marula trees >3 m in height after elephant utilisation in the Kruger National Park, based on values obtained from trees assessed between 2001 and 2010.

3.4.4. Spatial analysis 1: Ecozones (2001-2008)

3.4.4.1. Size class distributions

Almost half of the sampled marulas occurred in STT (n=226), while few represented DT (n=11) (Figure 12). The STT were dominated by individuals >5 m in height (96.9%) and >14 cm in diameter, while the other ecozones were all dominated by individuals <3 m in height and <14 cm in diameter (Figure 12). There is, therefore, wide spatial variability in demography.

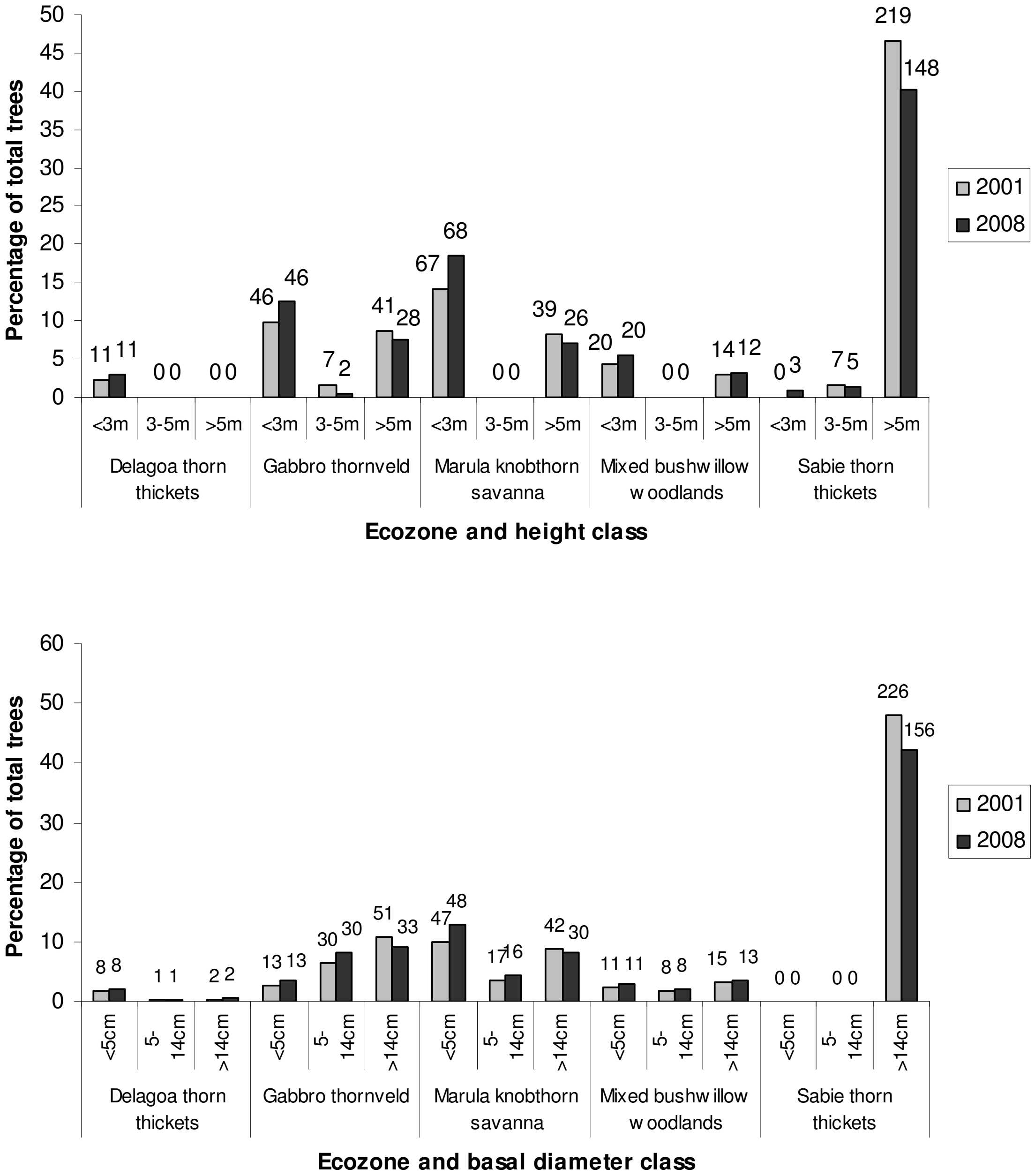
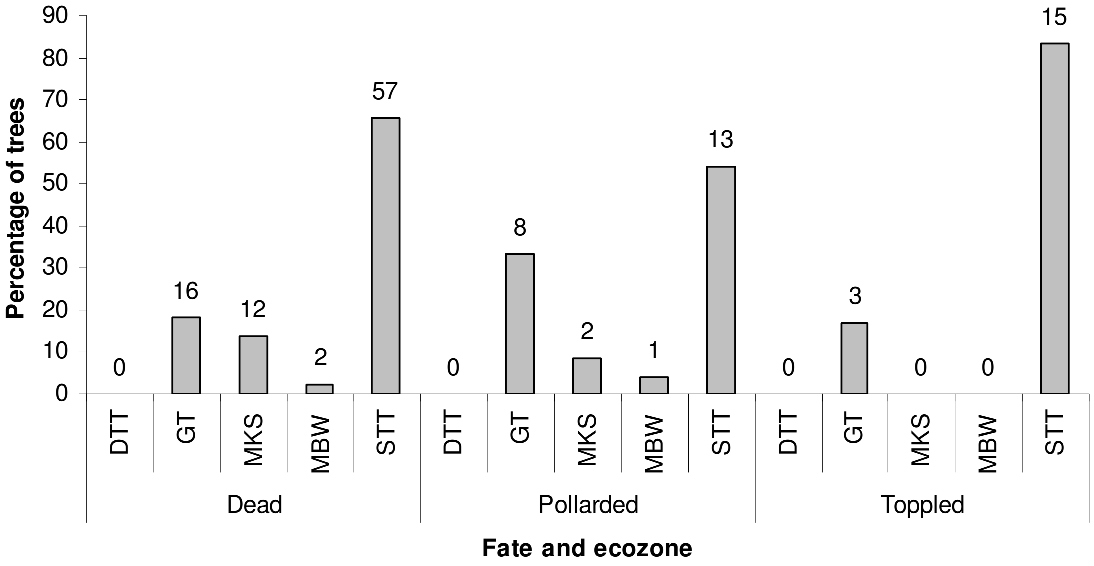


Figure 12: a) Height and b) diameter size class distributions of marulas in the five sampled ecozones in 2001 and 2008 in the Kruger National Park. Numbers above the bars represent the number of trees in each class.

3.4.4.2. Mortality rates

STT, GT, MKS and MBW had annual mortality rates across all size classes of 3.6%, 2.4%, 1.5% and 0.8% respectively. 58.5% of the dead individuals occurred in the STT (n=38). In the STT the highest mortality occurred in size class 5 (8-11 m; 40.4%). Most of the dead, toppled and pollarded individuals were in STT (Figure 13a). However, due to the variable number of individuals in each size class it is important to also determine whether an ecozone is being utilised in terms of the proportional availability of trees. GT had a greater proportion pollarded than STT once the effect of sample size had been removed (Figure 13b). The number of toppled individuals in the STT was higher than expected (χ^2_1=12.50; p=0.0004) while the number of pollarded individuals was not (χ^2_1=0.014; p=0.9065).



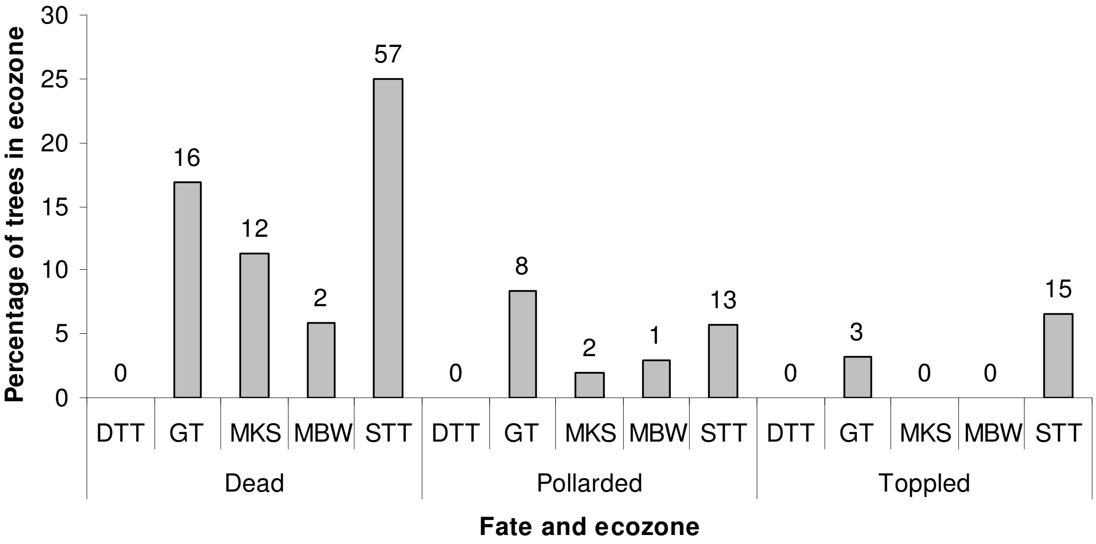
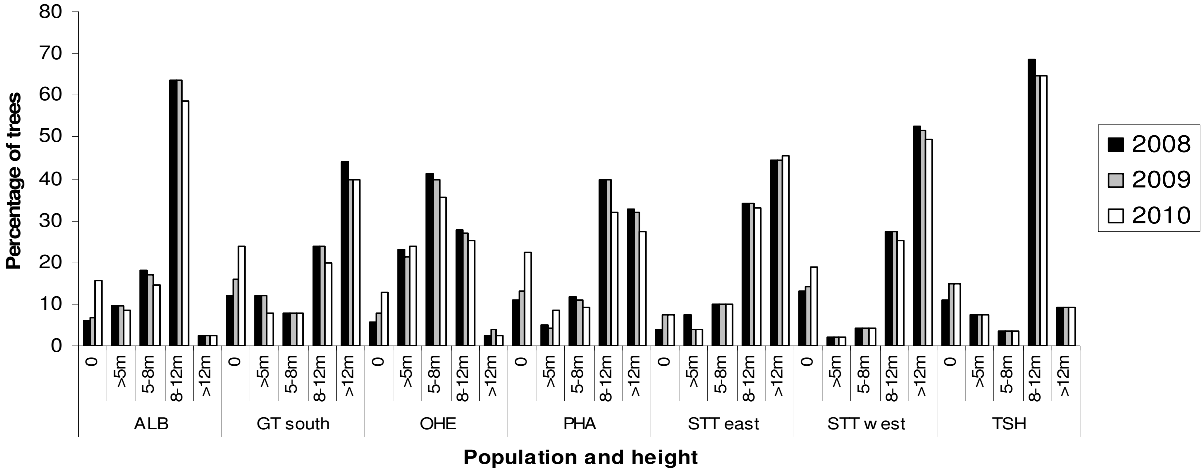


Figure 13: a) Percentage and b) percentage relative to number in ecozone of the dead, live pollarded and live toppled trees within each ecozone between 2001 and 2008 in the Kruger National Park (n=474). Numbers above the bars represent the numbers of trees in each class.

3.4.5. Spatial analysis 2: Adult populations (2008-2009 and 2009-2010)

3.4.5.1. Size class distributions

Kolmogorov-Smirnov tests indicated that there was no significant differences in the height distributions of the mature individuals in the seven populations monitored between 2008 and 2010 (p>0.05). ALB, PHA and TSH were dominated by individuals between 8-15 m in height, while OHE was dominated by individuals 5 to 8 m in height. The remaining populations were dominated by individuals >12 m in height (Figure 14a).



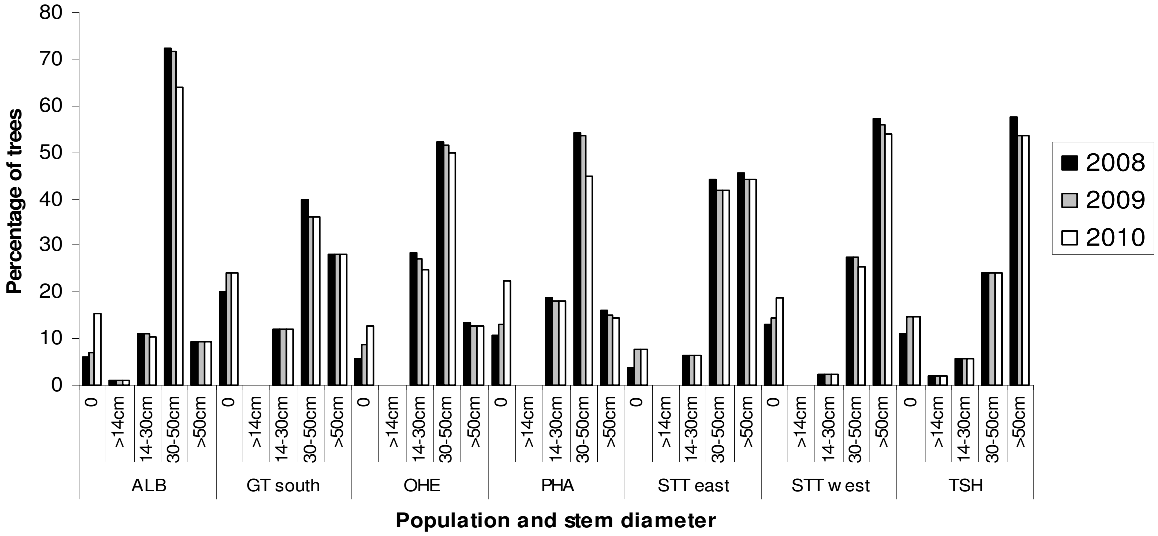


Figure 14: a) Height and b) diameter size class distributions of seven populations surveyed in the southern Kruger National Park between 2008 and 2010. Size class 0 represents all toppled and dead individuals.

3.4.5.2. Mortality rates

The mortality rates across the seven populations varied from 2.6% at ALB to 5.8% at PHA between 2008 and 2009, while between 2009 and 2010, mortality rates varied from 0% (TSH and STT east) to 8.8% at ALB (Table 5). Mortality rates tended to be associated with population between 2008 and 2009 (χ^2_6=11.51, p=0.07) and were associated with population between 2009 and 2010 (χ^2_6 = 13.06, p=0.04). Between 2008 and 2009, toppling rates were greatest at PHA (8.7%), while pollarding rates were highest at ALB (2.6%). Between 2009 and 2010, toppling rates were highest at GT south (4.2%), while pollarding rates were highest at PHA (3.1%) (Table 5). Toppling rates were significantly associated with population between 2008 and 2009 (χ^2_6 =26.74, p=0.0002), but not between 2009 and 2010 (χ^2_6 = 5.22, p=0.52), while pollarding rates were not associated with populations between 2008 and 2009 (χ^2_6 =3.56, p=0.74), nor between 2009 and 2010 (χ^2_6 =8.36, p=0.21).

Table 5: Fate (number of trees dead, pollarded or toppled) relative to damage type and score the previous year, of seven adult marula populations surveyed in the southern Kruger National Park between 2008 and 2010.

Albasini (ALB)											
2008-2009						**2009-2010**					
2008 state	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**	**2009 state**	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**
Pollarded	10		0 (0)			**Pollarded**	13		4 (30.8)		
Toppled	7		2 (28.6)			**Toppled**	5		1 (20)		
Standing	99		1 (1.0)	3 (3.0)	0 (0)	**Standing**	95		5 (5.3)	0	1 (1.1)
	24	**0**	0 (0)	0 (0)	0 (0)		21	**0**	0	0	0
	33	**1**	1 (3.0)	1 (3.0)	0 (0)		28	**1**	1 (3.6)	0	0
	32	**2**	0 (0)	0 (0)	0 (0)		36	**2**	2 (5.6)	0	1 (2.8)
	10	**3**	0 (0)	0 (0)	0 (0)		10	**3**	2 (20.0)	0	0
	0	**4**	0 (0)	0 (0)	0 (0)		0	**4**	0	0	0
Total	116		3 (2.6)	3 (2.6)	0 (0)	**Total**	113		10	0	1 (0.9)

Sabie thorn thickets east (STT east)											
2008-2009						2009-2010					
2008 state	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**	**2009 state**	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**
Pollarded	11		3 (27.3)			**Pollarded**	8		0		
Toppled	3		1 (33.3)			**Toppled**	2		0		
Standing	65		0 (0)	0 (0)	0 (0)	**Standing**	65		0	0	0
	8	**0**	0 (0)	0 (0)	0 (0)		10	**0**	0	0	0
	20	**1**	0 (0)	0 (0)	0 (0)		19	**1**	0	0	0
	24	**2**	0 (0)	0 (0)	0 (0)		27	**2**	0	0	0
	10	**3**	0 (0)	0 (0)	0 (0)		9	**3**	0	0	0
	0	**4**	0 (0)	0 (0)	0 (0)		0	**4**	0	0	0
Total	79		4 (5.1)	0 (0)	0 (0)	**Total**	75		0	0	0

Phabeni (PHA)											
2008-2009						2009-2010					
2008 state	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**	**2009 state**	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**
Pollarded	10		2 (20.0)			**Pollarded**	10		0		
Toppled	15		5 (33.3)			**Toppled**	22		2 (9.1)		
Standing	113		1 (0.9)	2 (1.8)	12 (10.6)	**Standing**	98		0	4 (4.1)	1 (1.0)
	26	**0**	0 (0)	0 (0)	3 (11.5)		20	**0**	0	1 (5.0)	0
	18	**1**	0 (0)	0 (0)	1 (5.6)		17	**1**	0	0	1 (5.9)
	32	**2**	0 (0)	0 (0)	6 (18.8)		46	**2**	0	2 (4.3)	0
	17	**3**	1 (5.9)	0 (0)	2 (11.8)		15	**3**	0	1 (6.7)	0
	0	**4**	0 (0)	0 (0)	0 (0)		0	**4**	0	0	0
Total	138		8 (5.8)	2 (1.5)	12 (8.7)	**Total**	130		2	4 (3.1)	1 (0.8)

Pretoriuskop (OHE)											
2008-2009						2009-2010					
2008 state	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**	**2009 state**	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**
Pollarded	16		2 (12.5)			**Pollarded**	16		0		
Toppled	8		1 (12.5)			**Toppled**	9		2 (22.2)		
Standing	103		2 (1.9)	2 (1.9)	3 (2.9)	**Standing**	96		1 (1.0)	3 (3.1)	1 (1.0)
	11	**0**	0 (0)	0 (0)	0 (0)		9	**0**	0	0	0
	17	**1**	0 (0)	0 (0)	1 (5.9)		17	**1**	0	0	0
	51	**2**	0 (0)	1 (1.9)	2 (3.9)		46	**2**	0	1 (2.1)	0
	24	**3**	2 (8.3)	1 (4.2)	0 (0)		24	**3**	1 (4.2)	2 (8.3)	1 (4.2)
	0	**4**	0 (0)	0 (0)	0 (0)		0	**4**	0	0	0
Total	127		5 (3.9)	2 (1.6)	3 (2.4)	**Total**	121		3 (2.5)	3 (2.5)	1 (0.8)

Sabie thorn thickets west (STT west)											
2008-2009						2009-2010					
2008 state	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**	**2008 state**	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**
Pollarded	2		0 (0)			**Pollarded**	3		1 (33.3)		
Toppled	12		4 (33.3)			**Toppled**	9		1 (11.1)		
Standing	77		1 (1.3)	1 (1.3)	1 (1.3)	**Standing**	74		1 (1.4)	1 (1.4)	1 (1.4)
	11	**0**	0 (0)	0 (0)	0 (0)		11	**0**	0	0	0
	29	**1**	0 (0)	0 (0)	0 (0)		28	**1**	0	0	0
	31	**2**	1 (3.2)	1 (1.9)	1 (3.2)		2	**2**	0	0	1 (50.0)
	6	**3**	0 (0)	1 (16.7)	0 (0)		6	**3**	1 (16.7)	1 (16.7)	0
	0	**4**	0 (0)	0 (0)	0 (0)		0	**4**	0	0	0
Total	91		5 (5.5)	1 (1.1)	1 (1.2)	**Total**	86		3 (3.5)	1 (1.2)	1 (1.2)

Gabbro thornveld south (GT south)											
2008-2009						**2009-2010**					
2008 state	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**	**2009 state**	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**
Pollarded	5		0 (0)			**Pollarded**	5		1 (20.0)		
Toppled	3		0 (0)			**Toppled**	3		0		
Standing	17		1 (5.9)	0 (0)	0 (0)	**Standing**	16		0	0	1 (6.3)
	3	**0**	0 (0)	0 (0)	0 (0)		3	**0**	0	0	0
	2	**1**	0 (0)	0 (0)	0 (0)		2	**1**	0	0	0
	11	**2**	1 (9.1)	0 (0)	0 (0)		10	**2**	0	0	1 (10.0)
	1	**3**	0 (0)	0 (0)	0 (0)		1	**3**	0	0	0
	0	**4**	0 (0)	0 (0)	0 (0)		0	**4**	0	0	0
Total	25		1 (4.0)	0 (0)	0 (0)	**Total**	24		1 (4.2)	0	1 (4.2)

Tshokwane (TSH)											
2008-2009						**2009-2010**					
2008 state	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**	**2009 state**	**n**	**Damage code**	**Dead (%)**	**Pollarded (%)**	**Toppled (%)**
Pollarded	1		0 (0)			**Pollarded**	1		0		
Toppled	6		1 (16.6)			**Toppled**	5		0		
Standing	47		2 (4.3)	0 (0)	0 (0)	**Standing**	45		0	0	0
	12	**0**	0 (0)	0 (0)	0 (0)		9	**0**	0	0	0
	9	**1**	0 (0)	0 (0)	0 (0)		12	**1**	0	0	0
	18	**2**	0 (0)	0 (0)	0 (0)		15	**2**	0	0	0
	6	**3**	0 (0)	0 (0)	0 (0)		9	**3**	0	0	0
	2	**4**	2 (100)	0 (0)	0 (0)		0	**4**	0	0	0
Total	54		3 (5.6)	0 (0)	0 (0)	**Total**	51		0	0	0

3.4.6. Spatial analysis 3: Distance to water analysis (2008-2009)

3.4.6.1. Mortality rates

All marula trees surveyed in this study occurred in the Sabie and Crocodile River catchment areas described by Smit and Ferreira (2010). Between 2008 and 2009, there was a significant negative relationship between mortality rate and long-term mean annual rainfall (R^2=0.79, df=6, p=0.008), mean annual rainfall for the previous three years (R^2=0.82, df=6, p=0.005), but not with the distance to either type of river (seasonal or perennial) (Table 6, Figure 15). Between 2009 and 2010 no significant patterns with rainfall or distance to water could be discerned.

Table 6: Mortality rates in relation to the shortest distance to large perennial and seasonal rivers and mean annual rainfall in the Sabie and Crocodile catchments of the southern Kruger National Park.

Population	**Long-term mean annual rainfall (mm) (rain station)**	**Mean annual rainfall over last 3 years (July 2006-June 2009 (mm))**	**Distance from large perennial river (km)**	**Distance from large seasonal river (km)**	**Mortality rate 2008-2009 (%)**	**Mortality rate 2009-2010 (%)**
ALB	737 (Pretoriuskop)	666.9	3	4	2.6	8.8
STT east	603 (Lower Sabie)	565.5	2	21	5.1	0
PHA	550 (Skukuza)	499.2	6	3.3	5.8	1.5
OHE	737 (Pretoriuskop)	666.9	21	2	4.0	2.5
STT west	550 (Skukuza)	499.2	2.5	3.3	5.5	3.5
GT south	603 (Lower Sabie)	565.5	5	22	4.0	4.2
TSH	562 (Tshokwane)	507.3	11	13	5.6	0

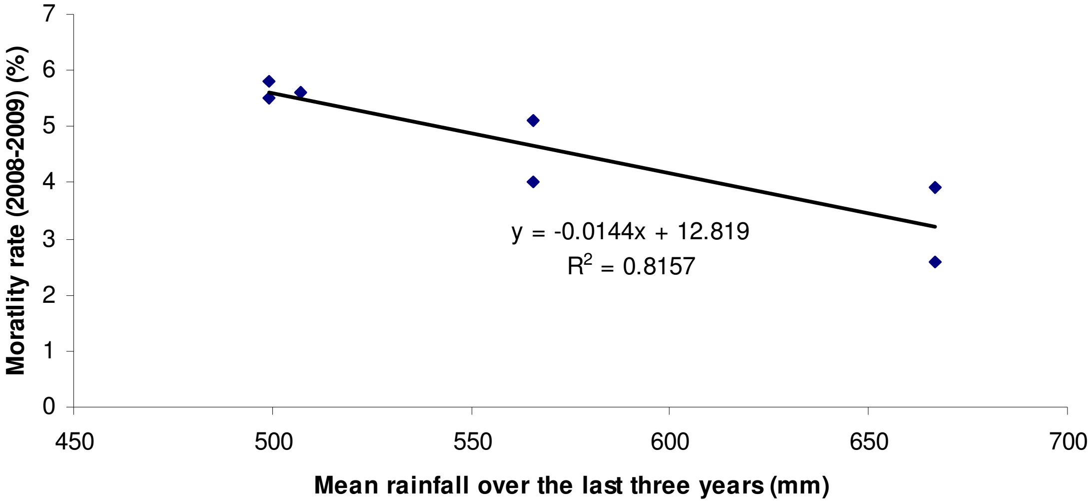


Figure 15: Relationship between mortality rate between 2008 and 2009 and mean annual rainfall over the previous three years in seven populations in the southern Kruger National Park.

3.5. Discussion

Annual mortality rates between 2001 and 2010 of the sampled >3 m in height marula population in the southern KNP ranged from 3.2 to 4.6%. In the much smaller Pongola Nature Reserve, elephants were killing marulas (a relatively rare species in the reserve) at a rate of 5.0% per annum (Duffy *et al.* 2002). The population surveyed in the KNP in 2001 by M. Hofmeyr consisted of only 1.8% dead, while by 2008 this had increased to 18.4% (including missing mature). These results indicate that mortality rates have increased since previous studies were conducted a decade ago. Adult tree annual mortality rates (3.7% between 2001 and 2008, 4.6% between 2008 and 2009, 3.2% between 2009 and 2010) over the study period appear to be relatively constant even though the sample of trees (but not the area surveyed) was decreasing with each progressive year. In comparison, annual tree mortality rates (all species combined) of 1% for Australian savannas (Andersen *et al.* 1998) and 5% for South African savannas (Shackleton 1997) have been reported. These, however, include the small size classes which are expected to have higher mortality rates. Higgins *et al*. (2000) modelled that <5% annual adult mortality is required for tree persistence.

Although marulas are able to recover substantially from damage through resprouting and bark regrowth, a high proportion is still succumbing to elephant utilisation.

Mortality was concentrated in individuals between 3 and 8 m in height. Since the prevalence of individuals between 3-5 m in the areas studied was already low in 2001 (n=12), the further loss of individuals between 5 and 8 m widened the gap between recruits <3 m in height and mature individuals >8 m in height between 2001 and 2008. Therefore, the "missing size class" phenomenon became even more noticeable in the intervening period in certain populations.

89.2% of the mature marula population sampled in 2008 showed some form of utilisation, compared to only 26.8% in 2001, indicating that the proportion of damaged trees has more than tripled. This value is also higher than figures presented by earlier studies. According to Gadd (2002), 62.1% of the marula trees sampled in the private reserves bordering the KNP in 1996 showed signs of elephant utilisation in the form of primary and secondary branch damage. Jacobs and Biggs (2002b) found that almost half of the surveyed population over four different KNP landscapes suffered damage from elephant activity in 2000. Similarly, Shannon *et al.* (2008) found 75% of marulas sampled in the southern KNP were utilised by elephants in 2006. In the Waza National Park, Cameroon, only 14% of marulas had been utilised (Tchamba 1995). Shannon *et al.* (2008) and Trollope *et al.* (1998) also found that marulas had a higher frequency of utilisation relative to other tree species in the KNP. This is important because marulas may show negative responses, despite their reported resilience (Coetzee *et al.* 1979; Gadd 2002; Jacobs and Biggs 2002b) to the elephant impact, long before other less utilised species, and hence could be used as an indicator species (for example, when setting thresholds of potential concern for elephant impacts on woody vegetation in the KNP).

Viljoen (1988) reported that, between 1944 and 1981, the number of large trees in the Satara section of the KNP had decreased by 93.4%. This area corresponds closely to the marula knobthorn savanna (MKS) sampled in this study. In 2001 only marula individuals <3 m in height were still dominant in this area and by 2008 none of these plants had grown above 3 m in height. In addition, mortality of the large trees sampled in this area between 2001 and 2008 was 10.5%. These results indicate that the large tree loss trend in this area has continued. However, there were a healthy number of recruits <3 m in height in this area which could, should conditions permit their escape, allow for the regeneration of a stable size class structure. In contrast, the 3.6% annual mortality of individuals >3 m in height within the STT, has resulted in an overall loss of 25.2% of the trees between 2001 and 2008.

The recruitment of new marulas into the seedling size class was very low in the sampled ecozones. Annual recruitment of trees will vary by species and region but it has been calculated to be in the range of 1-4% for savannas (Duffy *et al*. 1999). Thus, tree mortality >4% per year could eventually remove that tree population from an area (Duffy *et al.* 2002). Recruitment levels in the sampled area were below this theoretical range (approximately 0.2% per annum). These values suggest a population decline over the study period. Possible reasons for the lack of recruitment include drought, droughts within the wet season, or high inter-annual rainfall variability during the study period. However, according to the soil water model of Botha (2006) investigating how the interaction between rainfall, soil substrate and grass influences the probability of tree seedling establishment and based on data collected in the KNP, there was at least one year between 2001 and 2008 in the Satara area that would have been conducive to seedling establishment. Other possible explanations include browsing of the seedlings by impala or predation of the seed by rodents (Chapter 6).

In addition, only one of the individuals in size classes 1 and 2 (<2 m) in 2001 had recruited to a higher size class by 2008. In contrast, 60.5% of marulas <2 m in height had decreased in height as a result of fire. Jacobs and Biggs (2001) reached similar conclusions for marulas in the KNP in 2000. This suggests that the fire regime, or fire in combination with herbivory during the study period, continued to prevent the escape of these marulas from the fire trap.

Walker *et al.* (1986) found evidence of episodic recruitment in a very small marula population in the Nylsvley Provincial Nature Reserve. In contrast, Jacobs and Biggs (2002a) indicated that recruitment was taking place continuously in certain landscapes within the KNP. The lack of recruitment over the last ten years observed in the populations of this study points to an episodic recruitment strategy for marula. Episodic recruitment is most likely to occur either during the seedling establishment phase or during the phase where saplings are able to escape from the fire trap. Long-term suppression of both these phases may result in the absence of certain size classes.

Higher browsing pressure from impala in the STT ecozone could be causing the early death of emerging seedlings and hence the lack of recruiting individuals in this ecozone. It is postulated here that recruitment is prevented by suppression of marulas of 0.25 – 3 m in height by fire in areas with higher grass biomass (hence more frequent or intense fires (van Wilgen and Scholes 1997)), while recruitment is prevented through browser pressure on seedlings (<0.25 m in height) in areas with higher tree density and hence lower fire occurrence. Therefore, a number of different stages of vulnerability in the marula life history still need elucidation. In addition the spatial variability in recruitment and mortality rates indicates that in drier areas there is more mortality and less recruitment. Hence local extirpation is most likely to occur in the more arid parts of the KNP earlier than in the wetter regions.

It has been suggested that a decline in large trees is not necessarily an indication of ecological decline but rather a natural regression to a state that was prevalent before herbivores were "wiped out" from the area in the late 1800's due to hunting and the rinderpest epidemic (Prins and van der Jeugd 1993; van der Vijver *et al*. 1999; Skarpe *et al*. 2004; Shannon *et al.* 2008). If the present density of large trees is due to an episodic recruitment event in the late 1800s, it has been postulated that the ongoing loss of trees due to factors such as elephants would be overshadowed by mass large tree senescent mortality (Shannon *et al.* 2008). However, the adult size classes found to have the highest mortality rates were between 30 and 60 cm in stem diameter, no where near the maximum size that marula adults can reach (~1 m in stem diameter). This indicates that large tree loss is due to the selection of mid range size classes which are more easily toppled and pollarded. Therefore, although a large proportion of the trees established in the KNP may be due to this one large historic recruitment event, the loss of the adult trees is not due to natural senescence of this population cohort. An investigation into the ages of marula trees in the landscape will further aid in weighing the evidence for this hypothesis (see Chapter 4).

Certain populations, specifically STT east and west, PHA and ALB, may actually have established through the aid of early human settlement in the area now protected within the KNP. Before the establishment of the KNP, human settlements were common in the area (Meyer 1986; Joubert 2007). The marula fruit is highly favoured by local inhabitants for its flesh as well as its seed. Large collections of marula fruits may have been brought into the settlements for processing, resulting in favourable conditions for establishment. Once the population in the KNP was protected from humans, no recruitment has occurred. This is postulated to have been the case at Nylsvley (Walker *et al.* 1986), where the marula population was established around human settlements and is currently dying out due to lack of human dispersal and aided regeneration.

With both the recruitment bottleneck (either at the seedling stage due to browser pressure or at the Gulliver stage due to fire suppression) and elephant impact operating simultaneously, the marula population in the KNP may be under threat of significant reduction and possibly even local extirpation if the conditions prevalent between 2001 and 2010 continue. This is thought to have already occurred in the mopane dominated shrubveld on basalts in the northern parts of the KNP (Jacobs and Biggs 2002a), a dry landscape as predicted above. In stark contrast, an enclosure (NE) erected in 1968 excluding all herbivores greater than hares except for roan antelope, in this same landscape, contains a healthy population of marulas (Chapter 2; Jacobs and Biggs 2002a). Marula has also been lost from the Tuli Game Reserve in Botswana (O'Connor *et al.* 2007), after the reintroduction of elephants.

A current focus in the Kruger National Park has been on riparian trees due to the finding that elephant densities have increased the most around perennial and large seasonal rivers (Smit and Ferreira 2010). However, a study conducted in 2008 indicated that elephant impact on marulas along rivers is of little concern to the future persistence of this species (Hartmann 2008). Since marulas do not occur in large numbers along rivers but prefer well drained crests (Jacobs and Biggs 2002a, Hall *et al.* 2002) and since most marulas that do occur along rivers are very large and hence relatively immune to elephant impacts (the average size of trees along rivers was 60 cm in diameter), this study has little predictive capability in terms of the future dynamics of marula populations away from rivers. It is therefore not possible to state that elephant impacts are of little concern to the future persistence of adult marulas in the KNP. Although a number of other mortality agents such as lightning (Spinage and Guiness 1971), windthrow (O'Connor 2010), drought (Viljoen 1988), parasite infestation, disease and natural senescence are acting simultaneously, the present study has shown that elephant impact is most likely the greatest cause of adult marula tree mortality. However, the interaction of elephants and fire on adult size classes also needs further investigation (refer to Chapter 8).

3.6. An ecological narrative of the persistence of marula in the KNP

This study has attempted to explain a passage of ecological history. Such a study is not repeatable but can provide insights into the ecological processes taking place and can be used for predicting possible future states (O'Connor 2010). This study covered 10 years, which is a small window in the life of a long lived tree species. However, this small window has provided detailed data on what is currently happening to marula trees in the KNP. It indicates that there is a process of change occurring in terms of the future persistence of marula. The lack of recruitment in some habitats combined with the loss of reproductive trees indicates that marula may in future become a rarer species relegated to rocky refugia. The populations present in these areas today may even be remnants of populations established through the aid of early human settlements. However, in order to understand the life history strategy of marula, the reasons for why they are unable to establish within these areas without assistance still need elucidation. The responses of marula seedlings to defoliation, drought and fire are all explored in later chapters of this thesis. In other areas where recruitment is continuous but escape from the fire trap is hampered, further study into the vulnerability of marula seedlings to fire is required to determine whether the fire regime is the only cause (refer to Chapter 8). Overall, the current rate of loss of large trees in the KNP appears unsustainable and the future dominance of marula is unlikely. A simple thought experiment can verify that given an average loss of 4% per annum continuing linearly into the future and assuming zero recruitment, the sampled marula population (n=630) will drop below ten individuals within 100 years. However, given that ecological systems are complex, such a projection is best placed within a modelling framework where stochastic and deterministic factors are all taken into account (refer to Chapter 11).

3.7. References

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PART B: LIFE HISTORY CHARACTERISTICS OF MARULA

Chapter 4: Growth rates of *Sclerocarya birrea* subsp. *caffra* (marula) protected from fire, elephants and other browsers

How fast do marula grow when not exposed to fire or herbivory?

4.1. Abstract

Given that savanna woody plant growth rate data are rare and that such data provides useful information on escape opportunities from disturbances such as fire and herbivory, this study was undertaken to determine the growth rates of marula saplings under natural conditions. Basal stem diameter and height growth rates of marula trees in three populations (N'washitsumbe enclosure (NE), Hlangwine enclosure (HE), Wits Rural Facility (WRF)) protected from herbivory and fire, were monitored over three years (2007-2010). Overall, only 1% of the stems died over the study period. All stems that died (n=5) had shrunk in stem diameter by >8% the season before. This contrasts with annual mortality rates exceeding 4.6% in populations exposed to elephant herbivory and fire in the Kruger National Park. Termite activity decreased the AGR and RGR of trees affected. It did not, however, affect the relationships between these variables and tree size or competition indices. Annual RGRs were significantly different between sites, with those at WRF (4.8%) being the highest and NE (1.9%), the lowest. Overall RGRs decreased with increasing stem diameter, ranging from 8.5% for stems 2-5 cm in diameter to 0.4% for stems >30 cm. Annual AGR stem diameter increments ranged between 0.2-1.1 cm. Based on these rates, it takes marula approximately 46 years to reach reproductive maturity. These data also indicate that marula trees of 80 cm in diameter, relatively common in the KNP, are approximately 300 years old. Marula trees establishing in the 1880s at the time of low herbivore densities (due to rinderpest and hunting) would now be approximately 40 cm in stem diameter. This indicates that a large proportion of the marula trees present in the KNP today, predate the proposed rinderpest epidemic recruitment event. Continued monitoring is required to determine the range of variability in growth rates over time.

4.2. Introduction

The life history strategies and responses of savanna woody species to disturbance are still poorly understood. In particular, the growth rates of savanna woody trees are largely undocumented (Baxter and Getz 2005), and marula is no exception (Hall *et al.* 2002). This situation exists despite much research into and reviews on marula biology published in the literature (Shone 1979; von Teichman 1982; 1983; Hall *et al.* 2002; Shackleton *et al.* 2002; Wynberg *et al.* 2002). These studies have largely been motivated by the commercial importance of the marula fruit and conclude that much is known about the fruit and its uses, as well as growing marulas under artificial conditions. However, the available information on natural marula populations is fragmentary, based on a collection of observations which have not been integrated (Shackleton *et al.* 2002).

In disturbance prone environments such as African savannas, growth is a key variable regulating tree cover, as it determines the probability of escape from fire sensitive size classes (Holdo 2006). In the fire and elephant spatial model of Baxter and Getz (2005), one of the main outcomes was that information on the demography, growth rates in particular, of savanna woody species was needed, as the model was very sensitive to these parameters. It was suggested that growth rates have a significant effect on the grass tree dominance relationship as well as the response of the woody vegetation to utilisation by elephants and fire. Model parameterisation has until now relied on data from widely varying sources and species, often based on few individuals (Ben-Shahar 1996; Higgins *et al.* 2000, Baxter and Getz 2005). Hence, detailed growth and transition rate data between size classes is highly sought after information.

Since marulas are utilised by both humans and animals and have a high ecological, cultural and economic value, estimates of size specific growth rates are imperative for commercialisation ventures (Shackleton *et al.* 2002), as well as the management of natural populations. Growth rates are also dependent on a variety of extrinsic factors including soil type and rainfall (Shackleton 1997; Holdo 2006). Considerable uncertainty exists about the effects of extrinsic factors such as competition and site attributes on the growth of savanna trees and this has been attributed to the variability observed between individuals and between seasons (Holdo 2006). Many life history processes are thought to be highly stochastic, driven by spatiotemporal rainfall variability (Higgins *et al*. 2000). Estimates of the background growth rates of undamaged trees at a number of sites and their relationship to size, rainfall and soil type are the starting point for model parameterisation. Thereafter the effect of utilisation, in the form of fire or herbivores, on the growth rates of these trees is required to understand their dynamics.

It has been noted by various authors that at certain sites, especially those with megaherbivores (Owen-Smith 1992), a "missing size class" has become evident, whereby marula individuals between 2-8 m are missing (Jacobs and Biggs 2002a; Neke 2005). This has also been noted in some communal lands in Limpopo by Neke (2005). An analysis of the studied populations of marula in Chapter 2 indicated four main groups of frequency distributions. The groups appeared to be transitioning between each other, with the first group consisting of a stable size structure (negative J-shaped curve), followed by the group with the "missing size class", followed by the groups consisting entirely of juvenile or adult trees, being the end products of either ongoing lack of recruitment (at the seedling regeneration phase or at the escape from the fire trap phase), or due to removal of large trees by megaherbivores or humans. Jacobs and Biggs (2001) suggested that the previous fire management policy (before 2002; controlled triennial block burns) within the KNP was preventing the marula population from recruiting to adult size classes. Further evidence indicated that elephants were targeting individuals between 2 and 8 m in height, causing mortality in this size class (Jacobs and Biggs 2002b). In contrast, Lewis (1987) and Haig (1999) attributed marula mortality to be mostly in the seedling phase due to browsing by impala (*Aepyceros melampus*). Walker *et al.* (1986) suggested that recruitment of marula was highly episodic and hence limited by rainfall events resulting in bimodal population structures. An understanding of the growth rates of the individuals could aid in sorting through some of these conflicting hypotheses. The rate of throughput of the various size classes into the next largest size class could then be calculated and deductions on how quickly marula trees can become tall enough to escape the fire trap or estimates of the age of individuals within each size class, can then be made.

Finding undisturbed populations of marula is difficult. In addition, finding individuals in the so called "missing size class" is even more difficult. Fortuitously there are a few protected sites where the "missing size class" occurs in large numbers and where agents of disturbance such as elephants, other browsers and humans do not have access to the trees. In addition the effect of fire could also be controlled. These sites occur where herbivore exclusion has been practiced in the KNP and in some protected reserves outside the KNP.

Enquist *et al.* (1999) found that for tropical dry forest species, there was essentially a trade-off in growth rate with allocation to tissue density: species that allocate less biomass to their stems (light woods) increase in basal diameter faster than species that allocate more (dense woods). Marula has been categorised as having a relatively low wood density (590 kg/m^3) (Shone 1979; Goldsmith and Carter 1981), which makes the wood very popular for carving. Hence growth rates for marula are expected to be relatively fast (Gaugris *et al.* 2008)

This study aims to determine the growth rates of marula trees between 1 and 30 cm in basal stem diameter, and protected from fire, herbivory and elephants, over three growing seasons (2007 and 2010). Growth data for individuals >30 cm in stem diameter from KNP Scientific Services and C. Shackleton (unpublished data) were also obtained to increase the size range studied and for comparison. The data presented here represents the natural background growth rate of healthy undamaged marula trees. Early seedling growth was measured in the glasshouse to complete the growth dynamics of marula and is presented in Chapter 5.

The key objectives of this study were therefore to: a) characterise basal stem diameter increments as a function of stem size; canopy area and canopy dieback; b) characterise height increments for fire sensitive size classes; c) investigate the pattern of variation in growth across sites and between years; d) test for competitive effects on tree growth from neighbouring trees; and e) summarise all available data on marula growth rates in order to estimate size specific ages and longevity.

4.3. Methodology

4.3.1. Study Sites

Three sites in the eastern low altitude savannas of South Africa were chosen to assess the growth rates of marula. The sites were: (a) the 254 ha N'washitsumbe enclosure (NE) north of Shingwedzi in the northern part of the 18 998 km^2 KNP; (b) the 220 ha Hlangwine enclosure (HE) east of Pretoriuskop in the southern KNP, and (c) the 350 ha Wits Rural Facility (WRF) near Klaserie on the western boundary of the KNP (Figure 1). The N'watshishumbe enclosure was established in 1968 (Levick and Rogers 2008), while the Hlangwine enclosure was established in 1972 (Goodall 2006), both to exclude all animals larger than hares except rare antelope grazers such as roan (*Hippotragus equinus*) and sable (*Hipppotragus niger*). These sites were chosen due to their protection from disturbances such as elephants, humans, browsers and fire (Table 1).

Table 1: Characteristics and disturbance history of the three study sites in the low altitude savannas of South Africa (information from Grant *et al.* 2002; Kröger and Rogers 2005; Frandsen 2008).

	Hlangwine enclosure (HE)	**N'washitsumbe enclosure (NE)**	**Wits Rural Facility (WRF)**
Location	S25.20162 E31.29076 Southern KNP	S22.78049 E31.28185 Northern KNP	S24.56386 E31.10331 Western boundary of KNP
Management	Rare antelope breeding	Rare antelope breeding	Research
Long-term mean annual rainfall (mm)	737	515	682
Geology	Granite Sandy soil	Basalt Clay soil	Granite Sandy soil
Year of last fire	2006	2002	2002
Browsers	None	None	Few impala and kudu
Marula tree density (trees/ha)	88.9	37.7	8.7

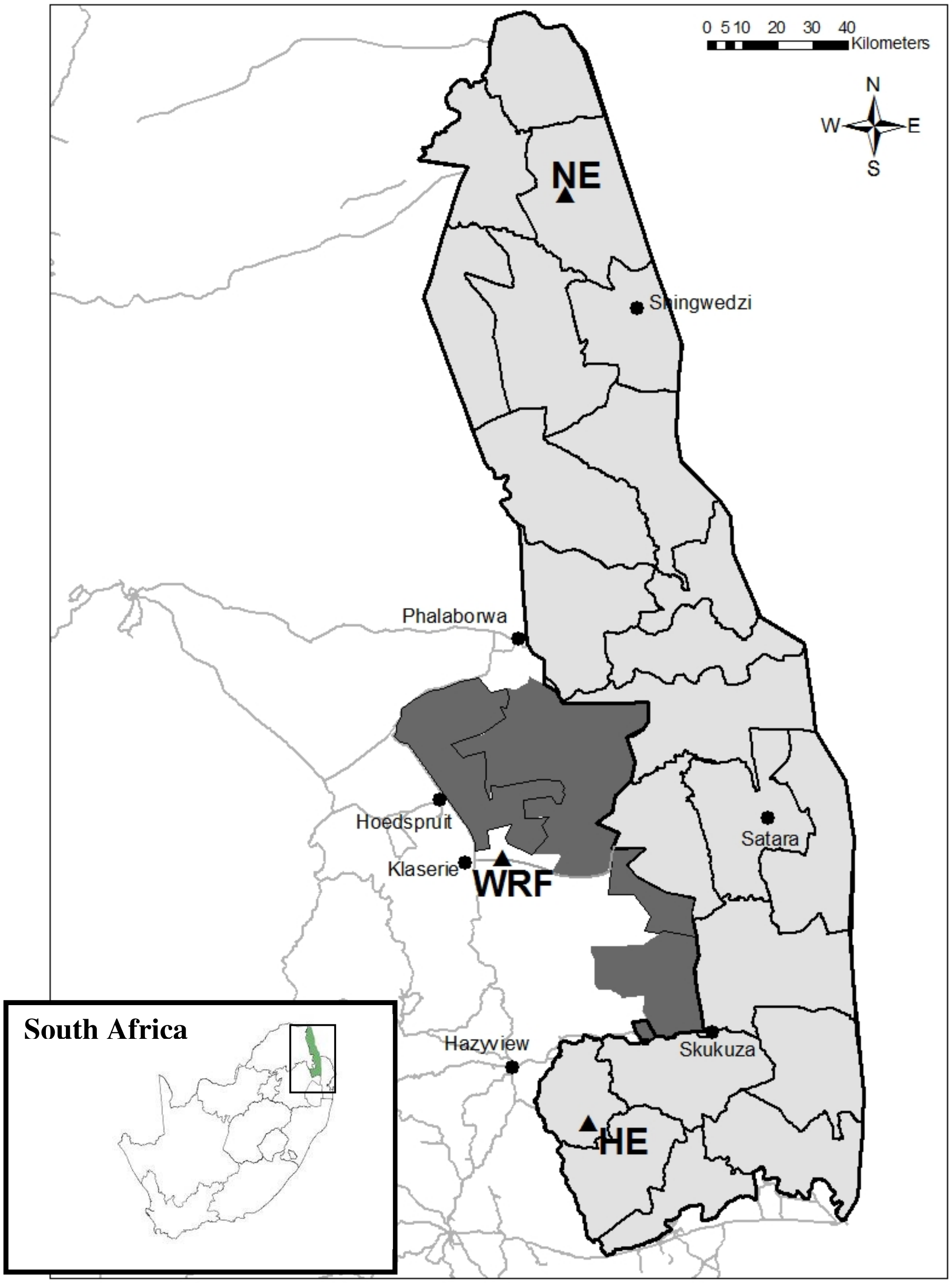

Figure 1: Location of the three study sites (triangles) in relation to the Kruger National Park (light grey) and within South Africa (inset). HE=Hlangwine enclosure in the KNP, NE=N'washitsumbe enclosure in the KNP and WRF=Wits Rural Facility.

4.3.2. Tree sampling and growth assessment

Trees were selected through walking a minimum of 4 randomly located wandering transects at each site until a minimum of 40 individuals in each of the following height size classes were obtained: 1-3 m, 3-5 m, 5-8 m. This was done in late October 2007. These individuals were concentrated in one management block to ensure that they would receive the same fire treatments, if any, and that they had the same fire history. In total 243 trees were assessed at WRF while at HE and NE 122 and 130 trees were assessed respectively. Each tree was marked with a metal tag, its GPS position taken, and the following parameters were measured: height, diameter above the basal swelling (usually 30 cm from ground when no swelling is present), and diameter at breast height (1.3 m). A dot using oil paint was made at the exact position at which the basal diameter was measured to ensure that future measurements were made in the same position. Diameters were measured with a tree calliper accurate to 0.5 mm. Height was measured with a tape measure for trees <3 m and with a digital height metre for trees >3 m. In addition percentage foliar herbivory, nearest neighbour species, its distance and height, nearest marula distance and its height, presence of termite activity (either on the stems or at the base of the tree) and percentage canopy dieback were assessed. In May 2008, November 2008, May 2009, November 2009 and May 2010, each tree was reassessed in terms of height, stem diameter at 30 cm, and percentage foliar herbivory. Each interval represented alternative growing and dormant seasons of six months.

Many previous studies have estimated growth rates from trees within a specified plot (Shackleton 1997; Holdo 2006). These plot samples select trees close together and hence the number of trees in each size class or of species cannot be controlled. Plotless sampling for growth rate analysis, as used here, enables the researcher to select a sample of trees from across all size classes and ensures a representative sample for the entire size range.

Tree trunks swell when wet and shrink during the dormant season dependent on the soil moisture levels (Baker *et al.* 2002). It was all but impossible to ensure that the trees were measured before soil moisture affected stem swelling at the beginning of the growing season. However, at the beginning of November when the early growing season was just starting, little enough rain had fallen to assume that the stem measurements taken would be essentially the seasonal minimum. Measurements taken in May towards the end of the growing season would be when the stems had swelled to their maximum capacity.

Data from other researchers was obtained for trees >30 cm in diameter. Data obtained from C. Shackleton (unpublished data) consisted of basal diameter measurements of 20 trees (15 marked in February 1994 and an additional 5 marked in Dec 2001) at WRF. These trees were reassessed in August 2008 (14 and 7 years respectively). Biannual growth data was also obtained from Scientific Services in the KNP for 10 trees in the Nkuhlu exclosure near Lower Sabie and 10 trees in the Buffalo enclosure near Satara, originally fitted with dendrobands in June 2006. These trees were reassessed in July 2008.

Monthly rainfall data was obtained from Vlakteplaas ranger station for NE, Pretoriuskop ranger station for HE and WRF Lodge for WRF (http://www.sanparks.org/parks/kruger/conservation/scientific/weather) (Table 2). Five independent soil samples from the A soil horizon (0-10 cm deep) from each site were collected and sent for analysis of various physical and chemical properties (eg. total N, C, cation concentrations, pH, texture, etc.) to the Soil Fertility and Analytical Services laboratories of the Kwa-Zulu Natal Department of Agriculture and Environmental Affairs at Cedara (Table 3; methodology: Appendix 1).

4.3.3. Data analysis

All data was tested for normality prior to analysis using the Shapiro Wilks Test. Soil properties were compared using one-way ANOVA and Fisher's LSD. The absolute (AGR) and relative growth rates (RGR) of trees in the following five size classes between each monitoring period were calculated at each site: <5 cm (established stems vulnerable to fire which will regress to resprouting basally after a fire, Chapter 8), 5-9 cm (stems which will be damaged by fire but which will be able to resprout epicormically, Chapter 8), 9-14 cm (non reproductive, fire resistant stems, Chapter 6 and 8), 14-20 cm (reproductive stems producing few fruit, Chapter 6), 20-30 cm (reproductive stems producing intermediate numbers of fruit, Chapter 6).

Table 2: Total rainfall during the growth monitoring periods between 2007 and 2010 at three sites in the low altitude savannas of South Africa.

Sites (see table 1 for abbreviations)		**HE**	**NE**	**WRF**
November-April	Long-term mean (mm)	652	463	655
May-October	Long-term mean (mm)	85	58	74
November-October	Long-term mean (mm)	737	521	729
Monitoring period 1 (Nov07-April 08)	Actual rainfall (mm)	460	579	503
	Deviation from mean (%)	-29	+25	-23
Monitoring period 2 (May 08-Oct08)	Actual rainfall (mm)	72	36	83
	Deviation from mean (%)	-15	-38	+12
Monitoring period 3 (Nov08-April 09)	Actual rainfall (mm)	803	248	869
	Deviation from mean (%)	+23	-46	+33
Monitoring period 4 (May 09-Oct 09)	Actual rainfall (mm)	194	54	62
	Deviation from mean (%)	-128	-7	-16
Monitoring period 5 (Nov09-April 10)	Actual rainfall (mm)	575	468	714
	Deviation from mean (%)	-12	+1	+9
Mean annual rainfall (May07-May10)	Actual rainfall (mm)	727	480	764
	Deviation from mean (%)	-1	-8	+5

Due to the large variation in the growth rate data, normality was not achieved for the NE and WRF data sets and transformations were unable to improve the fit. Where the data were not normal, equivalent non-parametric tests were used for the comparisons across sites. Comparisons between the relative growth rates of trees associated with termite activity and those not associated were made using a t-test. It was found that termite activity affected the growth rates of the stem (see results section). All plants that had termite activity were thereafter removed from the data set. This resulted in the remaining data being normally distributed and ordinary parametric statistical analyses of the data could follow. For regression analysis the assumption of homogeneity of variance was not met by the data. In an attempt to analyse the data using robust regression techniques, it was found that the results did not differ from the ordinary regression analysis, and hence normal least squares regression was used to analyse relationships in this chapter. Differences in basal diameter increments and RGR between size classes were calculated using one-way ANOVA and Fisher’s LSD. Coefficients of variation (CV) for the basal diameter increment were calculated for the same monitoring period across sites, and at the same site across individuals. Height growth rates for those individuals <3 m in height were assessed and compared using one-way ANOVA and Fisher's LSD. Trees with missing measurements for any monitoring period were removed before analysis.

Table 3: Comparison of soil texture and fertility (mean±SE, n=5/site) between basaltic and granitic soils from three sites in the low altitude savannas of South Africa. Significant differences between the two soil types were tested using one-way ANOVA and Fisher's LSD and represented with different letters ($p<0.05$).

Soil property	**NE**	**WRF**	**HE**	**$F_{2,12}$**	**p**
Geology	Basalt	Granitic gneiss	Granite		
Texture class	Sandy clay loam	Loamy sand	Loamy sand		
Clay (<0.002mm) (%)	32.8 ± 0.8^{a}	8.0 ± 0^{b}	8.0 ± 0.3^{b}	831.1	**<0.0001**
Fine silt (0.002-0.02mm) (%)	20.6 ± 0.5^{a}	3.8 ± 0.2^{b}	7.6 ± 0.2^{c}	646.8	**<0.0001**
Course silt and sand (0.02-2mm) (%)	46.6 ± 1.1^{a}	88.4 ± 0.2^{b}	84.2 ± 0.6^{c}	957.4	**<0.0001**
Bulk density (g/ml)	1.04 ± 0.01^{a}	1.33 ± 0.02^{b}	1.32 ± 0.03^{b}	66.4	**<0.0001**
Available P (mg/L)	5.4±1.7	4.0±0.8	3.4±0.2	1.5	0.26
Exchangeable K (mg/L)	855.0 ± 69.3^{a}	89.6 ± 9.1^{b}	173.8 ± 18.1^{b}	101.4	**<0.0001**
Exchangeable Ca (mg/L)	5763.0 ± 153.6^{a}	320.2 ± 40.3^{b}	555.2 ± 10.3^{b}	1121.5	**<0.0001**
Exchangeable Mg (mg/L)	481.0 ± 31.9^{a}	65.2 ± 7.9^{b}	114.0 ± 7.2^{b}	137.2	**<0.0001**
Total cations (cmol/L)	35.0 ± 1.0^{a}	2.5 ± 0.3^{b}	4.2 ± 0.1^{b}	920.7	**<0.0001**
pH (KCl)	6.7 ± 0.1^{a}	4.6 ± 0.1^{b}	5.1 ± 0.1^{c}	161.7	**<0.0001**
Total Zn (mg/L)	0.8±0.1	1.0±0.4	1.3±0.3	0.78	0.48
Total Mn (mg/L)	2.8 ± 0.2^{a}	17.6 ± 0.9^{b}	19.6 ± 1.3^{b}	98.7	**<0.0001**
Total Cu (mg/L)	3.3 ± 0.1^{a}	1.0 ± 0.1^{b}	0.8 ± 0.03^{c}	292.1	**<0.0001**
Total N (%)	0.15 ± 0.02^{a}	0.09 ± 0.01^{b}	0.11 ± 0.01^{b}	6.61	**0.012**
Organic C (%)	2.24 ± 0.16^{a}	0.83 ± 0.12^{b}	1.34 ± 0.09^{c}	32.4	**<0.0001**

The effects of neighbouring trees on marula RGR over the study period was investigated using two different measures of competition influence (Penridge and Walker 1986): nearest neighbour distance and Wiener's nearest neighbour interference (W). W was calculated as the height of the nearest neighbour divided by the square of the distance to the nearest neighbour. This was done for (a) the data set as a whole (b) for each site and (c) for each of the five diameter size classes. The relationship between RGR and the nearest neighbour distance and W was determined using regression analysis.

Given that a number of variables interact simultaneously to affect growth rates a linear multiple regression model was developed using best subsets to determine which variables affected annual growth increment. The variables initially used in the model included: initial stem diameter, canopy area, % canopy dieback, nearest neighbour distance and /or Weiner's neighbour index (W) and site. First the presence of autocorrelation in the growth data was explored by regressing current-year on previous-year growth (Brienen *et al.* 2006) and using the Durbin Watson statistic to determine the presence of autocorrelation. If significant temporal autocorrelation was found, the data were detrended by rotating data points around the mean (Berryman 1997) before input into the model using the equation:

$DDt = Dt - (a + bt) + d$

Where DDt is the detrended data point at time t, Dt is the observed data point at time t, d is mean of the observed data series, a is the regression intercept and b is regression slope following Chidumayo (2008).

4.4. Results

4.4.1. Absolute basal diameter growth rates

Stem diameter shrinkage occurred during the dormant season and growth during the growing season (Table 4). Overall, the greatest degree of shrinkage occurred during the May09-Nov09 dormant season and the greatest growth during the Nov09-May10 growing season (Table 4). Up to 5% of the stems showed overall shrinkage over the study period (Nov07-May10), with the highest degree of shrinkage occurring at the driest site, NE. The mean basal diameter increment (AGR) varied widely between monitoring periods for all sites (Figure 2). Inter-annual CVs were 51%, 92% and 171% for HE, WRF and NE respectively. Inter-site CVs within monitoring periods varied from 18% (Nov09-May10) to 521% (May08-Nov08). Variation across individuals within a site was highest at HE (248%) and lowest at WRF (114%).

The relationship between diameter increment over a monitoring period and rainfall for all sites combined (n=15) was strongly positive ($F_{1,13}$=22.33, p=0.0004, r^2=0.63) with a linear relationship of:

Basal diameter increment (cm) =0.0012*Rainfall (mm)-0.1636

At WRF, NE and HE, 43%, 43% and 0% of the trees respectively were associated with some form of termite activity. Mean annual AGR was 50% higher in trees without termite activity at NE, but remained the same for trees at WRF. A comparison of the growth rate in trees where termite activity was evident versus those where it was not, indicated that RGR is negatively affected by the presence of termites ($p<0.05$; Figure 3).

Table 4: Number (and percentage in parenthesis) of marked stems that grew, shrank, remained unchanged or died for each monitoring period. N=actual number of trees where a growth estimate was obtained for that monitoring period as it was not always possible to measure a stem.

Site	**Monitoring period**	**N**	**Grown**	**Shrunk**	**Same**	**Died**
Hlangwine enclosure (HE)	**Nov 07-May 08**	122	93 (76)	16 (13)	13 (11)	0 (0)
	May08-Nov08	121	89 (74)	14 (11)	18 (15)	0 (0)
	Nov08-May09	121	74 (61)	24 (20)	21 (17)	1 (1)
	May09-Nov09	120	39 (33)	53 (44)	27 (23)	1 (1)
	Nov09-May 10	118	101 (86)	11 (9)	6 (5)	0 (0)
	Mean (Nov07-May10)	121	107 (88)	9 (7)	3 (2)	2 (2)
N'washitsumbe enclosure (NE)	**Nov 07-May 08**	129	70 (54)	27 (21)	30 (23)	2 (2)
	May08-Nov08	127	34 (27)	59 (46)	34 (27)	0 (0)
	Nov08-May09	127	98 (77)	14 (11)	15 (12)	0 (0)
	May09-Nov09	127	8 (6)	107 (84)	12 (9)	0 (0)
	Nov09-May 10	126	125 (99)	0 (0)	1 (1)	0 (0)
	Mean (Nov07-May10)	128	108 (84)	11 (9)	7 (5)	2 (2)
Wits Rural Facility (WRF)	**Nov 07-May 08**	219	158 (72)	38 (17)	24 (11)	0 (0)
	May08-Nov08	217	38 (18)	153 (71)	26 (12)	0 (0)
	Nov08-May09	167	166 (99)	1 (1)	0 (0)	0(0)
	May09-Nov09	169	12 (7)	139 (82)	18 (11)	0(0)
	Nov09-May 10	227	216 (95)	3 (1)	5 (2)	3 (1)
	Mean (Nov07-May10)	229	220 (96)	2 (1)	4 (2)	3 (1)
Overall	**Nov 07-May 08**	470	320 (68)	81 (17)	67 (14)	2 (0.4)
	May08-Nov08	465	161 (35)	226 (49)	78 (17)	0 (0)
	Nov08-May09	415	339 (82)	39 (9)	36 (9)	1 (0.2)
	May09-Nov09	416	59 (14)	299 (72)	57 (14)	1 (0.2)
	Nov09-May 10	471	442 (94)	14 (3)	12 (3)	3 (0.6)
	Mean (Nov 07-May 10)	476	435 (91)	22 (5)	14 (3)	5 (1)

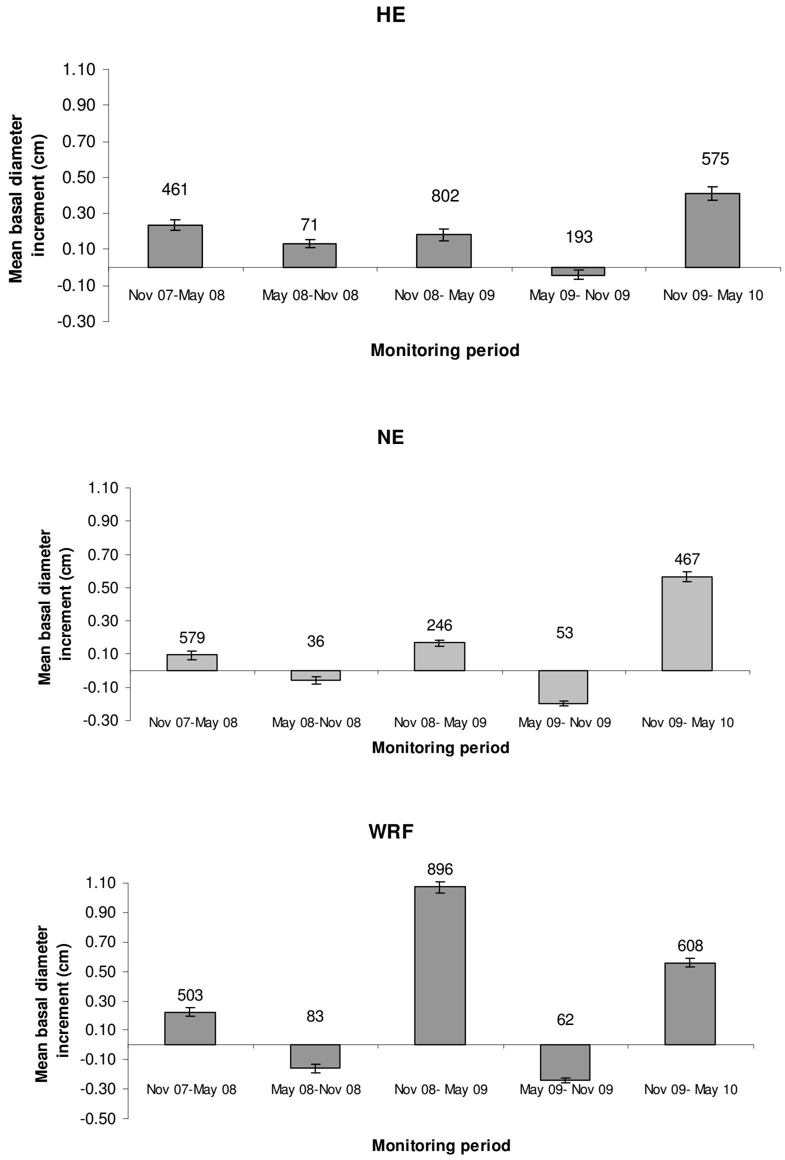

Figure 2: Absolute growth rates (mean±SE) between the monitoring periods from 2007 to 2010 at the Hlangwine enclosure (HE) (N=116), N'washitsumbe enclosure (NE) (N=125), and Wits Rural Facility (WRF) (N=155). Numbers above the bars indicate the rainfall (mm) that had fallen during the period of growth assessed.

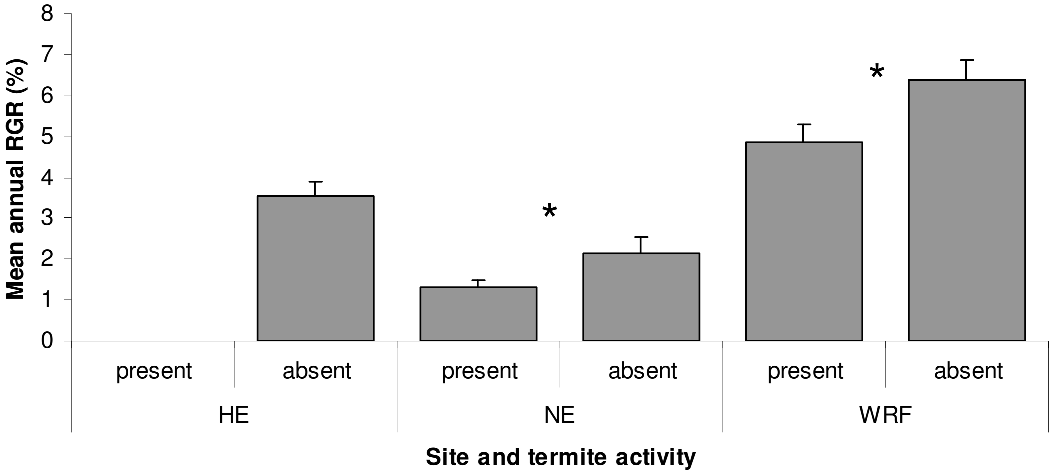

Figure 3: Effect of termite activity on the mean annual relative growth rate (RGR) of marula at three sites in the low altitude savannas of South Africa. * indicates significant differences within a site.

Significant differences in mean annual basal diameter increment were found between sites (Kruskall Wallis test: $H_{2,392}$=76.80, p<0.0001), with WRF higher than all other sites and HE higher than NE (Figure 4). When trees affected by termites were removed, the same patterns were observed (ANOVA: $F_{2,273}$=14.01, p<0.0001).

Absolute growth increment had no significant relationship with stem diameter at WRF ($F_{1,155}$=0.80, p=0.37), but did have a negative relationship for HE ($F_{1,115}$=16.36, p<0.0001) and a positive relationship for NE ($F_{1,124}$=37.60, p<0.0001) (Figure 5). Overall, for all sites combined, there was no relationship between mean annual basal diameter increment and initial stem diameter ($F_{1,395}$=0.79, p=0.79). Minimal within size class variation in AGR was observed (p<0.05). When trees affected by termite activity were removed, a weak negative relationship across all sites was observed ($F_{1,274}$=13.51, p=0.0003, R^2=0.047), but there was still no relationship at WRF ($F_{1,87}$=0.13, p=0.72), and the relationship at NE remained positive ($F_{1,69}$=11.58, p=0.0011, R^2=0.14).

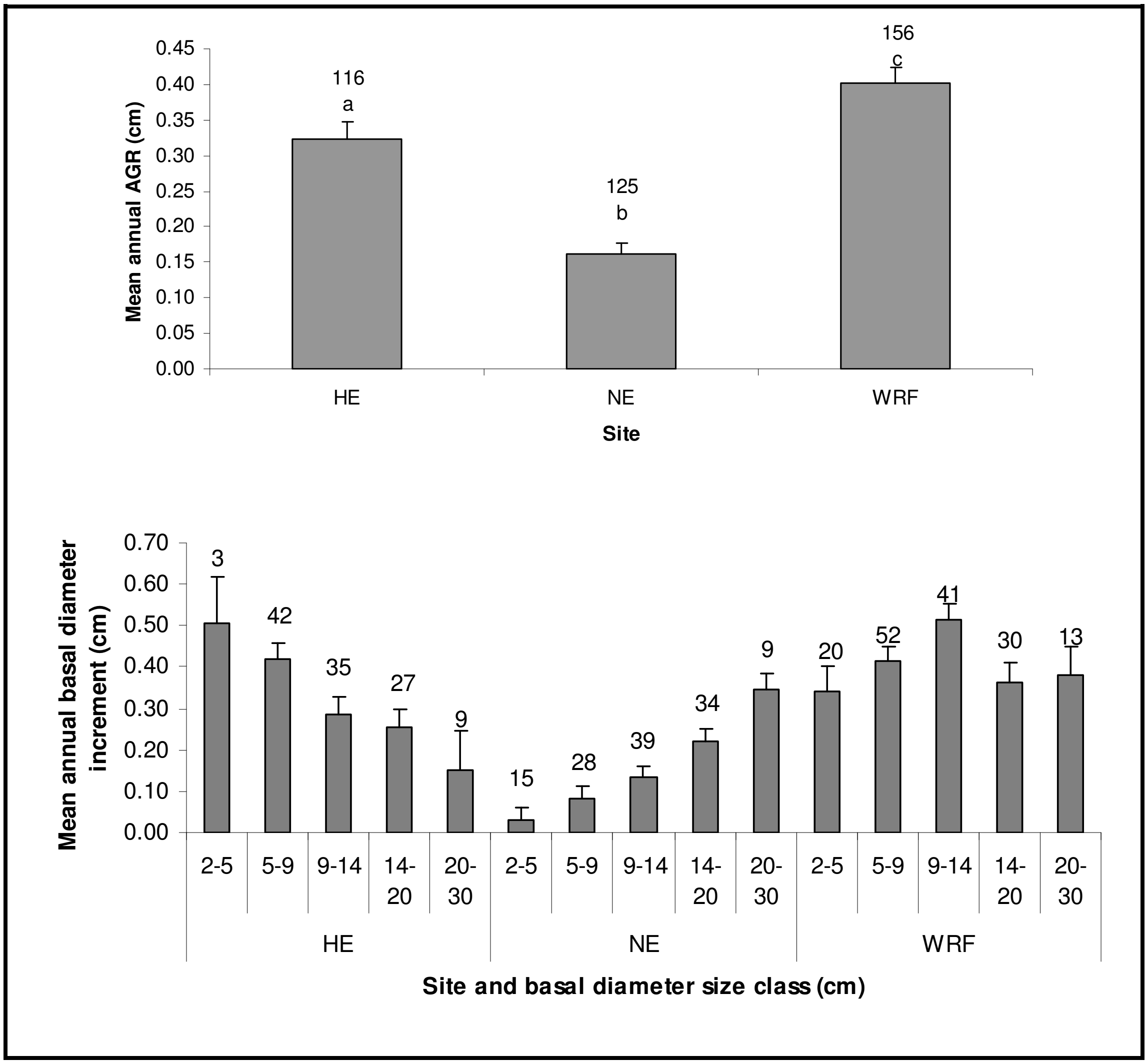

Figure 4: Mean annual basal diameter increment (AGR) (cm) at three sites combined in the low altitude savannas of South Africa. Numbers above the bars represent sample sizes and different letters indicate significant differences between sites.

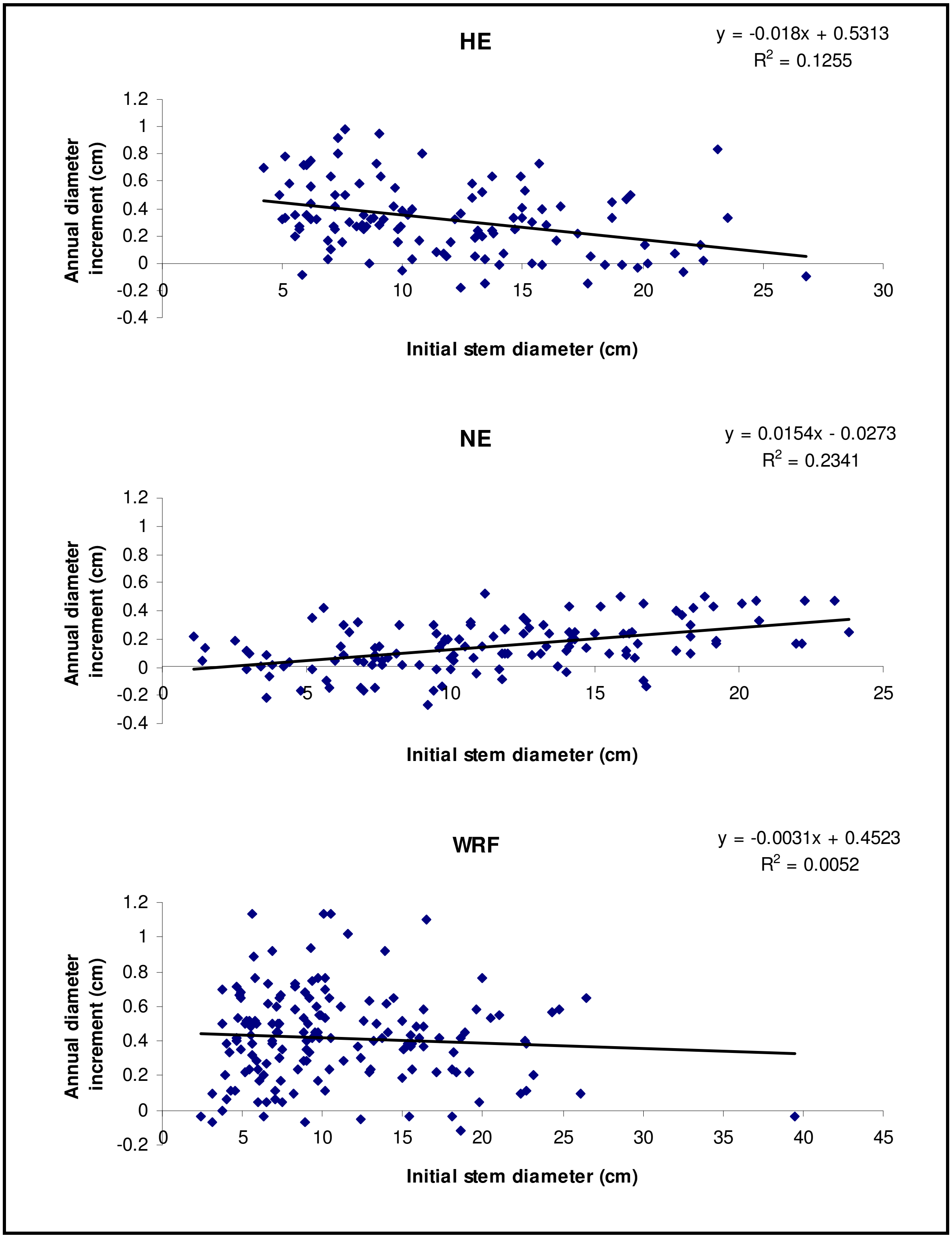

Figure 5: Relationship between mean annual growth increment (cm) (AGR) of individual trees and initial stem diameter for three sites in the low altitude savannas of South Africa.

The relationship between mean annual increment and canopy area as well as the percentage canopy dieback was highly variable across sites (Table 5). NE was the only site where the mean annual growth increment had a significant positive relationship with canopy area, while HE had a significant negative relationship. Only HE did not have a significant negative relationship with percentage canopy dieback. When trees with termites were removed, the same patterns were observed.

Table 5: Relationships between mean annual growth increment and canopy area and percentage canopy dieback at three protected sites in the low altitude savannas of South Africa.

Site	**Canopy area (m^2)**				**Canopy dieback (%)**			
	slope	**R^2**	**F**	**p**	**slope**	**R^2**	**F**	**p**
WRF	none	0.015	2.40	0.12	negative	0.06	9.75	0.002
HE	negative	0.04	4.77	0.03	none	0.002	0.26	0.61
NE	positive	0.25	41.07	<0.0001	negative	0.05	6.56	0.01
Overall	none	0.002	0.005	0.94	negative	0.05	19.87	<0.0001

4.4.2. Relative basal diameter growth rates

There was significant variability in RGR between monitoring periods (Figure 6), but 65% of this variability can be explained by rainfall alone ($F_{1,13}$=24.2, p=0.00028, RGR(%)=0.0111*Rainfall(mm)-1.9337, r^2=0.65). There were significant differences in RGR between sites ($F_{2, 393}$=43.33, p<0.0001), with WRF having the highest and NE the lowest (Figure 7a). The smallest size classes had the highest relative growth rates of up to 12% (Figure 7b). All sites had significant negative exponential relationships between RGR and initial basal stem diameter (Figure 8). When trees with termite activity were removed, the same patterns were evident. The relationships between RGR and both a) canopy area and b) percentage canopy dieback were highly variable across sites (Table 6). WRF and HE RGR had significant negative relationships with canopy area. Only NE had a significant negative relationship with percentage canopy dieback. Removal of the trees with termites resulted in the same pattern.

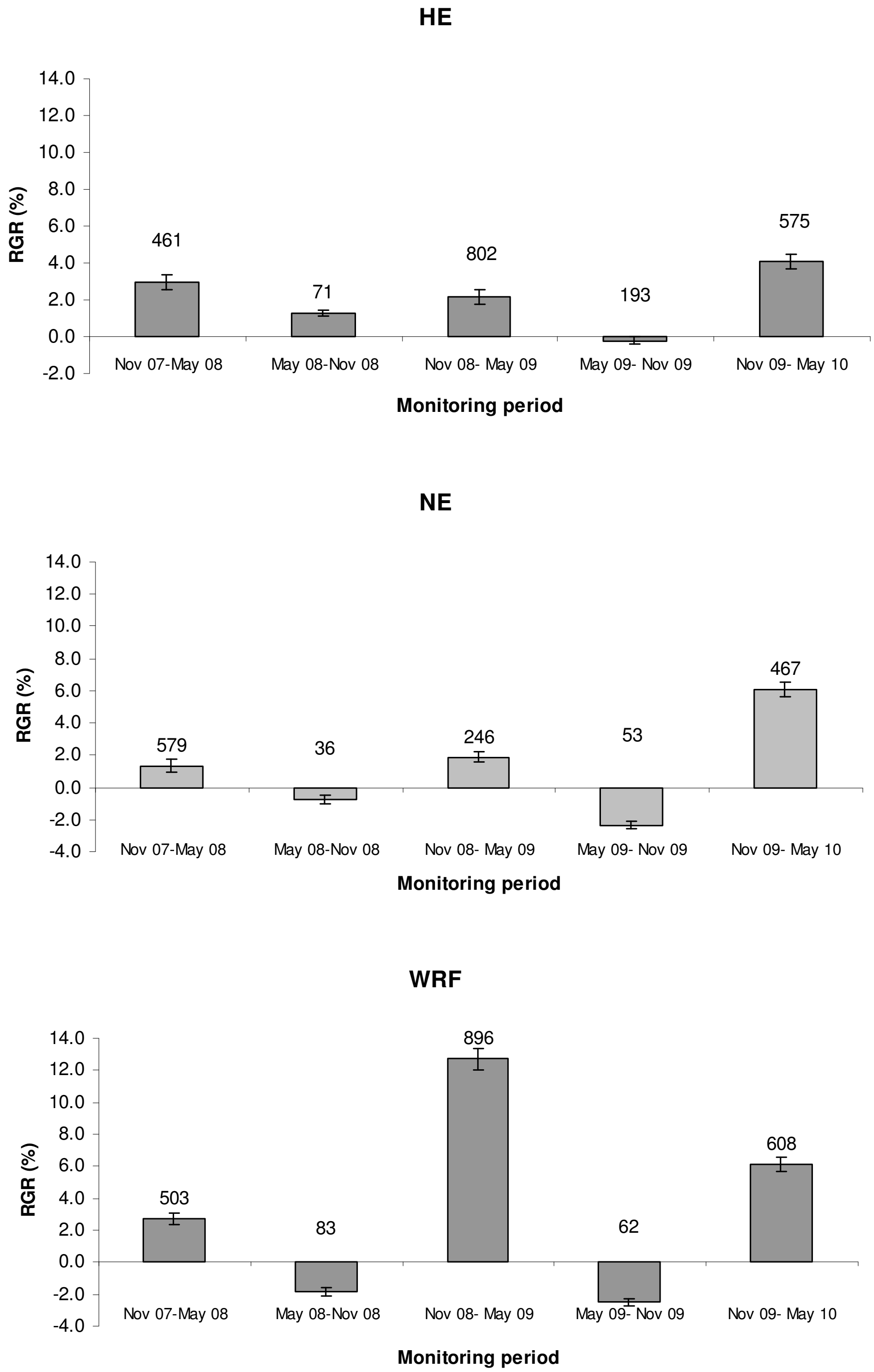

Figure 6: Relative growth rates (RGR) between the monitoring periods from 2007 to 2010 at the Hlangwine enclosure (HE) (N=116), N'waschitshumbe enclosure (NE) (N=125), and Wits Rural Facility (WRF) (N=155). Numbers above the bars indicate the rainfall (mm) that had fallen during the period of growth assessed.

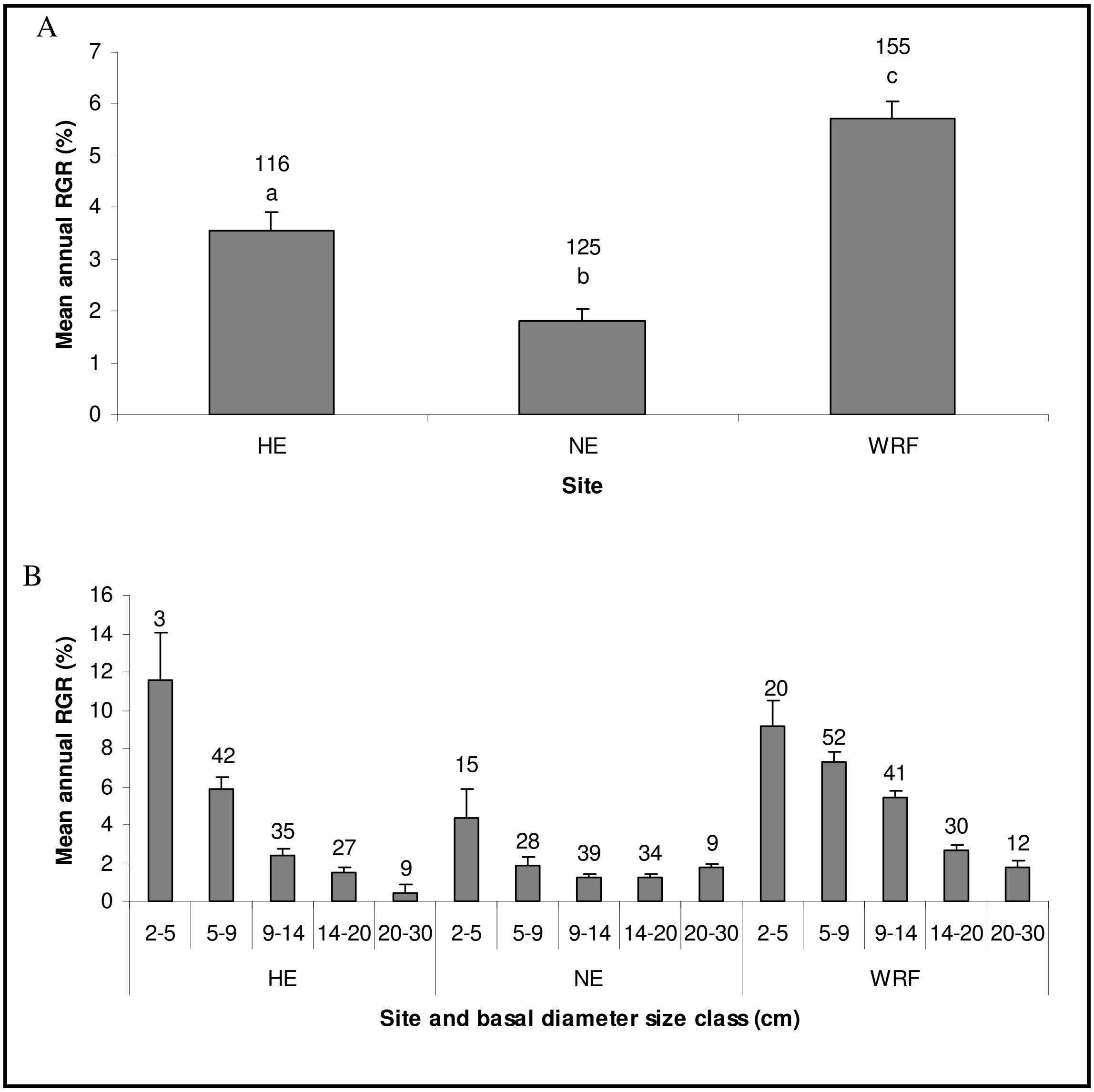

Figure 7: Mean (+SE) annual relative growth rates (RGR) A) overall and B) for five basal diameter size classes in the Hlangwine enclosure (HE), N'washitsumbe enclosure (NE) and Wits Rural Facility (WRF) between 2007 and 2010. Numbers above the bars indicate sample sizes and different letters indicate significant differences between sites.

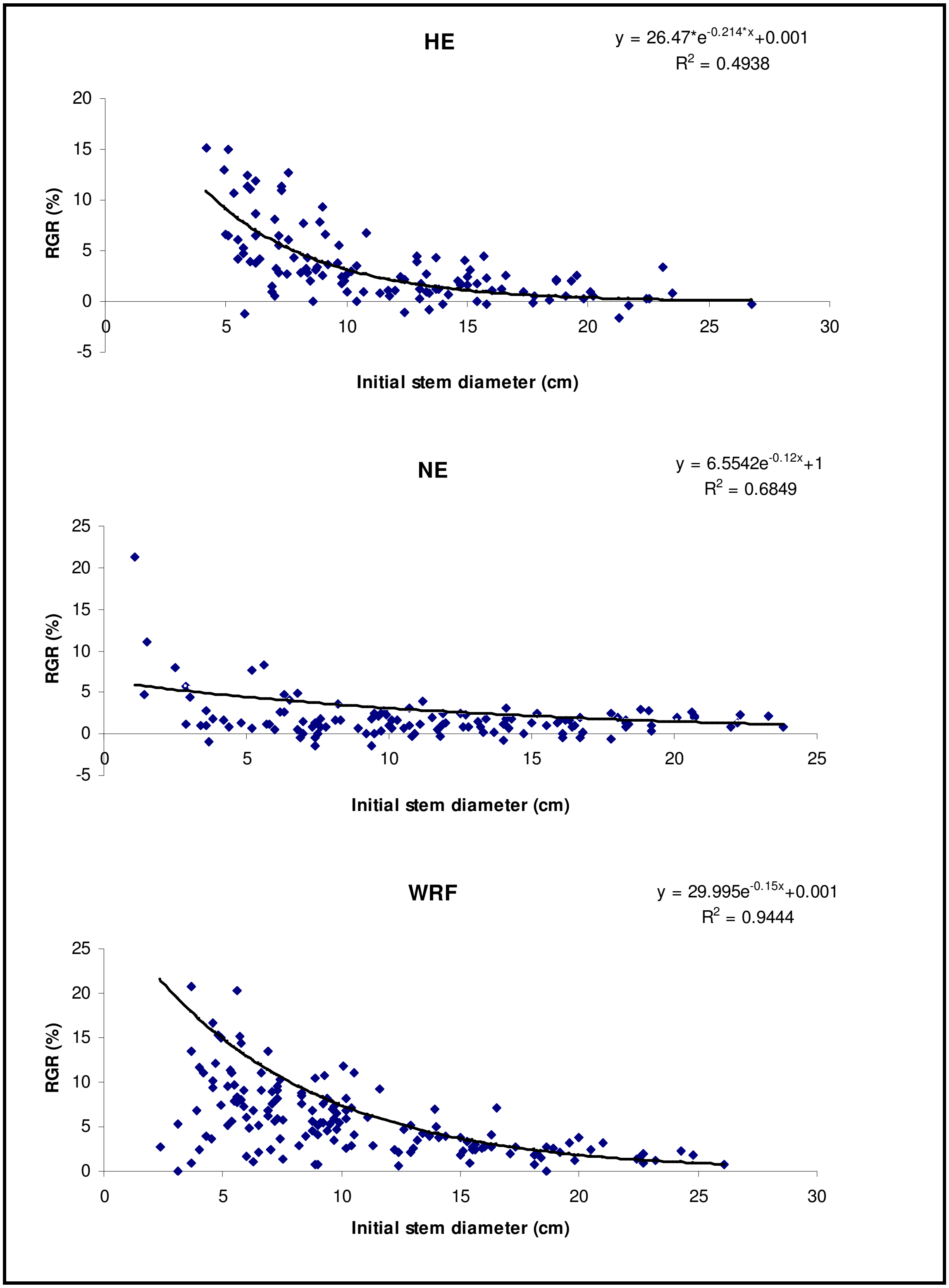

Figure 8: Relationship between mean annual relative growth rate (RGR) of individual trees and initial stem diameter for three sites in the low altitude savannas of South Africa.

Table 6: Relationships between RGR and canopy area and percentage canopy dieback at three protected sites in the low altitude savannas of South Africa.

Site	**Canopy area (m^2)**				**Canopy dieback (%)**			
	slope	**R^2**	**F**	**p**	**slope**	**R^2**	**F**	**p**
WRF	negative	0.23	45.72	<0.001	none	0.013	1.9647	0.163
HE	negative	0.16	21.03	<0.001	none	0.002	0.29	0.59
NE	none	0.01	2.21	0.14	negative	0.04	5.22	0.024
Overall	negative	0.11	50.26	<0.001	negative	0.026	10.51	0.001

4.4.3. Adult growth rates from other data sources

The mean annual relative stem diameter growth rate for individuals >30 cm at WRF from C. Shackleton's data was estimated at 0.42±0.07% or AGR of 0.21±0.03 cm. Growth rates did not differ between the two monitoring periods despite the lower cumulative rainfall during the second period (Figure 9). A negative relationship between stem diameter and mean annual diameter increment was found (R^2=0.20, $F_{1,18}$=4.48, p=0.049) (Figure 10).

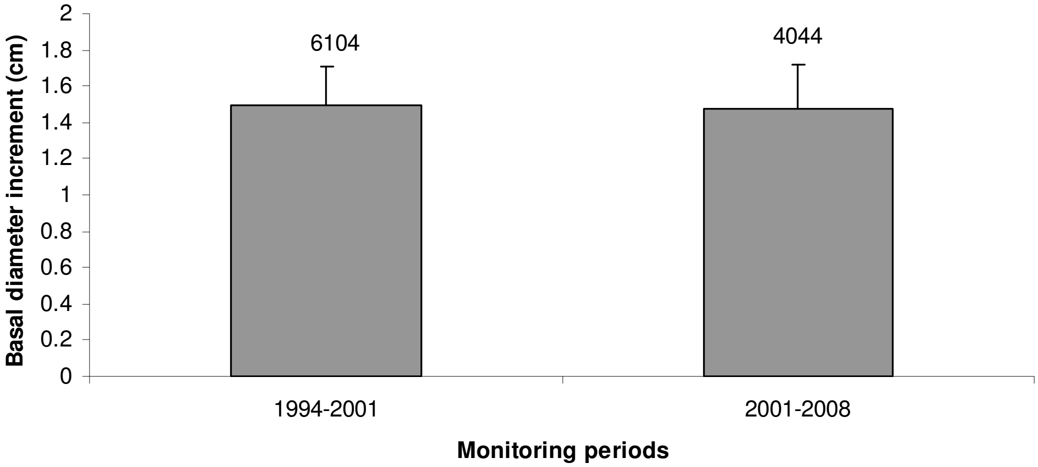

Figure 9: Mean (+SE) basal diameter increments (cm) (AGR) for the two monitoring periods. Numbers above the bars indicate the cumulative rainfall (mm) during the period.

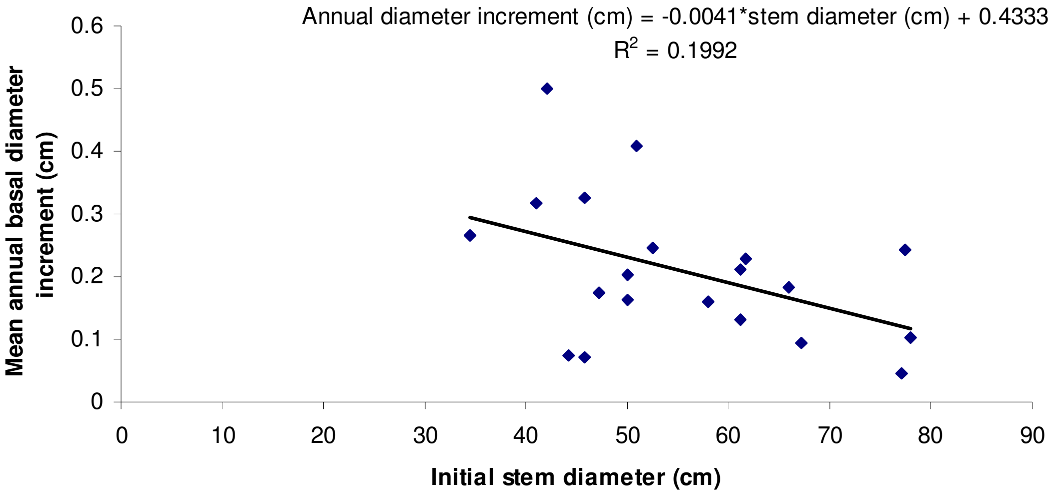

Figure 10: Relationship between stem diameter and mean annual diameter increment for trees >30 cm in diameter between 1994 and 2008 at Wits Rural Facility in the low altitude savannas of South Africa.

At the buffalo enclosure near Satara in the KNP, the mean annual diameter increment was 0.30±0.05 cm and the RGR was 0.63±0.10%, while at the Nkhuhlu exclosure near Lower Sabie in the KNP, the mean annual diameter increment was 0.10±0.04 cm and the RGR was 0.23±0.10%.

4.4.4. Mortality rates

Very few marked individuals died during the study (Table 4). Only 2% of the marked trees at HE died as a result of elephant utilisation after a bull elephant broke the fence of the enclosure and gained access to the protected trees in January 2009 (Figure 11a). At NE 2% of the trees died due to termite activity. During certain times of the year termites can cover the entire above-ground parts of a plant and consume the bark and, in some cases, the live wood of certain susceptible trees, resulting in mortality (Figure 11b). The 1% mortality at WRF can also be attributed to termite activity causing pipes in the heartwood. Such piping activity has been shown to decrease growth rates and cause mortality (Werner and Prior 2007) in savanna trees in Australian savannas.

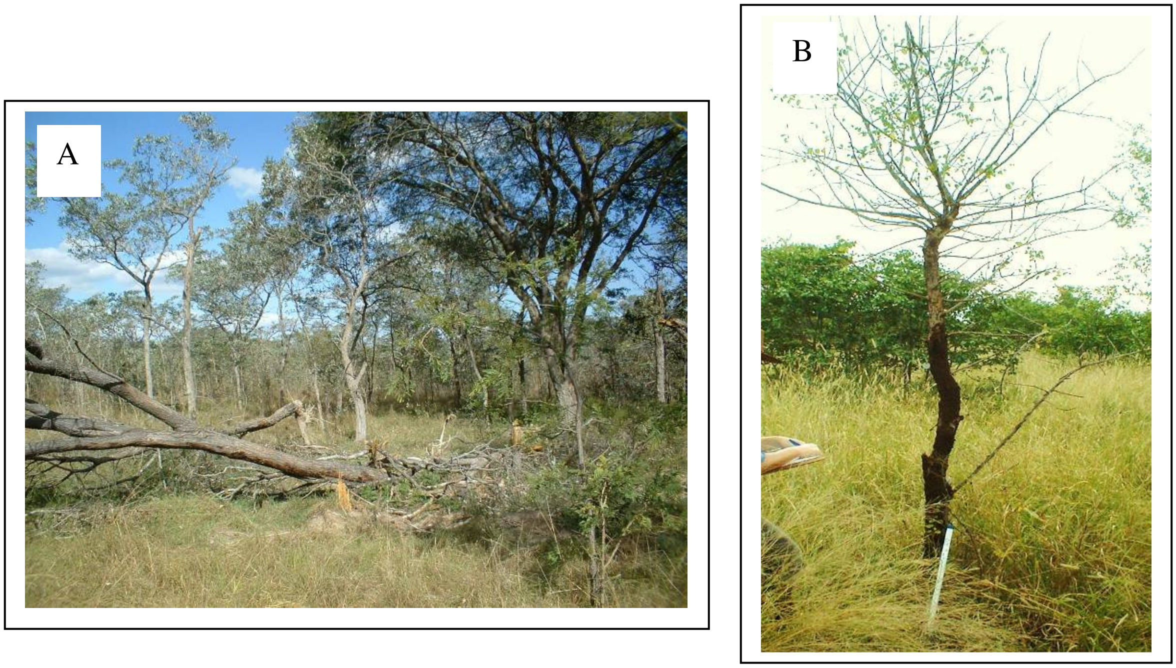

Figure 11: a) Destruction caused by a single bull elephant gaining access to the Hlangwine enclosure (HE, KNP) in January 2009, resulting in 2% mortality of the marked marulas therein. b) Extensive termite activity on the above-ground parts of a marula tree in the N'washitsumbe enclosure (NE, KNP) which resulted in the mortality of up to 2% of marked marulas therein.

4.4.5. Size class transition rates

Marula forward transition rates (Table 7) indicate that an average 2 cm recruit would take 42 years to become reproductively mature in the absence of fire or other disturbance. In addition an average 80 cm tree (average maximum size seen in the KNP) would be in the vicinity of 340 years if it had never experienced any form of disturbance.

Table 7: Years spent in each size class for all sites combined calculated from the mean annual relative basal diameter increments across sites. Comparisons between all the data and the data excluding the trees affected by termites have been made.

	Annual AGR (cm)		**Annual RGR (%)**		**Years spent in class**	
Size class	**All data**	**No termites**	**All data**	**No termites**	**All data**	**No termites**
2-5cm	0.23±0.04	0.37±0.06	8.49±1.7	9.73±1.28	12	11
5-9cm	0.34±0.02	0.41±0.03	5.00±0.5	6.54±0.45	13	11
9-14cm	0.32±0.03	0.30±0.03	2.79±0.3	2.86±0.28	17	17
14-20cm	0.28±0.02	0.25±0.02	1.75±0.2	1.54±0.14	21	25
20-30cm	0.30±0.04	0.28±0.05	1.34±0.3	1.29±0.29	32	33
30-80cm	0.21±0.03	0.21±0.03	0.42±0.07	0.42±0.07	235	235

4.4.6. Height growth rates

Height growth rates were unexpectedly low, but significantly different between sites ($F_{2,122}$=12.68, p<0.001), with WRF being over two fold higher than the two KNP sites (Figure 12). Maximum annual height gain over the study period was 52.0, 71.3 and 68.3 cm for HE, NE and WRF respectively. On average, annual height gain varied from 9-22 cm. This translates into a period of 12 years of vulnerability to fire (<3 m in height). If maximum height gain was achieved, a plant would stay in the flame zone for five years. Seventy five percent of the trees <3 m in height in the KNP sites could be classified as box plants (those forming a dense short stature Figure 13b) - an adaption related to herbivory (Archibald and Bond 2003), while only 25% at WRF could be classified as such, indicating that trees in the KNP have adapted to intensive herbivory, rather than height gain to escape the fire trap (characterised by a pole shape Figure 13a).

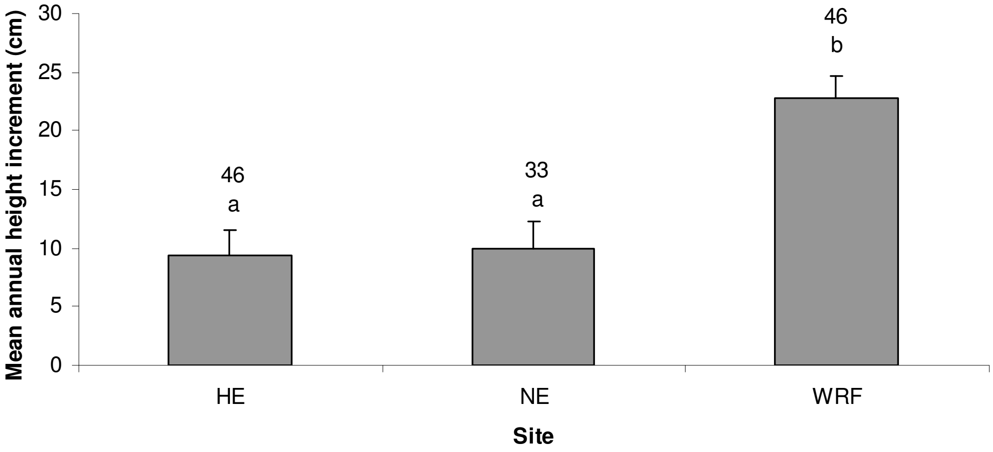

Figure 12: Mean (+SE) annual height increment (cm) for saplings <3 m in height at three sites in the low altitude savannas of South Africa. Numbers above the bars indicate the sample size while different letters indicate significant differences between sites.

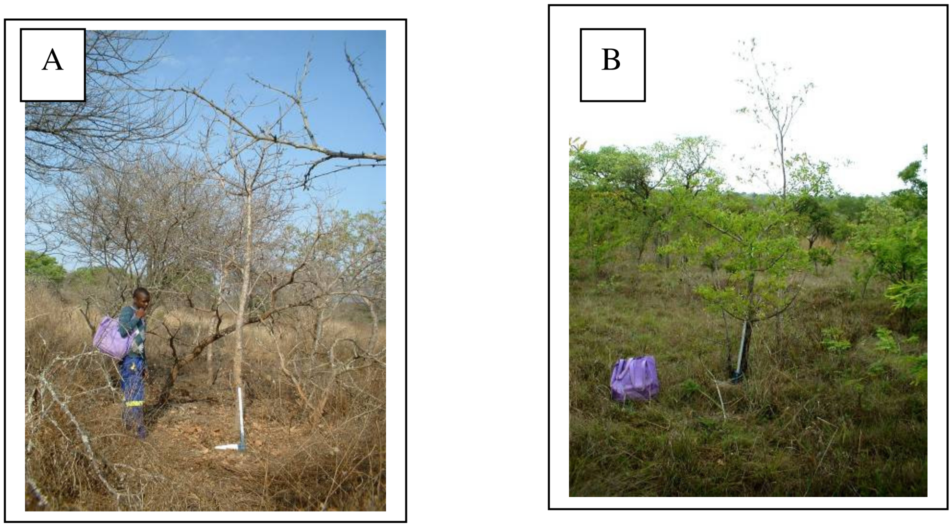

Figure 13: a) Pole and b) box forms of marula juveniles found at the different sites in the low altitude savannas of South Africa.

4.4.7. Nearest neighbour analysis

No relationships between nearest neighbour indices and growth rates for any sites could be discerned. Specifically, at WRF no relationship between the relative growth rate and nearest neighbour distance was found ($F_{1,224}$=0.9866, p=0.32), nor was a relationship between RGR and Wiener's nearest neighbour interference (W) found ($F_{1,223}$=1.90, p=0.17) (Figure 14). Additionally no relationships between RGR of various subsets of the data and any nearest neighbour index could be discerned, including splitting the data up by size class, nearest neighbour functional group (broad leaved or fine leaved nearest neighbours), nearest neighbour size or with removing outliers.

Competition as indicated by above-ground neighbourhood effects of all species appeared to have no effect on the growth rates of marula. At HE, NE and WRF the percentage of nearest neighbours that were also marulas was 10%, 8% and 18% respectively, while other broad leaved species made up 41%, 87% and 28% respectively. No relationships between RGR and the distance to the nearest marula or to Wieners nearest marula interference could be discerned either.

4.4.8. Growth model

Growth data for successive monitoring periods was significantly autocorrelated. Growth data was therefore adjusted before entry into the model using the methods described by Chidumayo (2008). The most parsimonious model (lowest AIC) to represent the relationship between the mean annual diameter increment and plant attributes and environmental variables (basal diameter, canopy area, percentage canopy dieback, rainfall, and competition indices) included only basal diameter and rainfall and their interaction, and explained 23% of the variance (Table 8).

Table 8: The most parsimonious model describing the relationship between mean annual diameter increment and environmental variables (R^2=0.232, p<0.001).

Parameter	**Estimate**	**Wald statistic**	**p**
Intercept	-0.9	-6.275	<0.0001
Basal diameter (cm)	0.05526	4.843	<0.0001
Rainfall (mm)	0.001841	8.755	<0.0001
Basal diameter*rainfall	-0.00008	-5.110	<0.0001

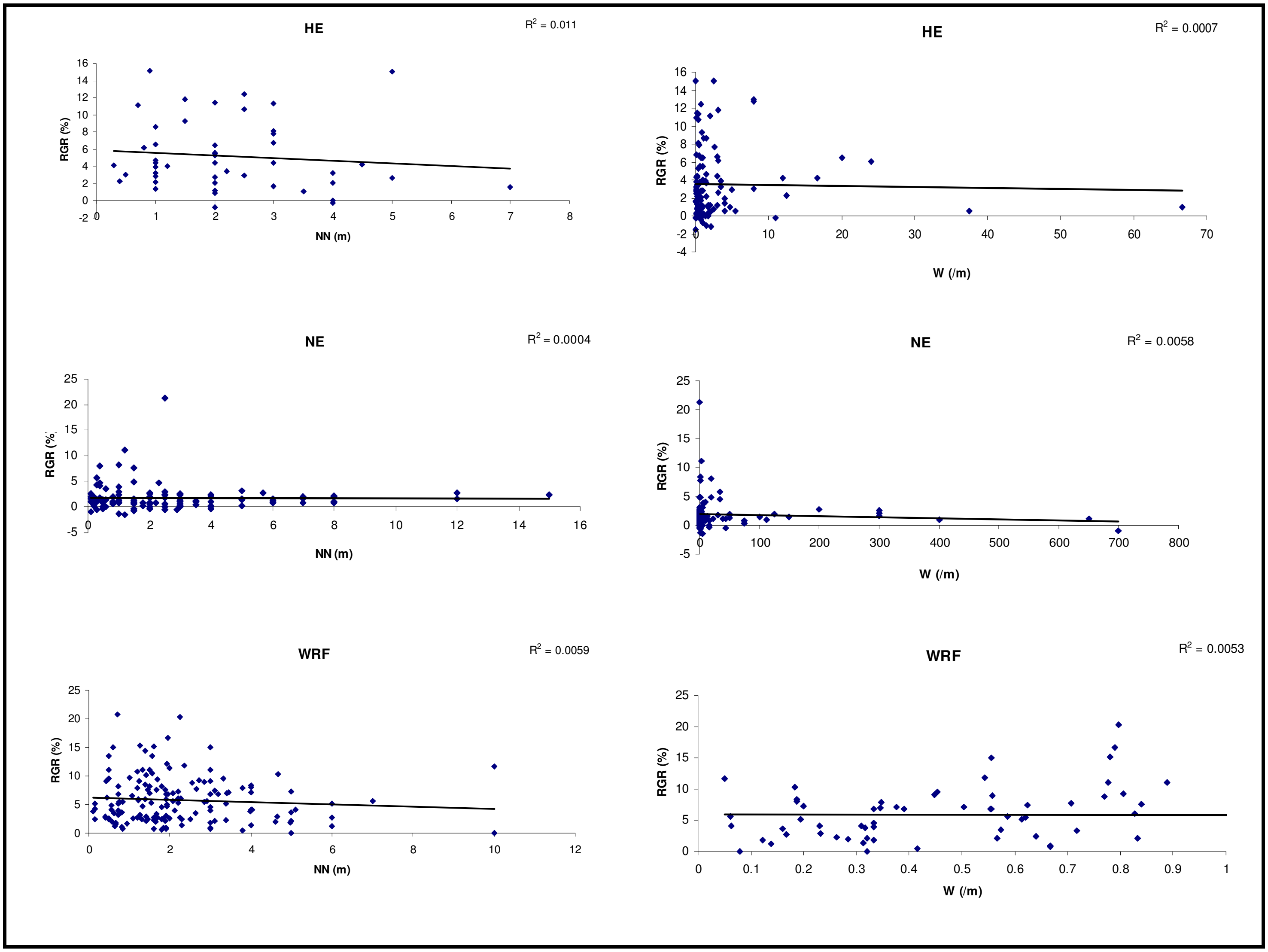

Figure 14: Relationships between mean annual relative growth rates (RGR, %) and both the distance to the nearest neighbour (NN) and Weiner's nearest neighbour interference index (W) at the Hlangwine enclosure (HE), N'washitsumbe enclosure (NE) and Wits Rural Facility (WRF).

4.5. Discussion

4.5.1. Stem/trunk shrinkage

Baker *et al.* (2002) warn against the bias introduced by growth estimates taken during the dry season when soil moisture availability is highly variable. They demonstrated significant stem shrinkage as soil matrix potential declines, with deciduous species showing larger contraction than evergreen species and larger trees showing more shrinkage than smaller ones. Baker *et al.'s* (2002) study offers an explanation for some of the observations in the present study. For example, the measurements taken towards the end of the dry season of November 2009 were after substantially less rainfall had fallen in October than in the previous 2 years, indicating the possibility of more severe shrinkage, specifically at WRF and NE. The diameters measured in November would have been influenced by the soil moisture and hence the rainfall in the previous month. To minimise the effect of inter-annual variation in dry season soil water availability on growth rate estimates, Baker *et al.* (2002) recommended that measurements should be taken during the wet season when variation is at its lowest. The mean annual growth rates provided in this study were derived from measurements taken towards the end of the wet season and these values should therefore not suffer from this shortcoming.

Shackleton (1997) found a relationship between the degree of shrinkage of a stem in a year and whether the stem dies the following year. Of the stems studied by Shackleton (1997) with >18% shrinkage in a year, 30% of them were found dead the following year. In this study the highest level of shrinkage was 36% after which time the stem died. The mean values obtained ranged between 2 to 8%. All stems that died (n=5) had shrunk by >8% the season before. Shackleton (1997) indicated that 15% of stems with 8% shrinkage would die the following year. Since the measurements of Shackleton (1997) were done during the dry season annually, his results could be due to stem shrinkage in relation to soil moisture content rather than due to stress and death (Baker *et al.* 2002).

4.5.2. Comparisons with other studies on marula

Other studies that have measured the growth rates of marula in natural populations are rare. Natural population growth rates of adult marula trees across sites in the savanna biome of South Africa have been studied by Shackleton (2002) and a strong relationship between mean diameter increment and stem diameter was found, summarised as: annual basal diameter increment (mm) = -0.068*basal diameter (cm) + 4.54 ($r^2=0.206$ $p<0.01$ $n=44$). This was based on trees ranging between 5 and 58 cm in basal diameter. The current study indicated different relationships for different sites with opposite patterns for HE and NE, based on trees ranging between 2 and 30 cm, but the relationship for the large trees (>30 cm) found at WRF was very similar to that presented by Shackleton (2002). The annual diameter increments of undamaged trees has been shown to follow a pattern of initial increase as a function of size followed by a decline as trees begin to reach maturity (Phillips *et al.* 2003; Holdo 2006). Other studies indicate that diameter increments remain constant with increasing size (Osho 1996). With increasing diameter increments with increasing size, NE appears to be anomalous. Growth rates were shown to be dependent not only on basal diameter, but also rainfall and termite activity. At NE rainfall is limiting, and hence those individuals with greater access to water (ie. deeper root systems) may be able to grow more than smaller individuals with shallower root systems. Indeed there is a nearby seep line at NE that may be contributing to the greater growth rates of the larger marulas (Levick and Rogers 2008). However, soil texture at NE consists of a high clay content which in turn relates to nutrient status as there is a positive correlation between clay content and cation exchange capacity (Shackleton 1997). Soil depth at clay sites such as NE is relatively shallow (Venter 1990), but the water holding capacity of the soil is greater. Scholes and Walker (1993) have indicated that the apparent relationship between growth rates and rainfall is related to the influence of rainfall on nitrogen mineralisation rates. Shackleton (1997) found that growth rates at sites with clay soils were largely determined by the overall woody vegetation density. Greater growth rates on sands than clays at low densities and the reverse at high densities. Shackleton (1997) also found that maximum productivity occurred at mean annual rainfall of 600-670 mm, supporting the higher WRF growth data in the present study. The finding that termite activity affects growth rates and mortality indicates that smaller stems may be more susceptible to termite attack and hence have lower growth rates than less susceptible larger trees, resulting in the pattern at NE (the driest site). The high level of termite activity at WRF could also be causing the lack of relationship between stem diameter and annual increment there.

The growth rates obtained for marula in this study are similar to those found by other studies in South Africa. From a sample of 44 marula trees ranging in diameter from 5 to 60 cm in stem diameter, an average annual diameter increment ranging between -2 to 9 mm was estimated for the years 1992-1996 by Shackleton (2002a). Gaugris *et al*. (2008) found that marulas ranging in diameter from 0.95 to 15.6 cm growing in northern Maputaland, grew at a rate of 0.5 to 9 mm per annum in diameter between 2000 and 2006. The results of the present study indicated a range of -2 to 10 mm for HE, -3 to 5 mm at NE and -1 to 11 mm at WRF between 2007 and 2010. In contrast, a growth potential of up to 24 mm per annum for a planted individual with an initial diameter of 8.1 cm has been reported (Hall *et al*. 2002).

The use of growth rings has not been found to be a reliable method to estimate the age of marula (Walker *et al.* 1986; Shackleton 1997). However, a regression for the relationship between basal circumference and age was postulated by Haig (1999): Basal circumference= 0.765 * age-1.47 (r^2=0.967, p<0.001). This regression can be used to obtain an approximate age for a marula tree. Therefore a reproductively mature tree of 14 cm in diameter (44 cm in circumference) would have an approximate age of 59 years. This relates well to the estimate of 46 years (assuming 4 years to reach 2 cm in diameter) obtained by this study based on the mean annual growth increments across sites. Hall *et al.* (2002) related ages of 4-5 years in Malawi and Israel and Shone (1979) noted an age of 7 years to reach 10 cm in diameter when fruit production started. These low values can only be from planted trees with irrigation and fertiliser additions and the fruit produced were probably few and small. However, planted trees in Botswana have produced large fruit crops at ages of 14-15 years (Hall *et al.* 2002). Shackleton (2002a) estimated an age of 35 years for a 1.5 cm recruit to reach a stem diameter of 15 cm, and 18 years to reach 9 cm in the South African savanna biome, but stressed that this could be an overestimate. Other anecdotal reports listed in Hall *et al.* (2002), indicate very young ages for large wild trees. For example a report on a 31 cm diameter wild tree indicated that it was 32 years old. These values are inconsistent with the data found by this study as well as Shackleton (2002) and Gaugris *et al.* (2008), which all indicate that wild trees take much longer to grow than what these estimates indicate. These widely varying ages of first reproduction indicate the substantial limitations that natural populations of marula encounter. However, Akinifessi *et al*. (2008) found that fertilisation and irrigation did not improve the growth rates of grafted marula saplings and mean annual diameter increments of 3 mm were found regardless of the fertilisation or irrigation treatment applied. This was thought to be due to its adaptation to infertile soils and a bimodal rainfall regime. Earlier anecdotal reports of marula growth therefore should be viewed with caution.

This study indicates that if marula trees manage to grow without disturbance, those trees of 80 cm in diameter or more (sometimes found in the KNP) are probably >334 years old. Most of the large trees in the KNP have an average stem diameter of 50 cm, which translates into an approximate age of 221 years, indicating a large recruitment event in about 1780. Prins and van der Jeugd (1993) indicated that even aged stands of *Acacia tortilis* (approximately 50 cm in diameter) with a mean annual diameter growth rate of 5.4 mm in Tanzania were probably due to a recruitment event in the 1880s due to the extermination of herbivores by the rinderpest pandemic. Marula trees currently 130 years old establishing at that time would be only 34 cm in stem diameter, assuming they had been undamaged by elephants and other major disturbances.

4.5.3. Comparisons with other savanna tree species

Growth rate data for forest tree species are abundant, however data for savanna species is harder to come by. Growth rate estimates for other similar savanna species in northern Maputaland in South Africa such as *Terminalia sericea, Combretum molle, Spirostachys africana, Strychnos spinosa* and *Strychnos madagascariensis* have mean annual diameter increments of 3 mm, 2 mm, 1 mm, 1 mm, and 2 mm respectively (Gaugris *et al.* 2008). Chidumayo (2007) reported growth rates of 2 to 21 mm for *Bauhinia thonningii* in a savanna site in Zambia. In a Kalahari sand woodland savanna in northern Zimbabwe overall growth rates ranging between -2 to 11 mm per annum were observed between 2001 and 2003 (Holdo 2006). More specifically mean annual growth rates for *Burkea africana* and *Terminalia sericea* ranged between -2 and 8 mm and -2 and 6 mm respectively. Rutherford (1978) reported a mean of 5 mm/yr for six species (range from 2–8 mm/yr) in a *Burkea africana* savanna on nutrient-poor sandy soils. Other savanna species have also been examined with reports of 3–4 mm/yr for *Pterocarpus angolensis* (Stahle *et al.* 1999) and 4 mm/yr for *Baikiaea plurijuga* (Rutherford 1978). Growth rates for *Acacia drepanolobium* in arid and semi-arid regions in Africa have been reported at 7 mm/yr (Okello *et al*. 2001) and 12–14 mm/yr for fifteen African *Acacia* species covering over 14 degrees of latitude (Gourlay 1995). On more fertile soils at two sites in Zambia, Chidumayo (2005) reported growth rates from 8 to 25 mm/yr. Gaugris *et al.* (2008) found marula to have one of the highest growth rates across a number of woodland and forest tree species in northern Maputaland. However, marula does appear to have slower growth rates than *Acacias* which are leguminous species. This could be due to *Acacias* preferring more nutrient-rich soils and having the ability to fix nitrogen. The relatively fast growth rates for marula therefore follow from that predicted by its low wood density.

4.5.4. The role of competition

There is little support for the effect of neighbouring trees regardless of species on the growth of marula. Inter-tree competition is thought to be an important factor affecting growth (Scholes and Walker 1993; Grundy *et al.* 1994), though recent studies have challenged this theory (Shackleton 2002b). Similar results to the current study have been found by Holdo (2006) for savanna trees in the Kalahari and by Shackleton (2002b) for trees at 44 sites scattered across the savanna biome in South Africa. The mixed opinions on the role of competition may be due to the interacting effect of competition and disturbance (Holdo 2006). When disturbance is high, competition may only play a limited role, as populations are prevented from achieving their maximum productive capacity by density-independent factors such as fire (Sankaran *et al.* 2005). Different types of competition have been found to be more important than others. Shackleton (2002b) found that intra-specific competition was more prevalent than inter-specific competition. The results of the current study did not bear this out for marula. No study has quantified the lateral distribution of the marula root system, but Rutherford (1983) indicated that the lateral spread can be up to four times the extent of the canopy for a variety of other savanna tree species. This indicates that the roots could be interacting with trees within a very large zone and perhaps only the larger trees within that zone would affect the growth of the focal tree rather than the nearest neighbour. The lack of an inter-tree competitive effect found by this study and others could also be due to problems with the nearest neighbour methodology (Shackleton 2002b).

4.5.5. Variability in tree growth rates

Shackleton (2002) indicated that rainfall was one of the most important variables explaining savanna woody plant growth. Monthly relative humidity and cumulative rainfall were found to explain 94% of the variation in grafted marula sapling growth at a miombo site in Malawi (Akinifessi *et al.* 2008). Significant relationships with rainfall were also found by the current study, but explaining only 65% of the variation.

However, a large amount of the variability in growth rates between individuals and between years could not be explained (70%). This is common for similar savanna systems (Holdo 2006). One explanation is the short duration of this study, covering only three growing seasons. An alternative explanation is that the important site variables were not assessed or that growth rates are inherently stochastic (Fox *et al.* 2001; Holdo 2006). Higgins *et al.* (2000) postulate that this stochasticity could be due to the patchiness of rainfall in semi-arid savanna systems and due to the fact that they are non-equilibrium event driven systems. The dependency of growth rates on certain site characteristics confounds the comparison across sites since the high variability between individual trees makes the use of mean growth rates spurious due to differing genetic potentials and microsite factors across individual stems (Shackleton 1997). Holdo (2006) postulates that small scale patchiness could be responsible for the low predictive power of growth rate models. However, deep-rooted species may be able to exploit more reliable (less variable) soil moisture layers and thus be less susceptible than shallow-rooted species to random variation in rainfall and other factors that affect soil moisture availability (Scholes and Walker 1993). Marula is known to have a relatively shallow (taproot of up to 2.4 m) but wide spreading root system (exact values not reported) (Hall *et al.* 2002). This would indicate that its growth potential is highly reliant on soil moisture content and hence spatiotemporal rainfall variability.

Trollope *et al.* (1998) found that in the KNP, marula has higher growth rates on the sandy granitic soils than on the clayey basaltic soils. This has been suggested to be because sandy soils do not hold moisture in the upper soil layers but permit the penetration of moisture down to greater depths than clay soils, thus favouring the deeper rooted woody vegetation more than the shallow rooted grass component of savannas, and hence reducing competition between grasses and trees (Jacobs 2001). In addition clay soils inhibit the penetration of deep root systems and hence favour grasses over trees. Soil type appeared to influence marula growth rates in the current study when comparing WRF and HE (sandy soils) growth rates with those at NE (clay soils). This was, however, confounded by the rainfall differences between sites.

4.5.5. Opportunities for escape from fire

This study has provided detailed information on the natural background (with minimal disturbance) basal diameter growth rates for marula trees between 2 and 30 cm in diameter at three different sites. However, in disturbance-prone environments, woody plants need to frequently recover from damage caused by disturbances such as fire and herbivory, and an understanding of tree height growth rates is thought to be key for predicting the frequency of escape opportunities from the inhibiting effect of such disturbances on the size of individual woody plants (Higgins *et al.* 2000; Maze 2001; Archibald and Bond 2003). Annual height growth rates for marula <3 m in height, ranging between 9 and 22 cm, are relatively lower than what has been predicted for height growth rates in a number of recent ecological models for African savanna dynamics in relation to fire (Higgins *et al.* (2000) – (35-80 cm/year); Baxter and Getz (2005) – 30 cm). Published height growth rate data for African savannas show highly variable estimates. Pellew (1983) found mean annual growth of *A. tortilis* in the Serengeti, Tanzania, to be 16.5 cm and 44.2 cm, with and without giraffe browsing respectively. Lewis (1987) measured mean annual growth in coppiced *Colophospermum mopane* trees in Luangwa Valley, Zambia, as 13.4-19.2 cm. Knoop and Walker (1985) measured shoot growth at irregular intervals at Nylsvley, finding growth in the range of 3-33 cm for *A. tortilis* and 3-15 cm for *A. nilotica*, over intervals of approximately 3-10 months, with this growth almost doubling where herbaceous vegetation had been removed. Herlocker (1979) estimated annual height growth of young *A. tortilis* as 30 cm.

Marulas appear to invest more in diameter increase than in height gain at small sizes which lends support to the alternative idea that escape from the fire trap may not be through height gain but rather through thickening of the stem with concomitant bark thickening (Chapter 8). Alternatively, marula may be better adapted for herbivory at some sites (taking on a box structure) and growing little in height. It would be assumed that a switch between fire adaptation (pole) and herbivory adaptation (box) would not have been possible in the time since the KNP enclosures were erected. Hence the box strategy may be extrapolated to individuals in the KNP outside the enclosures. A box strategy implies that the effects of herbivore utilisation are greater than fire (Archibald and Bond 2003). The plasticity of this response requires further investigation. If marulas become resistant to fire at 3 m (Jacobs and Biggs 2001), then a fire free period of 12 years would be required at WRF. An understanding of the resprouting response of marulas following a fire and the rate at which prefire height is achieved is fundamental in obtaining an estimate of the fire interval required for marula saplings to escape the fire trap. This has been explored in Chapter 8.

4.5.6. Growth rates of seedlings

Knowledge on the innate mean relative growth rates (the rate of dry mass increase per unit of plant dry mass) of seedlings is also crucial for a predictive understanding of the performance of species in their natural environment (Grime and Hunt 1975; Cornelissen *et al.* 1996). This has been undertaken for marulas in the seedling stage up to 1 m in height under glasshouse conditions (Chapter 5).

4.6. Conclusions

Marula trees have relatively fast stem diameter growth rates compared to other species but have relatively slow height growth rates in the sapling stage, indicating that diameter growth is more important than height gain in escape from fire. Escape from fire can be achieved in 12-15 years and reproductive maturity achieved in 46 years if not disturbed. Growth rates are related to stem diameter and are faster for smaller individuals. Absolute growth rates were highest for individuals between 5 and 9 cm in stem diameter compared with other size classes, indicating that once escape from the fire trap is achieved the plant grows rapidly. Stem shrinkage due to water loss needs to be taken into account when assessing growth rates and hence the times of measurement of stem diameter are very important and need to coincide with rainfall events. Termite activity significantly affected stem diameter growth rates but did not affect the relationships between growth rates and plant size. Competition appeared to have little effect on marula growth rates, however the indices used in this study may not be appropriate. Continued monitoring is required to determine the range of variability in growth rates over time.

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Chapter 5: Allocation patterns of *Sclerocarya birrea* subsp. *caffra* (marula) seedlings: trade-offs between growth, storage and defence

5.1. Abstract

The allocation patterns of marula seedlings in terms of biomass, growth, storage and defence were investigated to describe the overall resource allocation strategy of marula seedlings and how it varies with ontogeny throughout the seedling phase, as well as with soil type and provenance. Seedlings were grown under glasshouse conditions in natural soils and growth, biomass allocation, root starch and leaf phenolics were detemined from periodic harvests. Seedlings grown in basalt derived soil (high nutrient content) did not differ from those grown in granite derived soil (low nutrient content) in terms of plant size after 11 months of growth, but relative stem biomass allocation was greater in higher nutrient soils. However, considerable differences in the growth curves for plants grown in the two soil types were observed in the first season of growth. Effects of provenance on growth rates and plant size were evident, with higher rainfall provenances having taller seedlings than lower rainfall provenances. Chronosequences, from germination until the end of the third growing season, indicated seasonal patterns in leaf and starch allocation. Starch content peaked (35%) at the end of the growing season once growth had ceased, but dropped considerably after leaf flush. Allocation to roots increased exponentially over time (making up 65% of the overall biomass at 3 months of age), indicating a strategy of high below-ground allocation early on, to ensure recovery from disturbances such as fire and herbivory, consistent with observations for other savanna species. Defence allocation in the leaves remained relatively constant during the growing season, peaking at 18% total phenolics. RGR peaked at 20% per week just after germination and did not exceed 5% per week thereafter. There was a strong linkage between LAR and RGR. Rooting depth affected overall plant mass but not stem height. These results indicate a high storage allocation coupled with a high defence allocation strategy. Trade-offs were observed between storage and growth but not with storage and defence. Overall marula appears well adapted to recovery from fire during the seedling stage due to high root and storage (starch) allocation and is probably extremely drought resistant due to very high root: shoot ratios.

5.2. Introduction

Woody plants demonstrate a high potential for biomass production (Kozlowski 1992). The dry weight of woody plant tissues, however, represents only a small proportion of the total carbohydrates synthesized, the rest is removed by herbivores, parasites and fungi, and shedding of plant tissues (which is pronounced in deciduous trees), or allocated to respiration, leaching, exudation, secretion and translocation (Kozlowski 1992). Resources obtained from the environment and manufactured in the plant over and above these losses, are allocated to various plant parts in the form of structural biomass components and functions such as growth, reproduction, storage, maintenance and defence (Bazzaz 1997). This chapter focuses on the allocation patterns of juvenile marula plants in terms of their allocation to structural biomass components, growth, storage and defence.

5.2.1. Biomass and growth allocation

Optimal partitioning theory predicts that plants respond to environmental variation by partitioning biomass among various plant organs to optimize the capture of essential requirements in a manner that maximizes plant growth rate (McConnaughay and Colman 1999). Disturbances such as fire and herbivory may further modify this partitioning behaviour. Plants with relatively high proportions of structural materials such as long-lived trees are relatively inflexible in allocation and redeployment of certain compounds in response to disturbance, but seedlings of woody species are expected to be more plastic in their allocation patterns than mature trees (Bazzaz 1997). An explanation of many of the responses of the above-ground parts of plants to environmental factors can be explained only if the growth of the roots is also understood (Rutherford 1983). Therefore a study of the biomass allocation to all plant parts is required for a complete understanding of the biomass allocation patterns of a species.

Knowledge on the innate mean relative growth rates of seedlings is crucial for a predictive understanding of the performance of species in their natural environments (Grime and Hunt 1975; Cornelissen *et al.* 1996). In African savannas, this is particularly important to predict escape opportunities from disturbances such as fire and herbivory.

5.2.2. Storage allocation

Storage refers to carbohydrates that accumulate in plants that can later be mobilized to support biosynthesis for metabolism and growth (Kozlowski 1992). Woody plants accumulate nonstructural carbohydrates during periods of excess production and deplete carbohydrates for regrowth after a disturbance or a seasonal spurt of growth. Accumulation of reserve carbohydrates is particularly sensitive to late season stresses, which may result in reduced accumulation and can greatly influence metabolism and growth in the following year (Loescher *et al.* 1990). Starch is considered to be the most important reserve carbohydrate because it indicates where there is a carbohydrate surplus over current needs (Kozlowski 1992). Starch contents are often used as sole indicators of carbohydrate status, however a number of other compounds such as sucrose, polyols, amino acids, polysaccharides and lipids also accumulate in woody tissue (Kozlowski 1992). Carbohydrates accumulate in a variety of tissues and organs including buds, leaves, branches, stems, roots, seeds, fruits, and tracheal sap (Kozlowski 1992). Most carbohydrates are, however, in the stem, coarse roots and foliage. The proportion of the total carbohydrate pool in roots is usually as high and often higher in seedlings than in mature trees because the root: shoot ratio declines progressively with increasing tree age as progressively greater proportions end up in stem structural material (Kozlowski *et al.* 1991).

5.2.3. Defence allocation

Herbivores exert a major impact on plants, both in ecological and evolutionary time scales, by increasing plant mortality and by removing biomass that might otherwise be allocated to growth or reproduction (Harper 1977). In response, a proportion of the resources gained by a plant will be allocated to defence (Bryant *et al.* 1983). Optimal defence theory assumes that defence comes at a cost (Bazzaz 1997). Not all plant parts will be equally valuable to the plant and therefore will not be equally defended. The costs of defensive chemicals can be considerable, not only in terms of production and storage, but also in terms of opportunity costs, as energy allocated to defence cannot be concurrently used to capture more energy or for further growth (Bazzaz 1997).

There are large interspecific differences in both the type and amount of chemical defences in the different parts of a plant. There have been many attempts at identifying the selective forces which determine this interspecific variation (Feeny 1976; Rhoades and Cates 1976; McKey 1979; Bryant *et al.* 1983; Coley *et al.* 1985). Theories on allocation of resources to defence have developed from the evolutionary perspective of life history strategy, which have considered optimality models of allocation. These stress that the optimal allocation is that which maximizes the plant's lifetime reproductive value (optimal defence hypothesis) (Bazzaz 1997). Mechanistic models have come from the field of environmental physiology where biomass is the quantity maximized. The resource availability hypothesis extends the optimal defence hypothesis, predicting that the optimal level of plant defence will vary with the growth rate of the plant. It is based on indications that plants in resource poor environments grow slowly and have low capacities to photosynthesise and absorb nutrients (Coley *et al*. 1985). In contrast plants in nutrient-rich environments have the potential for rapid plant growth. Therefore because the relative impact of herbivory increases as inherent growth rate declines, we would expect higher defences in slower growers (Coley *et al.* 1985).

The development of a unifying theory on plant defence, has been hindered by the complexity of the subject, because any treatment of plant defence theory must cover both a number of disciplines and levels of biological organization (Herms and Mattson 1992). In addition, plant secondary metabolites are not only used for defence (Close and McArthur 2002). Other uses for these compounds include: attraction of pollinators; protection from ultra violet light; structural support; temporary nutrient storage; phytohormone regulation; drought resistance; facilitation of nutrient uptake; and protection of roots from acidic and reducing environments, amongst others (Herms and Mattson 1992). These additional functions may reduce the costs of secondary metabolites as defensive compounds and complicate their evolutionary importance, as well as the trade-off required with growth and storage. Recent biochemical work suggests that a trade-off of defence with growth as predicted by the various hypotheses described above, is unlikely to occur as the main role of many plant secondary metabolites may actually be to protect leaves from photodamage and not herbivores and will vary with the risk of photodamage (Close and McArthur 2002).

This hypothesis still requires further validation. For the purposes of this study it is accepted that these compounds play an ecological and evolutionary role in plant defence theory as suggested by Coley *et al.* (1985), Bryant *et al.*(1983) and others.

Phenolics (consist of both tannins and nontannins), especially tannins, have received the most attention from among all possible defence chemical compounds, as they reduce the activity of digestive enzymes of herbivores (Bernays *et al.* 1989). Tannins can be divided into condensed tannins and hydrolysable tannins (Makkar 2003). Negative correlations between feeding preferences of mammalian herbivores and concentrations of condensed tannins have been found for many woody species (Cooper and Owen-Smith 1985). Since terrestrial mammalian herbivores are restricted by feeding height, and assuming that high selection pressure by herbivores has driven the evolution of plant defences and that defences are costly, they should be more abundant and effective in plants and plant parts exposed to the highest browsing pressures (Feeny 1976). Therefore juvenile trees are expected to be more vulnerable to browsing and hence more defended than mature trees and tall trees are expected to differentially allocate defences to different heights with different exposure to browsers (Rooke *et al.* 2004). Alternative explanations for these patterns, other than selection pressure from herbivores, could be ontogenetical and physiological aging of plants related to maturation and reproduction (Fontanier and Jonkers 1976). Physiological requirements of plants such as light and water, rather than herbivore selection pressure may also determine allocation to defence (Rooke *et al.* 2004).

5.2.4. Trade-offs between growth, storage and defence

Under resource limiting conditions, allocation to one process, e.g. growth, can occur at the expense of other processes, e.g. storage or defence (Chapin *et al.* 1990; Herms and Mattson 1992; McConnaughay and Colman 1999). Since plants have limited resources, investment in one set of particular traits can carry trade-offs, which can make plants vulnerable to other disturbance agents (Chapin *et al.* 1990; Herms and Mattson 1992; Bond and Midgley 2003). Adaptations and trade-offs will be a function of the most important selective agent during the evolutionary history of the species (Iwasa and Kubo 1997). In African savannas, fire, herbivory and rainfall variability, are the most important top down controls for juvenile woody plants (Sankaran *et al.* 2005). Hence, investment in below-ground biomass and storage, fast regrowth (resprouting) capacity after disturbance and defence (structural or chemical) will be selected for, but the relative importance of each will depend on the life history strategy of the plant.

5.2.5. Aims and objectives

Little is known about the allocation patterns of juvenile savanna trees, including marula. Anecdotal reports indicate high growth rates, as well as high levels of defence and storage in marula (Shone 1979). This appears to contradict the optimal partitioning theory which predicts a trade-off between these processes. Different resource allocation strategies may also be applicable at different ages throughout the juvenile phase. In addition, herbivory by impalas and other browsers has been implicated in the lack of regeneration of marula populations in South Africa and elsewhere in Africa (Lewis 1987; Haig 1999). Therefore an understanding of the strategy of resource allocation in juvenile marula plants with respect to soil type, provenance and plant age (from germinants to 2-3 year old juveniles) is an essential starting point from which a further understanding of the response of marula individuals to disturbances such as browsing, fire and moisture stress may be obtained.

The aim of this study was therefore to investigate the allocation patterns of juvenile marula by focusing on the following specific objectives:

1) Investigate how soil type affects growth and biomass allocation
2) Investigate how provenance affects growth
3) Determine the growth rates from germination to 28 months of age
4) Determine the levels of starch in the root tissue of plants ranging in age from 3 to 28 months
5) Determine the levels of phenolics and tannins in the leaf tissue of plants ranging in age from 3 to 28 months
6) Determine whether a trade-off between growth, storage and defence is evident
7) Investigate the effect of pot size (rooting depth restrictions) on growth and biomass allocation

5.3. Methodology

Growth trials were conducted in the glasshouse at the University of the Witwatersrand between December 2007 and March 2010. Two separate experiments, one investigating the differences in growth rates and biomass allocation of plants grown in two soil types and the other investigating the same but in plants from four different provenances, were conducted in the 2007/2008 growing season. In the 2007/2008, 2008/2009 and 2009/2010 growing seasons, plants germinated from seeds of a single provenance were planted in a single soil type and used to investigate the effects of seedling/juvenile age on allocation patterns.

5.3.1. Glasshouse environmental conditions

The glasshouse was exposed to natural light and the incident photosynthetically active radiation (PAR) ranged between 440 and 1 750 μmol/m^2/s on cloud free days at midday at different positions. Temperatures were moderated by a heating and cooling system. Temperature and humidity were measured at 30 min intervals using DS1921 Thermochron iButtons (Dallas Semiconductor, Texas, USA) (www.ibutton.com/ibuttons/thermochron.htmli-buttons) (Figure 1). Temperatures were measured throughout the study period (December 2007 to March 2010), while humidity was only measured from December 2008 to March 2010. The highest relative humidity experienced in the glasshouse over the course of the study was 110% and the lowest was 20%, while the highest temperature was 54.6°C located in areas directly in the sun and close to the glass and hence extreme, and the lowest was 7.0°C.

The temperature regime in the glasshouse came close to those experienced in the southern Kruger National Park (KNP) (Figure 1) with differences in the minimum temperatures up to 4.2°C and differences in the maximum temperatures up to 9.8°C, while differences in the mean temperatures ranged between -0.3-3.6°C. However, these differences were well within the range of temperature variation found in the KNP and indeed the entire lowveld of South Africa when assessing changes over decades.

However, the mean monthly humidity at midday in summer in the KNP ranges from 50 to 53% and in winter from 37 to 42% (Venter *et al.* 2003). Hence the mean daily humidity in the glasshouse was up to 100% and 40% higher than in the KNP, in summer and winter respectively. The possible effects of the higher humidity in the glasshouse relative to in the field on plant growth include: higher growth rates, higher photosynthetic rates, higher transpiration rates (Woodward 1977) and decreased net assimilation rate (Ford and Thorne 1974). These need to be considered when extrapolating the results obtained here to field situations.

Plants were grown on bench surfaces, approximately 1 m above the ground, constructed of a mesh of steel bars which allowed water to drain freely from the bottom of the planting containers.

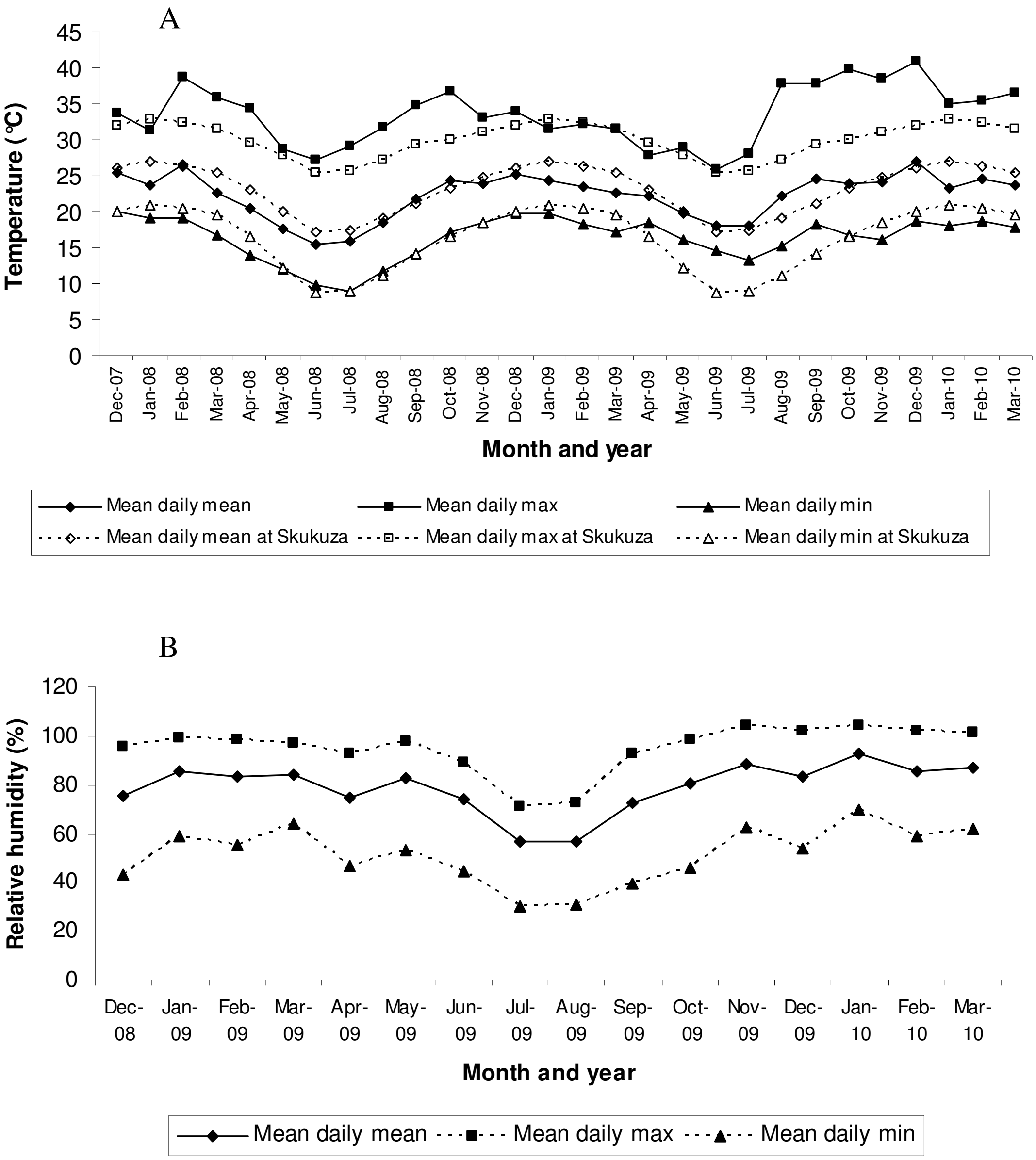

Figure 1: Mean daily A) temperatures (°C) and B) relative humidity (%) experienced between December 2007 and March 2010 in the glasshouse at the University of the Witwatersrand. Temperatures are compared to the equivalent long-term mean daily temperatures experienced at Skukuza in the southern KNP.

5.3.2. Initial seed preparation and germination

Marula fruit was collected from various localities in the north-eastern low altitude savannas of South Africa. The outer fleshy mesocarp was removed and the seeds were dried in the sun and stored in a cool, dry place in the laboratory. Before planting, the seeds were soaked for 48 hours in water which was initially 60 °C. The seeds were planted in seedling trays in a heavy clay soil. Seeds were buried at least the diameter of the endocarp to ensure that the possibility of light inhibiting germination was minimised. The seed trays were watered to saturation every second day. Since marula germination is unpredictable in the sense of when they germinate, a large number of kernels were planted to ensure that enough plants of equal age for each experiment (germination date varying by no more than seven days) could be grown at the same time.

5.3.3. Seedling transplant and conditions for growth

Germinated seedlings were transplanted into either root observation chambers or 11.25 litre volume (30 cm depth) black nursery bags after 5 to 7 days. The seedlings were planted so that the root crown was 1 cm below the soil surface where possible. The wooden root observation chambers each contained five separate chambers which were approximately 41 cm in depth with a glass surface (inclined at approximately 13°) on the front side through which plant roots may be observed (Wilson and Witkowski 1998). Rooting chambers had a width of 12 cm, and a length at the base of 13 cm and at the top of 23 cm, with a depth of 40 cm, giving a total volume of approximately 9.3 litres. The glass side of all rooting chambers were subsequently covered using pieces of cardboard cut to size and attached using prestick. Black plastic was then used to cover the entire front of the rooting box to ensure that no light reached the soil beneath the glass. It was found that the transplanted seedling roots did not follow the glass plate as expected and hence root length could not be measured at each interval. Nevertheless the root observation chambers were used for all experiments described in this chapter (except for the plants grown in nursery bags and PVC pipes), to ensure consistency and comparability.

A drip irrigation system was installed to water the seedlings in the rooting chambers three times a week for 6 min. Each dripper released water at a rate of 2.5 litres per hour and therefore each plant received 250 ml per watering period. This ensured that the chambers were always well-watered and that the same amount of water was given to each plant. Older plants grown in nursery bags were manually watered three times a week with 250 ml water. Plants started losing their leaves when mean daily temperatures dropped below approximately 18-19°C by May 2008. To simulate a dry period (typical seasonal dry conditions) during the winter months of 2008, watering was reduced by 15% per week until no water was given for a period of 6 weeks (June to August). Most plants lost all their leaves during this period. On 11 August 2008 plants were watered again according to the original schedule (6 minutes three times a week). However, regrowth only occurred once mean daily temperatures were above 18-19°C at the beginning of September 2008. In 2009 watering was decreased by 15% per week until no water was given for 12 weeks (June to September). Watering was started again on 7 September 2009. Overall, plants were dormant (without leaves) for approximately 3.5 months for each winter period. Adult marula trees tend to lose their leaves in May and regain them in October in the lowveld of South Africa (Grant and Thomas 2006; pers. obs.), and are leafless for approximately 4 months of the year in their natural habitat (Grant and Thomas 2006).

After 3 months of growth, a sustained release organic plant food (Nitrosol®, Fertilizer Grade 1, Registration Number: K2673, Envirogreen (Pty) Ltd.) was given to each plant in their allocated water at monthly intervals to ensure that nutrient levels were not depleted. Aphids and anthracnose fungus were controlled using an organic insecticide (Kirchhoffs Margaret Roberts organic insecticide, Registration number: L7149, Ballstraathoff (Pty) Ltd.) sprayed on the leaves of seedlings when necessary, and ensuring that it did not make contact with the growing medium. A broad based fungicide and bactericide (Virikop, registration number: L0527, Efekto (Pty) Ltd.) was also used to treat the anthracnose problem on the older plants when necessary.

Each week after transplant the seedlings were measured for stem height, stem diameter at the cotyledon node and number of leaves, until harvest. During the dormant period all plants were measured for stem height and stem diameter only once. Weekly measurements recommenced once the watering was returned to normal. At harvest the plants were extracted from the soil by removing the front glass panel of the root observation chamber and washing away the soil using a low pressure hose. All root material, including fine roots, was removed from the soil using this method. At harvest the number of leaves, stem height, stem diameter at the cotyledon node, leaf area and fresh weight of leaves, stems, and roots were measured. Leaf area was determined by taking scaled digital photographs of the flattened leaves. The leaf area was then measured off the photographs using the Simple PCI software package (C-Imaging, Compix Inc., Cranberry Township, PA). This was done for 50 plants covering the entire range of plant sizes. A regression relationship between leaf weight and leaf area was then determined and this was used to calculate the leaf area of all the other plants (Appendix 1). Harvested plants were dried according to the following protocol: Root samples were dried in a convection oven at 60 °C for 10 days or until constant mass was achieved and leaf samples were dried in a convection oven at 50 °C for five days. Dried material was reweighed and stored in a cool dry place until further analysis.

5.3.4. Soil type effects on allocation physiology

Twenty seedlings of a single provenance (Potgietersrus) were transplanted into root observation chambers in December 2007 in each of two soil types. Ten plants were planted in clay soil derived from basalt collected from the N'washitsumbe (NE) enclosure near Shingwedzi in the Kruger National Park (KNP) in 2007, and ten plants were planted in sandy soil derived from granite collected from Wits Rural Facility (WRF) in 2007. Analysis of these soil samples was decribed in Chapter 4. Plants were harvested in October 2008 (11 months old).

5.3.5. Provenance effects on growth allocation

Ten seedlings from each of four provenances were grown in root observation chambers using seeds collected from a) WRF in February 2007, b) Potgietersrus in February 2007, c) Klaserie Private Nature Reserve in January 2000, and d) Jejane Private Nature Reserve in February 2007. The Potgietersrus seeds were purchased from Lifestyle Seeds (P.O. Box 37486, Langenhovenpark, 9330) and had been collected from the Potgietersrus vicinity from below wild trees, while the remaining seeds had been collected by various researchers at the University of the Witwatersrand while on field trips. Seeds were stored in paper bags or cardboard boxes in a cool dry placed until used. Seedlings were planted into granite sandy soil collected from WRF, in December 2007. Some plants overlapped with the soil type comparison and hence could not all be harvested at the same time. Hence growth, stem height and diameter and leaf number comparisons between provenances were made at 6 months of age (24 weeks; May 2008). No comparisons could therefore be made in terms of biomass allocation patterns.

5.3.6. Age-based allocation patterns

5.3.6.1. Experimental design

To assess the affect of age on allocation patterns and to cover the entire age range from germination to 1 m in height (~2-3 years old) with sufficient replication, a large number of seedlings had to be grown (~150). Initial space limitations resulted in the decision to plant seedlings over two separate growing periods 2007/2008 and 2008/2009 (Figure 2). In addition germination of marula seeds was sporadic and hence germination date could not be controlled. Hence plants grown in 2008/2009 could not all be transplanted at the same time. Transplants were therefore done in two sub-stages between September and November 2009, whereby seedlings with the same germination date were transplanted in groups of ten (Figure 2). Harvesting date took cognisance of the age of the plants in each sub-stage. It was assumed that the conditions separating the sub-stages had negligible effects on the growth of the plants. This did however, have implications for how the data was analysed across size classes and these are discussed in the data analysis section below.

At the beginning of the 2007/2008 growing period, marula endocarps were planted in large black nursery bags (11.25 litres) in granite sandy soil collected from WRF in 2007. All seeds that germinated in December 2007 (n=33) were allowed to continue growth through 2008 and 2009. The position of the bags in the glasshouse was randomly rotated at monthly intervals to reduce positional effects. Typical seasonal (winter) plant dormancy was simulated by decreasing watering between June and August 2008 and between June and September 2009 following the protocols outlined above. Ten plants were randomly harvested in July 2009 during the dormant period (19 months old), a further eight plants in

December 2009 (24 months old) and the remaining fifteen plants in March 2010 (28 months old). The oldest plants experienced three growing seasons: 2007/2008, 2008/2009, 2009/2010.

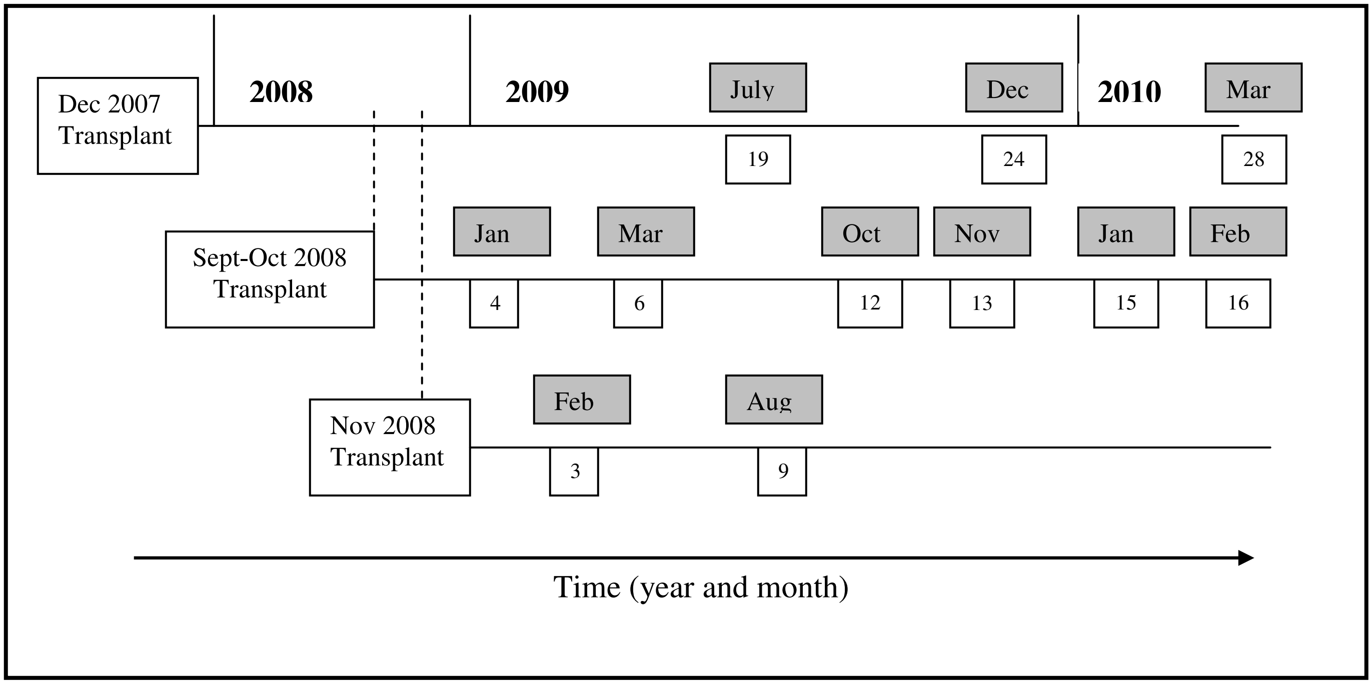

Figure 2: Conceptual diagram of the temporal sequence of seedling transplants and harvests between 2007 and 2010 under glasshouse conditions. Month of harvest is indicated by the grey boxes above the line and seedling age (months) is indicated by the small white boxes below the line. The horizontal axis is not exactly to scale.

Towards the end of August 2008, marula endocarps were germinated in seed trays as described above. In total, 80 seedlings were transplanted into rooting boxes with five root observation chambers each, with granite sandy soil collected from WRF, throughout September to November 2008. Two seedlings were planted into each root observation chamber so that each rooting box had ten seedlings of the same age. Plants were watered and measured as described above. Harvesting of these plants was done in groups of ten at the following ages: 3, 4, 6, 9, 12, 13, 15, and 16 months. The rooting boxes were unfortunately too heavy to move around the room to reduce positional effects on the growth of the plants.

In total 11 harvests were conducted, representing the following ages: 3, 4, 6, 9, 12, 13 15, 16, 19, 24 and 28 months (including all plants). These ages represent biologically meaningful plant stages to assess allocation patterns, namely: 1) 3 and 4 months: just after germination and during the first season of growth; 2) 6 months: at the end of the first growing season; 3) 9 months: during the first dormant season; 4) 12 and 13 months: at the beginning of the second growing season; 5) 15 and 16 months: at the end of the second growing season; 6) 19 months: during the second dormant season; 7) 24 months: at the beginning of the third growing season; 8) allocation patterns at the end of the third growing season.

Five to ten of the root samples from each age group were randomly selected for total starch and total phenolics analyses. These analyses were conducted using analytical grade reagents following the procedures outlined below.

5.3.6.2. Analysis of total starch in the roots

Dried root samples were then ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, USA) using a 1 mm mesh, and then sieved through a 0.5 mm sieve. Subsamples of 0.1 g of dried, ground root material were then used for total starch determination.

Total percentage root starch was measured using the Megazyme starch kit (K-TSTA) (Megazyme International Ireland Ltd., Ireland) using the enzymatic amyloglucosidase/ α-amylase methodology (McCleary *et al.* 1994a) (AOAC Method 996.11 and AACC Method 76.13).

D-glucose and maltodextrins were removed from the sample before starch analysis. The possible presence of resistant starch was investigated by comparing assays where the sample was pretreated with DMSO with those not pretreated. No difference in the starch content was observed and hence all subsequent samples were assumed not to contain resistant starch. The methodology described in the documentation supplied with the kit was followed and absorbance was read at 510 nm using a Helios Gamma UV-Vis spectrophotometer (Thermo Scientific, Inc, Waltham, MA, USA). Detailed descriptions of the assay and exact procedures followed are laid out in Appendix 2.

Total starch measured as the glucose derived from hydrolyzed starch was expressed as a percentage of total sample weight using the following formula:

$$\text{Total starch} = \Delta E \times F \times 1000 \times 1/1000 \times 100/W \times 162/180 = (\Delta E \times F)/\mathrm{W} \times 90$$

where ΔE is the absorbance read against the sample blank, F is a factor for the conversion of absorbance values to micrograms of glucose, 1000 is a volume correction factor, 1/1000 is a conversion from micrograms to milligrams, W is the sample weight, 100/W is a factor to express total starch as a percentage of sample weight and 162/180 is a factor to convert free glucose to anhydroglucose as occurs in starch (McCleary *et al.* 1994b).

5.3.6.3. Analysis of total phenolics and tannins in the leaves

The total phenolic content of the leaf samples was determined using the Folin Ciocalteau method. The methodology used was adapted from Makkar (2003) and Waterman and Mole (1994). Since no standard universal technique is applicable for all species, initial experimentation with extraction methods, initial sample weight, types of extractants (and dilution), levels of dilution and the assay procedure was undertaken and a standard methodology adopted based on the precision of the results obtained. A number of factors unique to marula leaves were also considered, including: 1) the high levels of ascorbic acid possibly present in the leaves due to the extremely high levels present in the fruit (Ojewole *et al.* 2010). Ascorbic acid is known to interfere with the Folin Ciocalteau method (Waterman and Mole 1994); and 2) the relatively high levels of phenolics reported in the leaves (Moyo *et al.* 2010) resulting in the need for greater dilution of the sample to confine the absorbance values obtained to within an acceptable range. A detailed description of the methodology adopted is described in Appendix 2.

Dried leaf samples were ground in a mill to pass through a 0.5 mm sieve. Three replicate 0.05 g subsamples per sample were weighed and used for the assay. Acetone (70%) was used to extract the phenolics from the leaf samples. A double extraction procedure was used and each extraction was conducted at 4 °C (Appendix 2).

The sample extract was then assayed using 1 N Folin Ciocalteau reagent and sodium carbonate. The absorbance was read at 760 nm using a Helios Gamma UV-Vis spectrophotometer (Thermo Scientific, Inc, Waltham, MA, USA).

The value of a quantitative assay is immeasurably increased if calibrated according to a reference standard (Waterman and Mole 2004). A tannic acid standard curve (plot of absorbance against the quantity of substance assayed) was thus prepared using the same methodology (Figure 3).

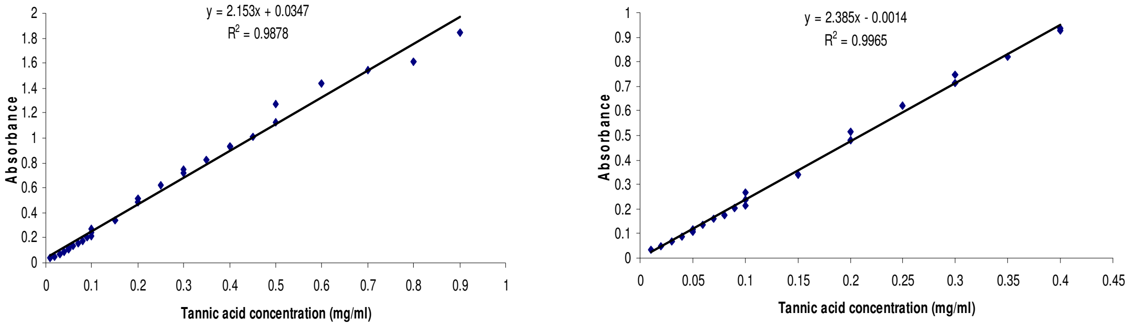

Figure 3: Tannic acid standard curves (mg/ml) for a) all concentrations of tannic acid assessed and b) only those tannic acid concentrations reflecting absorbance values <1 and from where most absorbance values obtained were restricted through dilution.

5.3.7. Effects of restrictions on rooting depth on plant growth and allocation

To determine whether restrictions in rooting depth affected the plant traits measured in this study, ten plants were planted in October 2008 in 50 cm long by 15 cm diameter PVC pipes in soil collected from WRF in 2007, making up a total volume of 8.8 litres, similar to the soil volume in the root observation chambers, but with a soil depth almost 0.5 fold deeper. Each plant received 250 ml of water three times per week and otherwise treated exactly the same as the plants grown in the rooting chambers. The plants were harvested after 16 months of growth and compared with the 16 month old harvested plants grown in the rooting chambers.

5.3.8. Data analysis

From the primary data the following variables were derived: % roots (root mass/total plant mass), % stems (stem mass/total plant mass), % leaves (leaf mass.total plant mass), leaf area ratio (LAR; leaf area/total plant mass, in m^2/g), leaf area root mass ratio (LARMR; total leaf area/root mass, in m^2/g), root to shoot ratio, mean leaf size (MLS, total leaf area/total leaf number, in mm^2) and specific stem length (SSL; stem length/stem mass, in mm/g). These variables refer, respectively, to biomass allocation (% roots, leaves and stems), leaf display (LAR), the balance between investment in light intercepting organs versus water and nutrient up-take organs (LARMR and root: shoot ratio) and the efficiency of biomass investment for height gain (SSL) (Poorter 1999). Whole plant mean above-ground RGR was calculated using the formula ln(final stem diameter^2*final stem height)-ln(initial stem diameter^2*initial stem height). Height and diameter relative growth rates (RGR) were calculated using the adjusted formulae. RGR based on weights could not be calculated because plants of different ages were not grown simultaneously as described above. Only the ten 16 month old plants were used to analyse weekly height RGR since germination. RGR between ages was calculated using the size of the plant at harvest (stem diameter^2* height) versus the size of the same plant at the previous age class. Mean RGR was calculated by averaging all the replicates within an age class.

All data were tested for normality with the Shapiro-Wilks test and with plots of studentized residuals versus predicted values. Homoscedasticity was tested using Bartlett’s and Levene’s tests. Data was analysed using Statistica V6.0 (Statsoft, Inc. 2004) and R freeware v12.1 (R development core team 2010). One-way ANOVA and Fisher’s LSD tests were used to compare growth characteristics and biomass allocation between soil types and growth characteristics between provenances. Growth curves were fitted to the height growth data measured at weekly intervals using polynomial and non-linear regression analysis. The mean of the replicate plants per treatment was used to fit the curves. A repeated measures ANOVA was used to compare the growth data between soil type and provenance treatments. One-way ANOVA and Fisher's LSD was used to test for differences in growth parameters, starch content, phenolic content and biomass allocation between age groups.

5.4. Results

5.4.1. Soil type effects on allocation physiology

There were no significant differences in terms of plant size for plants grown in basalt clay compared to granite sandy soil at the time of harvest (11 months old). The only difference was that the relative biomass allocation to stems, was higher in basalt soils ($F_{1,18}$=4.47, p=0.049) (Figure 4 and 5).

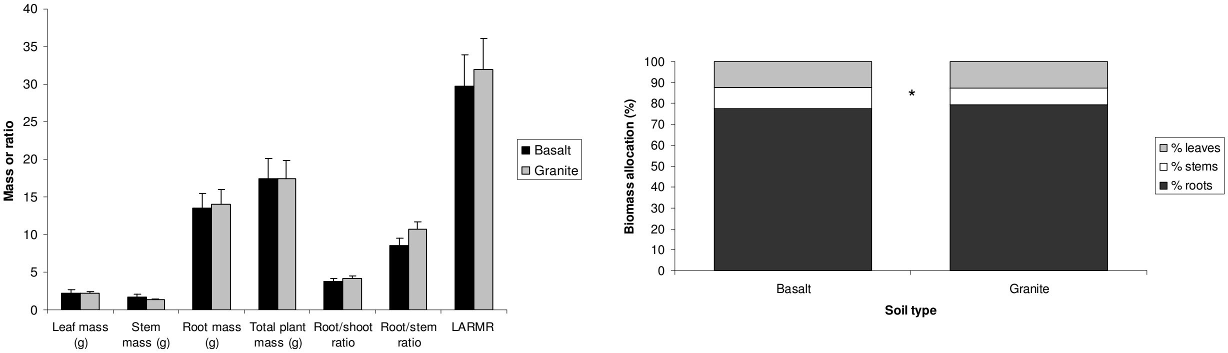

Figure 4: A) The balance between investment in light intercepting organs versus water and nutrient uptake organs (organ mass, root: shoot ratio, root:stem ratio, and LARMR (leaf area: root mass ratio cm^2/g)) and B) biomass allocation of 11 month old marula seedlings grown under glasshouse conditions in two soil types (mean±SE). * indicates significant differences between soil types.

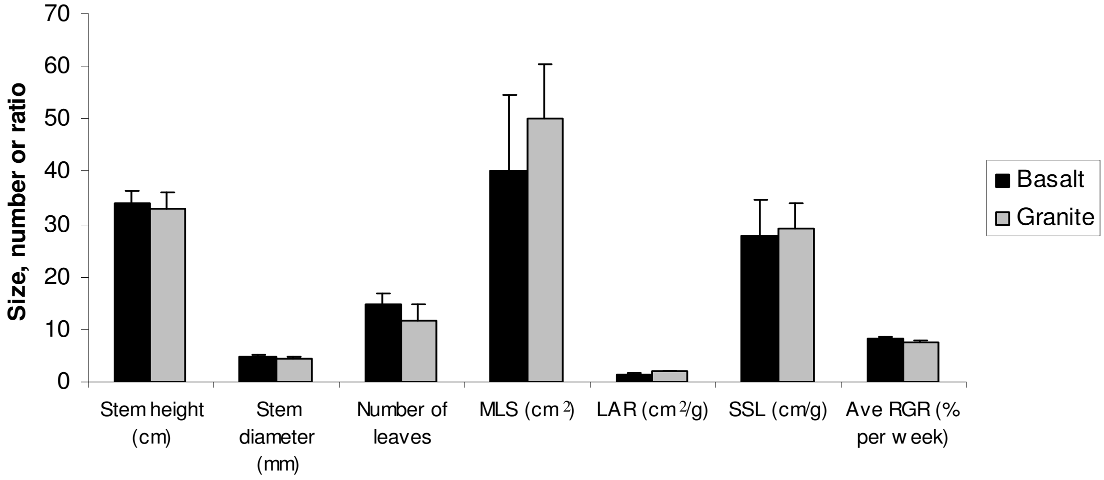

Figure 5: Plant size, leaf display (LAR, MLS), efficiency of biomass investment for height gain (SSL) and average weekly growth rates (RGR) of 11 month old marula seedlings grown under glasshouse conditions in two soil types (mean±SE). No significant differences between soil types were detected.

However, regardless of the lack of difference at 11 months of age, the height growth curves for the two soil types indicated considerable differences earlier in the growth period (Figure 6). A third order polynomial growth curve was the best fit for the growth of marula seedlings up to 11 months of age for both soil types (Figure 6a) ($p<0.00001$). A repeated measures ANOVA indicated that the heights in basalt tended to be higher than in granite ($F_{1,18}=3.31$, $p=0.0856$), time had a significant effect ($F_{30,540}=83.99$, $p<0.0001$) as did the interaction between time and soil type ($F_{30,540}=3.71$, $p<0.0001$). These results indicate that marula plants grow taller in basalt than in granite soils during at least the first growing season. However, this difference appears to decrease as the plants get older. No effects of soil type on stem diameter over time could be discerned (Figure 6b).

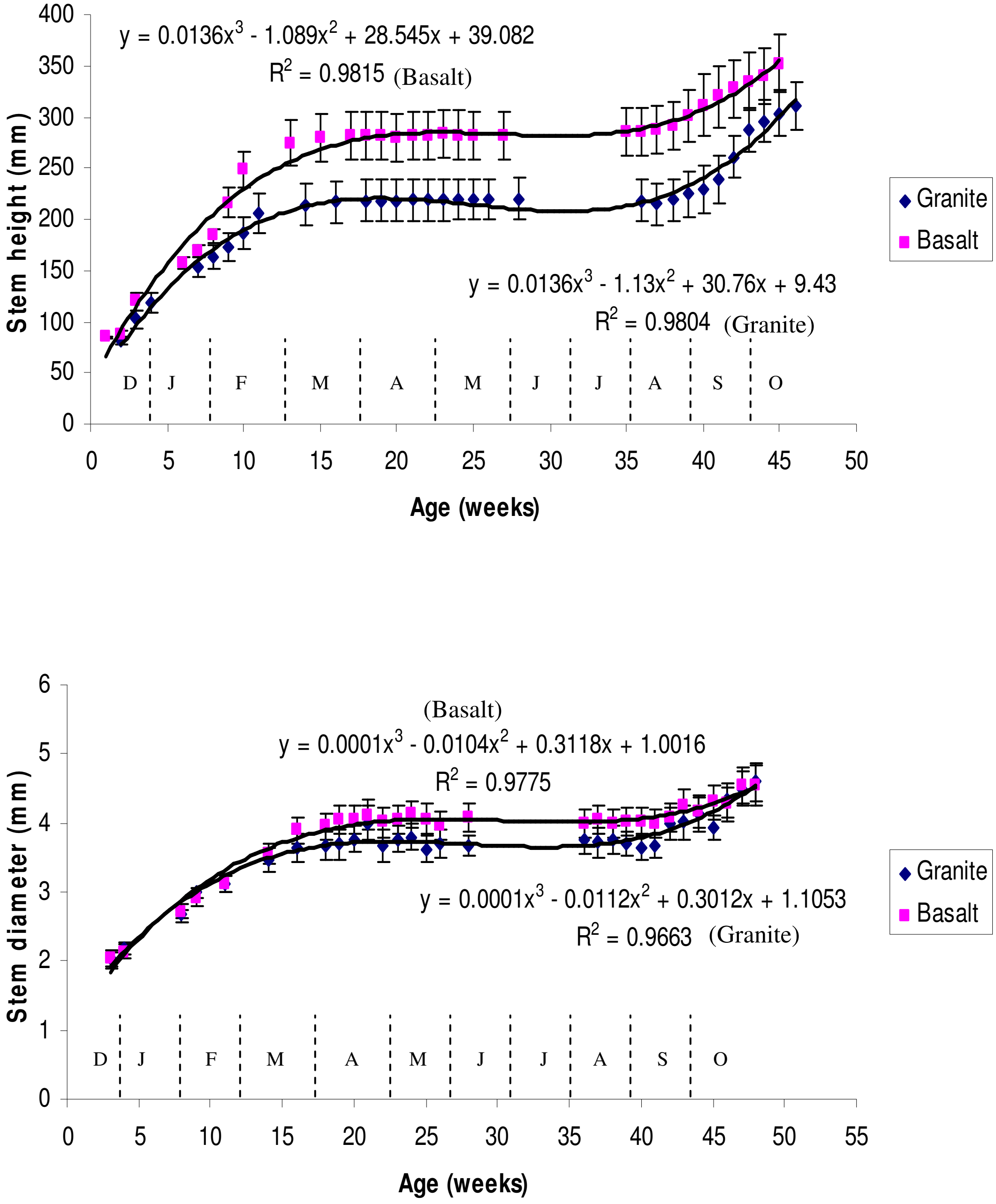

Figure 6: Growth curves using mean (±SE) a) stem height (mm) and b) stem diameter (mm) of marula seedlings grown in granite sandy soils and basalt clay soils under glasshouse conditions for 11 months between Dec 2007 and Oct 2008.

5.4.2. Provenance effects on allocation patterns

Height gain after 6 months was significantly affected by provenance ($F_{3,\ 36}$= 7.47, p=0.00052), with WRF and Potgietersrus seedlings taller than JPNR and Klaserie seedlings (Figure 7). Stem diameter was also significantly affected by provenance ($F_{3,\ 36}$=2.99, p=0.043), with Potgietersrus and Klaserie seedling stems thicker than those of JPNR and WRF. Hence there may be a trade-off between height and stem diameter growth. Neither leaf number nor mean weekly height RGR were significantly affected by provenance.

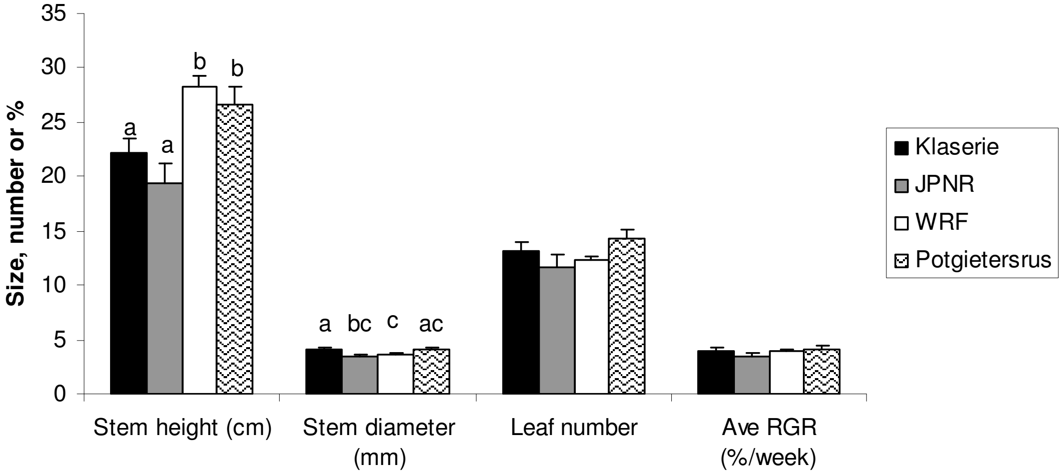

Figure 7: Plant size and average weekly height growth rates (RGR) of 6 month old marula seedlings from four different provenances, grown under glasshouse conditions (mean+SE). Different letters above the bars indicate significant differences between provenances.

The growth curves indicate that seedlings reached an asymptote in height gain after 11 weeks of age and the third order polynomial growth functions describe the weekly data best, except for WRF and Klaserie where a quadratic relationship was best (p<0.00001) (Figure 8). Although these curves are good statistical fits, they are not necessarily particularly biologically meaningful and therefore should not be used for interpretation of any ecological processes, nor can they be extrapolated outside the data range. Asymptotic functions would best serve as functional growth curves from which meaningful biological insights may be derived. According to the repeated measures ANOVA, stem height was significantly effected by provenance ($F_{3;36}$=6.47, p=0.0013), time $F_{15;540}$=219.42, p<0.0001) and the interaction between provenance and time ($F_{45,540}$=5.44, p<0.001).

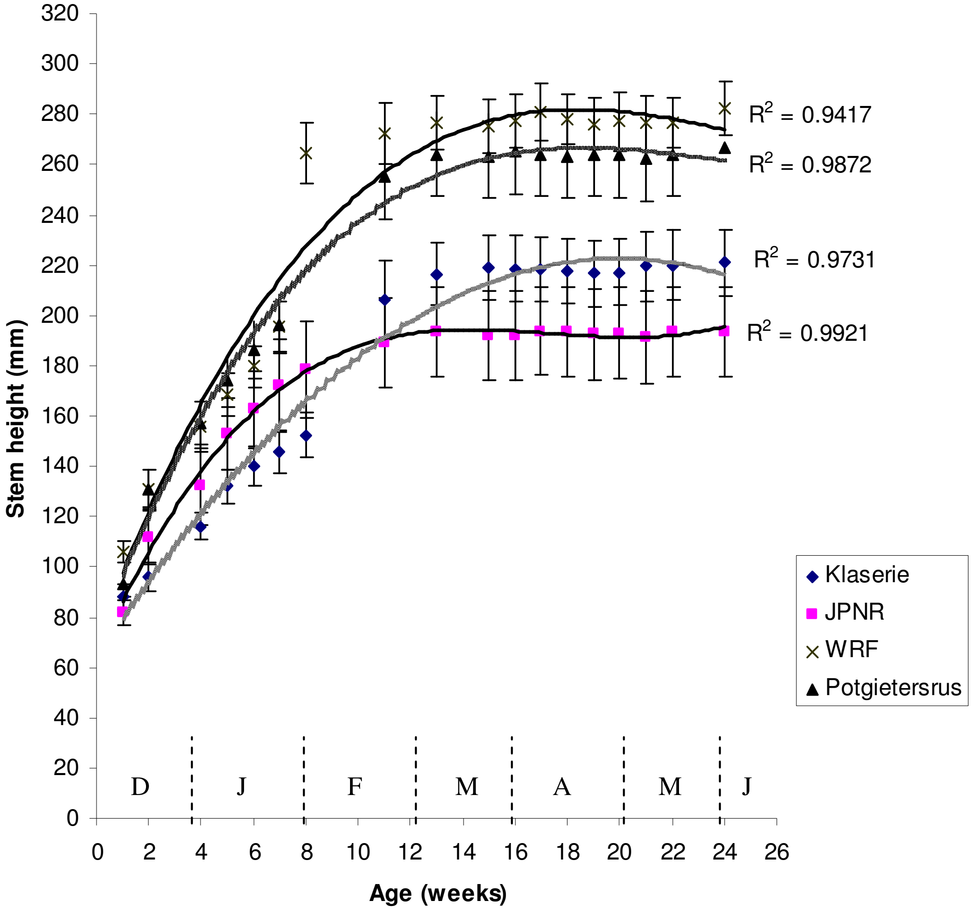

Figure 8: Growth curves using mean (±SE) stem height (mm) of marula seedlings from four provenances grown in granite sandy soils under glasshouse conditions for 6 months between December 2007 and June 2008.

5.4.3. Age-based allocation patterns

5.4.3.1. Plant size and biomass allocation

All size parameters differed between age classes (p<0.00001) (Figure 9). There was a general trend of increase in stem height ($F_{1,9}$=38.68, p<0.0001, R^2=0.81, y=2.41x+7.59) and diameter ($F_{1,9}$=35.18, p=0.0002, r^2=0.80, y=0.30x+1.95) with age. The efficiency of biomass investment for height gain decreased with age ($F_{1,9}$=56.18, p<0.0001, r^2=0.86, y=-

0.94x+30.14) (Figure 10). The high SSL at 9 months indicates possible reallocation to root starch in the dormant season.

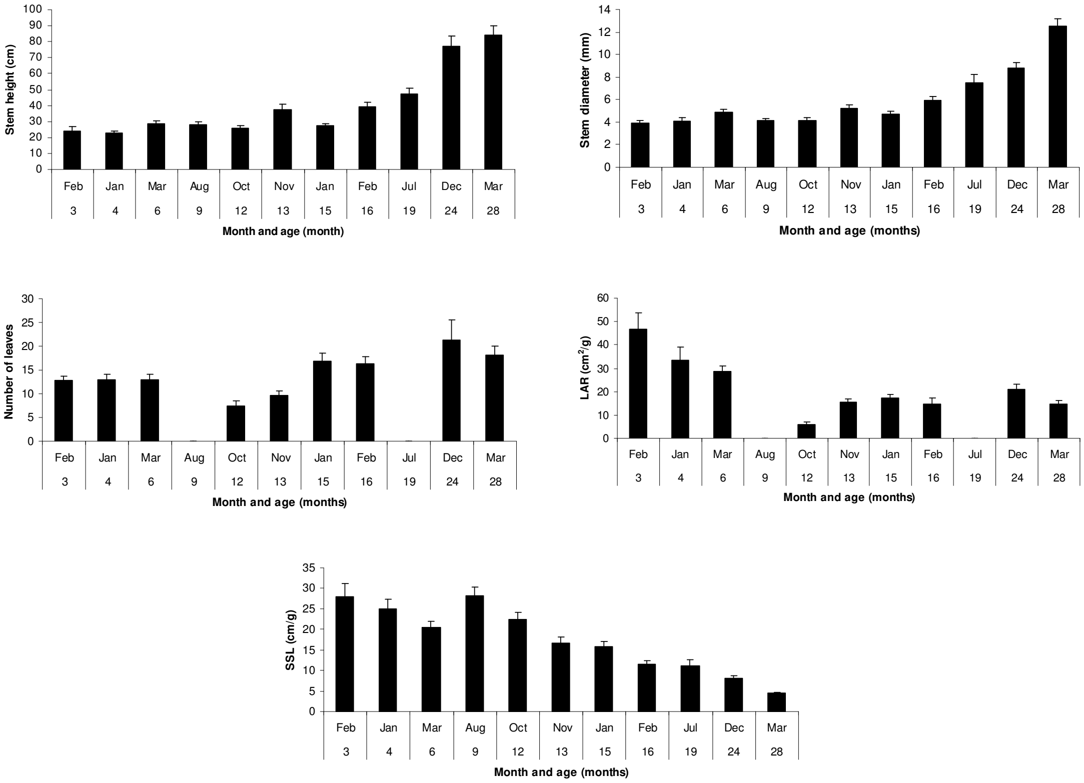

Figure 9: Comparisons of plant size attributes (mean±SE) of marula plants of different ages grown under glasshouse conditions between December 2007 and March 2010.

For plants grown for 16 months, the height growth curve was best fitted by a third order polynomial growth curve over the two seasons of growth assessed (Figure 11).

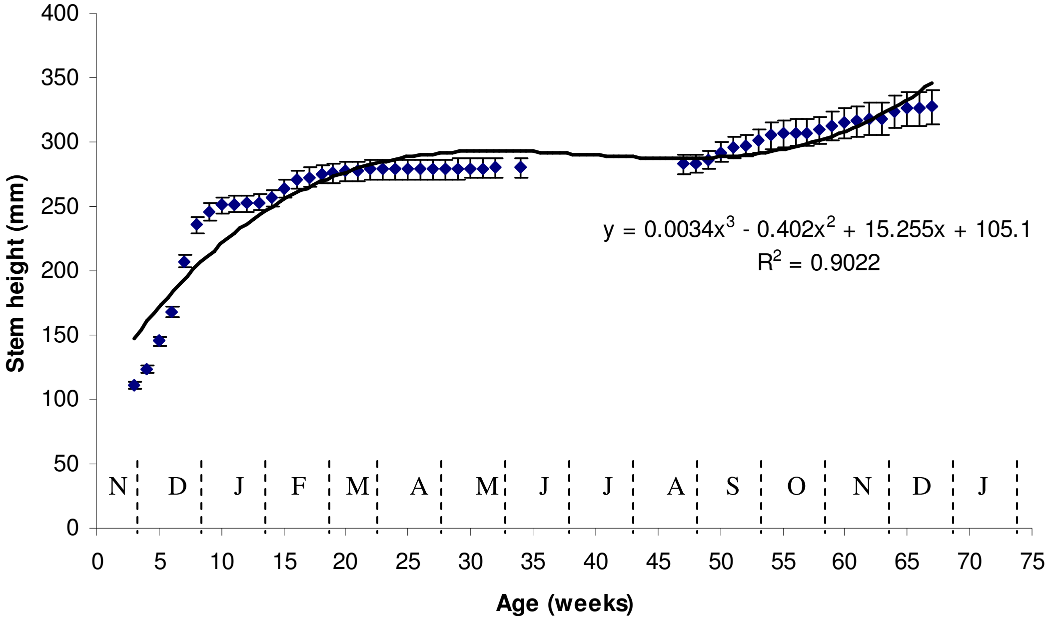

Figure 10: Growth curves using mean (±SE) stem height (mm) of ten marula seedlings/juveniles grown for 16 months under glasshouse conditions between November 2008 and February 2010.

There was a significant difference in the relative allocation of dry mass to roots ($F_{10,102}$=13.49, p<0.0001), leaves ($F_{10,102}$=20.24, p<0.0001) and stems ($F_{10,102}$=16.28, p<0.0001) in relation to plant age (Figure 11). There were strong seasonal effects when leaves were shed (Figure 12). Dry mass relative stem allocation increased with age, which is generally expected due to incremental stem support. Significant differences in absolute root mass ($F_{10,102}$=67.10, p<0.0001), stem mass ($F_{10,102}$=45.94, p<0.0001) and leaf mass ($F_{10,102}$=18.63, p<0.0001) in relation to plant age were also found. Absolute root dry mass increased nine fold between 3 months and 28 months of age, while stem dry mass remained below 5 g until 19 months of age (Figure 12), indicating substantial below-ground storage allocation during the first two seasons of growth (2007/2008 and 2008/2009). The root/shoot ratio initially increased with age and then decreased to below 5 at the beginning of the third growing season (19 months of age) (Figure 13).

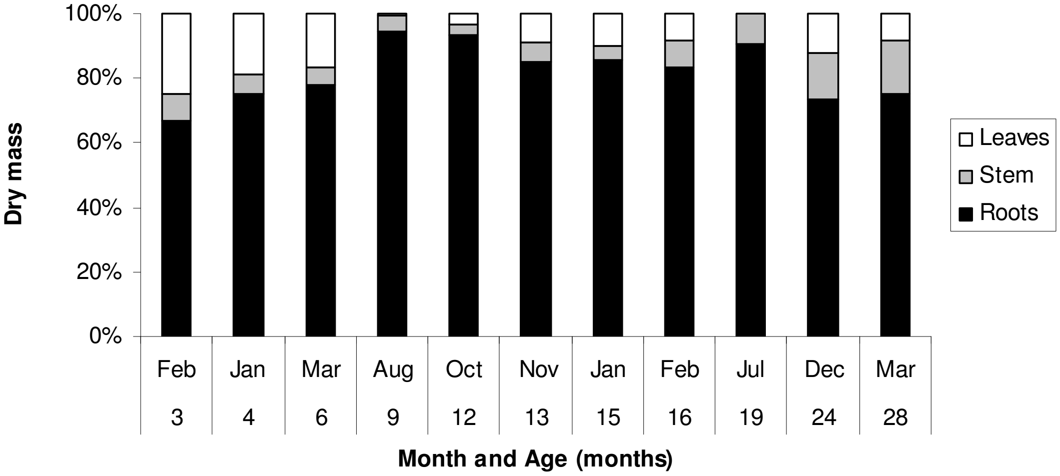

Figure 11: Relative allocation of dry mass to leaves, stem and roots in marula seedlings/juveniles of different ages (months) grown under glasshouse conditions between December 2007 and March 2010.

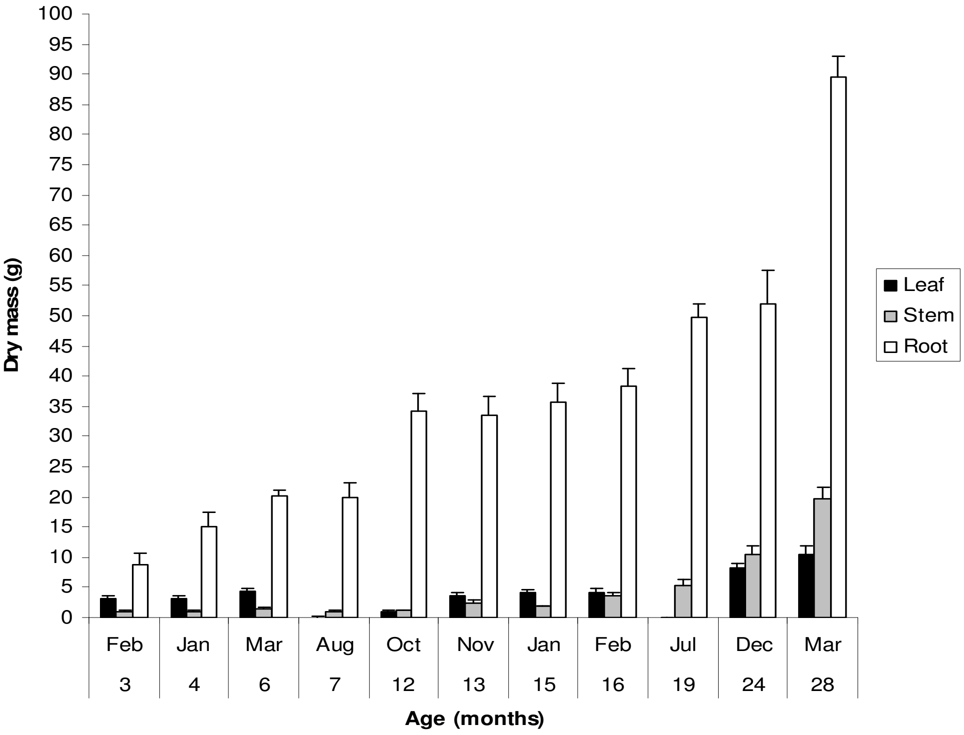

Figure 12: Absolute leaf, stem and root dry mass (mean+SE) of marula seedlings/juveniles of different ages (months) grown under glasshouse conditions between December 2007 and March 2010.

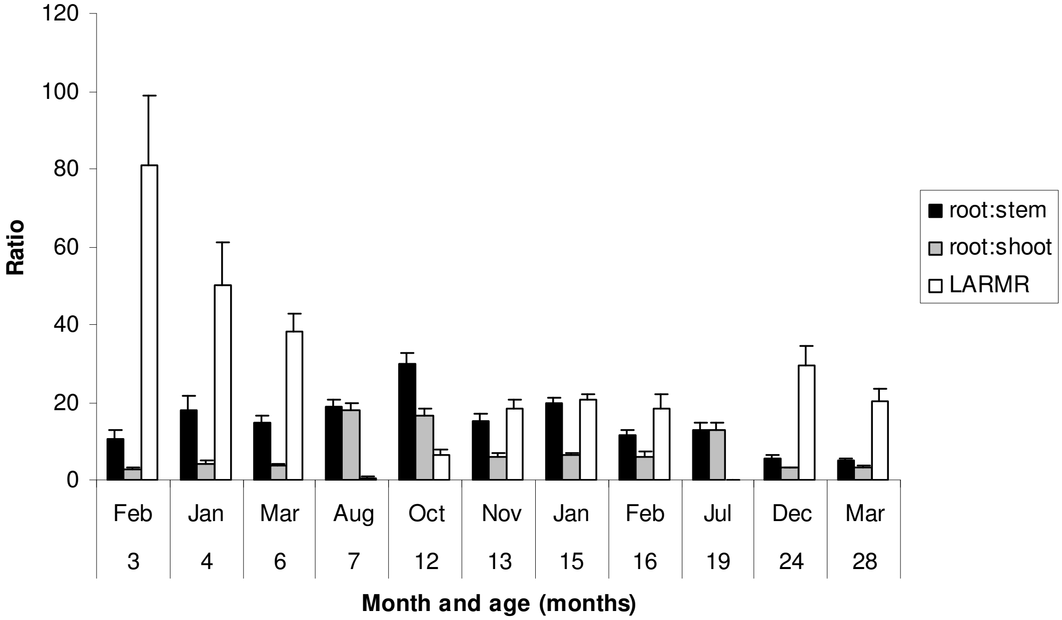

Figure 13: Balance between investment in light intercepting organs versus water and nutrient up-take organs in terms of root/shoot and root/stem ratio and LARMR (leaf area root mass ratio (cm^2/g)) (mean+SE) of marula seedlings of different ages grown under glasshouse conditions between December 2007 and March 2010.

5.4.3.2. Allocation to growth

Weekly measurements of the height of the ten 16 month old plants harvested in February 2010 indicated that the innate RGR was highest directly after germination, peaking at just above 20% per week (Figure 14). RGR did not exceed 5% per week thereafter. Maximum height for the first growing season had already been achieved by most plants after 10 weeks, although some experienced a small growth spurt after 12 weeks. No growth occurred between March and September (20-34 weeks). Growth of approximately 2% per week occurred throughout the second growing season until harvest (September-February).

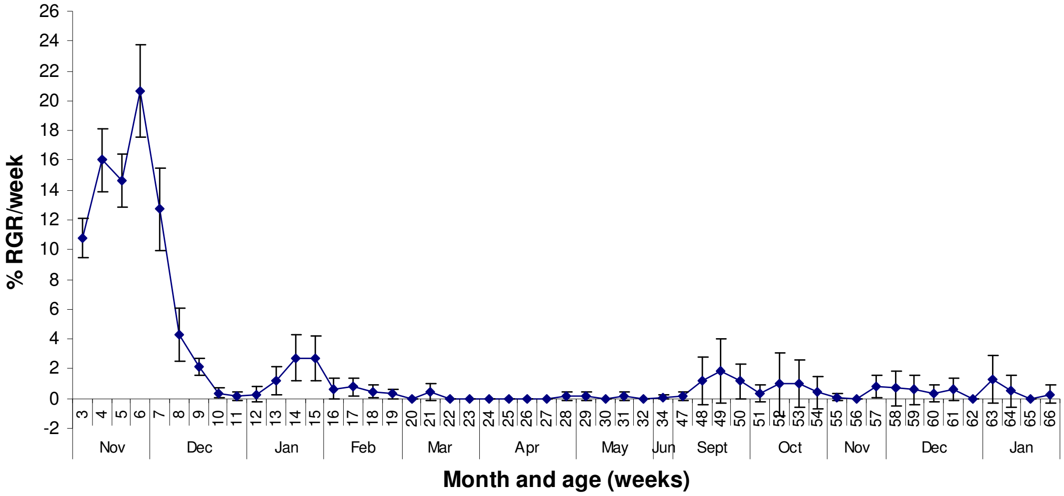

Figure 14: Mean (±SE) height relative growth rate (%, RGR) per week of ten plants grown for 16 months under glasshouse conditions between October 2008 and February 2010.

Plant mass increased exponentially over the first 28 months (Figure 15). RGR peaked early in the growing season and then dropped to zero during the first dormant season. There was a strong linkage between LAR and RGR ($F_{1,9}$=40.07, p<0.0001, R^2=0.80) (Figure 16). The non-linkage (Figure 15) between weight gain and above-ground RGR indicates that towards the end of the second growing season, above-ground growth tapers off but allocation to below-ground biomass probably continues.

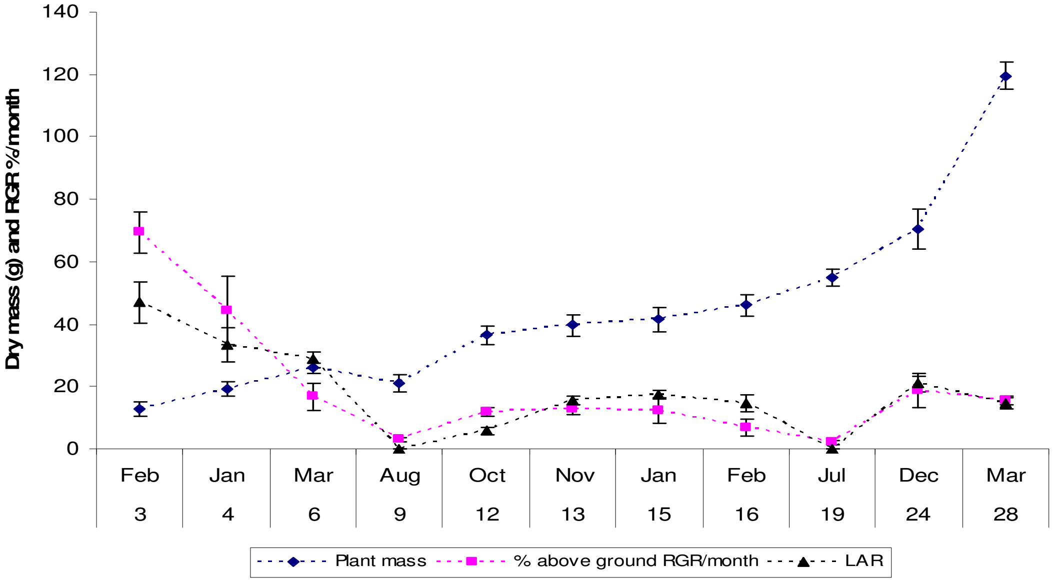

Figure 15: Above-ground RGR (%/month), total plant dry mass (g) and LAR (cm^2/g) for marula plants ranging in age from 3 to 28 months grown under glasshouse conditions between December 2007 and March 2010.

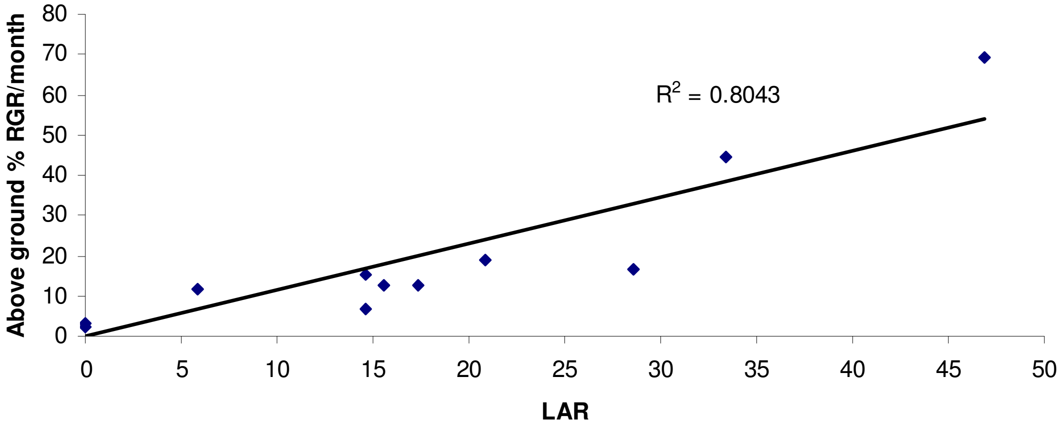

Figure 16: Relationship between above-ground monthly % RGR and LAR (cm^2/g) for marula seedlings ranging in age from 3 to 28 months grown under glasshouse conditions between December 2007 and March 2010.

5.4.3.3. Allocation to storage

Starch concentration increased to a peak at 9 months (35%) and then suddenly dropped after 13 months, probably coinciding with the increase in allocation to growth after the dormant period (Figure 17). Starch concentration then gradually increased throughout the second growing season, again peaking at 35% in March. Starch content followed a similar pattern with maximum starch being present at the end of the second growing season (March 2010).

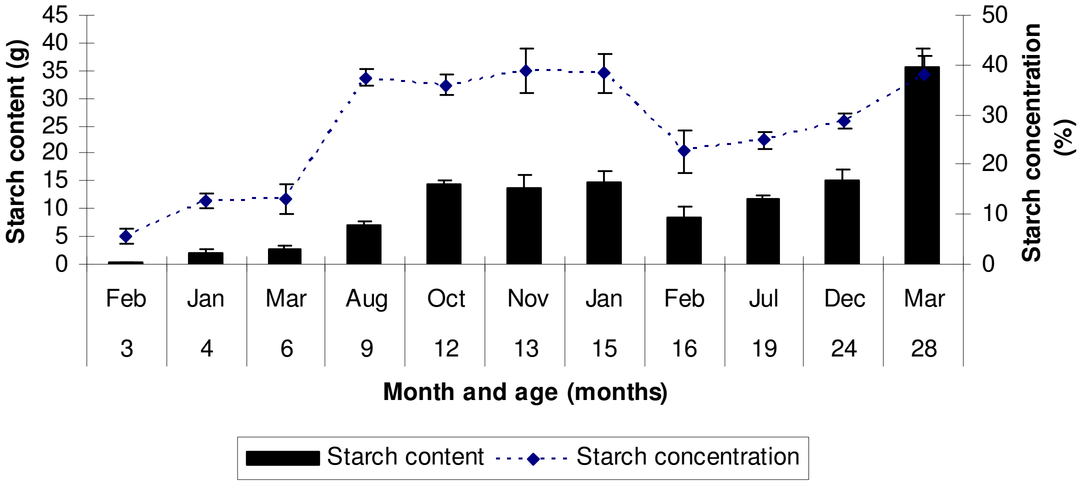

Figure 17: Root starch content (g) and concentration (%) (mean ± SE) of marula plants of different ages grown under glasshouse conditions between December 2007 and March 2010.

5.4.3.4. Allocation to defence

There was a significant effect of age on phenolic concentration ($F_{8,76}$=4.53, p=0.00016) and content ($F_{8,76}$=3.81, p=0.00083) (Figure 18). The phenolic concentration ranged between 8 to 18%, most of which consisted of tannins (Figure 18). Thirteen month old plants had the highest levels of phenolics ($p<0.05$) and 24 month old plants the lowest ($p<0.05$). Most of the phenolics consisted of tannins (range: 73-99%) and of these up to72% were condensed tannins (range: 25-72%) (Figure 19).

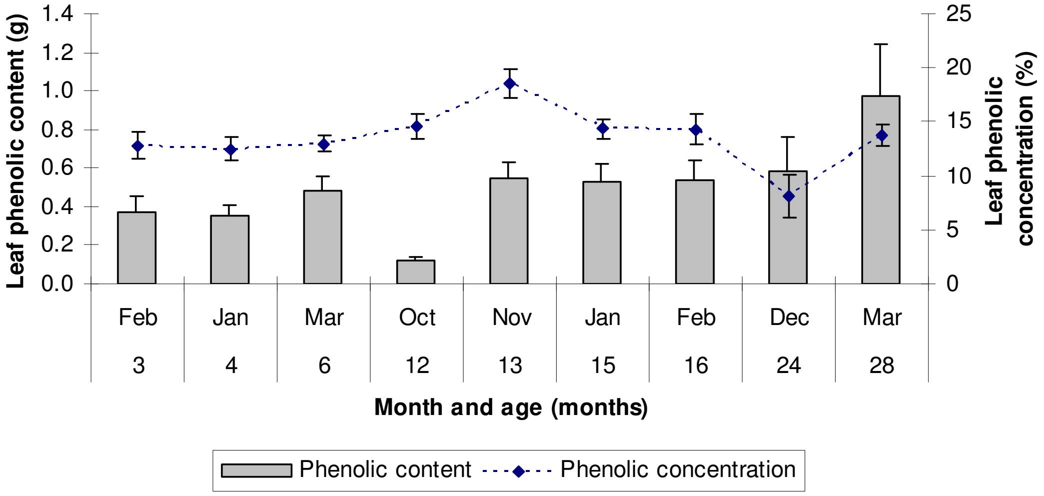

Figure 18: Leaf phenolic content (g) and concentration (%) (mean±SE) of marula plants of different ages grown under glasshouse conditions between December 2007 and March 2010.

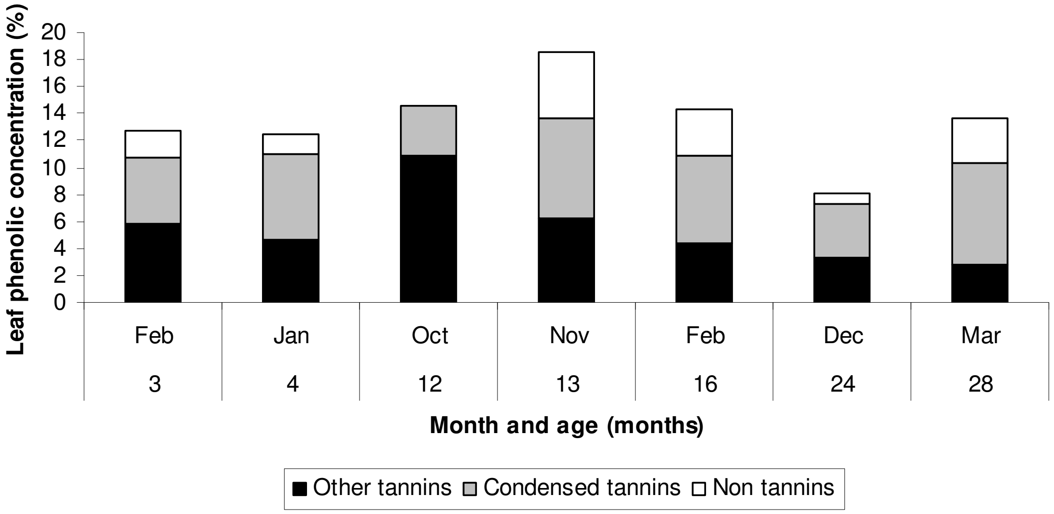

Figure 19: Breakdown of leaf phenolic concentration (%) of marula plants of different ages grown under glasshouse conditions between December 2007 and March 2010 into tannins (condensed and hydrolysable) and non tannins.

5.4.4. Effects of restrictions of rooting depth on plant growth and allocation

Root mass of 16 month old plants grown in PVC pipes was more than double ($F_{1,18}$=161.5, p<0.0001) and stem diameters were more than 50% greater ($F_{1,18}$=55.5, p<0.0001) than those grown in the rooting chambers. However, no significant difference was found in the root: shoot ratio ($F_{1,18}$=0.015, p=0.90) or plant height ($F_{1,18}$=0.33, p=0.57).

5.5. Discussion

5.5.1. Soil type effects on allocation patterns

Even with the large differences in fertility and soil texture, soil type did not appear to have much effect on the growth or allocation patterns of marula seedlings up to 11 months of age. However, there was evidence that the differences were greater during the first growing season. Plants grew exponentially in height until reaching a maximum height within 13 weeks of age, after which they remained the same height, allocating resources into structural biomass (increased stem diameter) and storage rather than vertical growth. Growth began again soon after termination of the dormant period and increased exponentially. Hall *et al.* (2002) indicate that marula prefer well drained sandy soils and heavy clay soils are regarded as unsuitable. However, a wide range of soil fertilities support established populations of marula throughout its range (Hall *et al.* 2002). Trollope *et al.* (1998) found in the KNP that marula have higher growth rates on the sandy granitic than clayey basaltic soils. This has been suggested to be because sandy soils do not hold moisture in the upper soil layers but permit the penetration of moisture down to greater depths than clay soils, thus favouring the deeper rooted woody vegetation more than the shallow rooted grass component of savannas (Jacobs 2001). Root growth has been shown to be favoured in soils where N, P and S are limiting, while the opposite pattern was obtained when K, Mg and Mn restricted growth (Ericsson 1995). It appears that soil texture and fertility may be less limiting for marula growth than moisture availability due to a wide tolerance for nutrient availabilities. Low plasticity in response to soil type was observed, therefore marula may have a fairly fixed growth allocation pattern, with a very high allocation to root growth regardless of soil type.

5.5.2. Provenance effects on allocation patterns

Provenance affected stem height and diameter of 6 month old marula seedlings, indicating that ontogenetic influences are important in marula growth and its responses to environmental variables. Taller seedlings corresponded to provenances from higher rainfall sites (WRF >Potgietersrus> Klaserie> Jejane). The fact that some provenances perform better than others, indicates evolutionary differentiation in response to geographic distribution and local habitats (Jia *et al.* 2010), which requires further investigation. However, given the lack of observed differences in plant traits of seedlings grown in two soil types after 11 months and the observed difference during the first growing season described above, these provenance differences may become less prevalent as the seedlings age.

5.5.3. Seedling age-based allocation patterns

5.5.3.1.Biomass allocation

Overall throughout the 28 months, the results of the chronosequence study suggests a significant effect of juvenile marula age on biomass partitioning allocation, and specifically there is a high allocation to root mass, which varies from 67 to 93 %. The effect of age on biomass partitioning may result from both physiological and physical changes at the plant level. The contribution of root biomass to total plant biomass is high throughout and initially increased with age during the first growing season but decreased with age into the second and third growing seasons. The first year of marula growth involved rapid root extension and build up of root starch stores which are greatly depleted by up to 42% during the second growing season. The root starch stores then increase gradually through the third growing season. The root: shoot ratio gives an indication of a plants ability to withstand moisture stress and often increases with decreasing soil fertility (Gerhardt and Frederiksson 1995). Hence these results suggest that marula has an ability to withstand water stress, but its growth does not necessarily appear to respond to soil fertility.

5.5.3.2. Allocation to growth

As has already been noted, little is known about the growth rates of marula. Studies that have measured growth indirectly indicate that height growth of newly germinated stems is approximately 0.7 m within a year (Shackleton *et al.* 2002). Shone (1979) found that growth after one year could range between 0.3 to 2 m. Venter and Venter (1996) describe marulas as growing easily from seed and being one of the fastest growing trees in South Africa, with a growth rate of up to 1.5 m per year. These values are generally for plants growing in gardens. The results from the current study indicate much slower height growth rates.

This study found that seedling heights ranged between 0.15 and 0.3 m within the first growing season. Maximum height within the first growing season was reached rapidly, between 11-15 weeks of age, while heights did not increase by more than 30% in the second growing season. The maximum height of the oldest plants (28 months – 2.3 years of age) studied was 1.3 m (range: 0.5-1.3 m). These results indicate that the seedlings grown in these experiments would only be able to escape the fire trap after approximately 5 years without fire (2.75 m; Jacobs and Biggs 2001). These estimates are much faster than those obtained in Chapter 4, where escape from the fire trap was expected after 12 years based on growth rates of individuals between 2 and 30 cm in diameter. On the other hand, the maximum stem diameter reached by the same plants was 1.7 cm (range: 1.1- 1.8 cm). In Chapter 8 it was found that the minimum diameter of resistance to fire was 3.4 cm and complete resistance occurred from 5 cm. This translates into the time taken for the plant to become resistant to fire to be approximately 7 years. Once again this estimate is much faster than that predicted in Chapter 4. These height and diameter gain values are still much lower than that predicted by other studies of marulas grown under nursery conditions mentioned above, indicating that the growth of these plants could have been restricted by the growth chambers and nursery bags used. The provenance used was also one of the slowest growing types available and hence this could be indicative of the slow growth of seedlings in the low rainfall JPNR. The faster growth rates found in this study compared to those presented in Chapter 4 are expected as the plants grown in the glasshouse were grown under optimal conditions with no water or nutrient limitations whereas the plants growing under natural conditions as decribed in Chapter 4 would have grown under suboptimal conditions.

The question of whether the growth patterns observed in these glasshouse trials accurately represents plants growing in natural communities requires addressing (Mukany and Ash 2008). Mukany and Ash (2008) found that for most traits, plants grown in pots were significantly different to those grown under field conditions. Plants growing in the field were found to be significantly shorter and with significantly smaller leaves and lower SLA than those grown in pots. Container size may also restrict root growth and hence affect plant growth. Cucumber plants grown in smaller containers had lower root and shoot weights and lower starch accumulation rates (Robbins and Pharr 1988). Root: shoot ratio was not altered by the container volume in the current study, suggesting coordination of root and shoot growth due to rooting volume. Plants generally respond to restrictions on rooting depth by reducing shoot and root growth (Robbins and Pharr 1988). This was mostly borne out by the study comparing plants grown in PVC pipes versus those grown in rooting chambers, with the exception that height gain appeared not to be affected by the rooting depth restriction in marula. Therefore the estimate of 5-7 years to escape the fire trap under optimal conditions is still reasonable.

RGR and LAR have been found to be tightly linked in tree seedlings by a number of studies (Cornelissen *et al.* 1996), including the current one, hence the RGR of marula may be determined from the LAR (leaf area- plant weight ratio) using the relationship defined in the results section. A higher LAR indicates a higher growth rate along with other traits such as low wood density and large seed mass.

5.5.3.3. Allocation to defensive chemicals

Marulas tend to grow in nutrient-poor environments. According to the carbon-nutrient balance hypothesis, defences in marula are expected to be carbon based. Biomedical reports have found evidence of tannins (especially procyanidins/condensed tannins) and other polyphenols in the leaves of marula (Ojewole *et al.* 2010). There are contradictory scientific opinions on the utilisation of marula foliage by browsers and the allocation to defences, with some scientists indicating that marula foliage is palatable and others unpalatable (W. Bond pers. comm.). There is, however, evidence that members of the Anacardiaceae tend to be outliers in their defence allocation characteristics, having both fast growth rates and high allocation to defence. Extracts of marula provide substantial amounts of secondary metabolites (Moyo *et al*. 2010). Young marula stems have higher phenolic contents than the leaves and bark (Moyo *et al.* 2010). Phenolic concentrations in leaves are reported to be approximately 13.95% (gallic acid equivalents) and condensed tannins to be in the range of 1.25% (Moyo *et al.* 2010). In the current study, condensed tannin levels of up to 9% were recorded and levels of up to 18% total phenolic concentration were encountered in marula leaves. Furstenberg and van Hoven (1994) found levels of condensed tannins in marula tree leaves in the KNP to range from 16-46%, which is much higher than the levels found by this study and any other published study. These plants were, however, exposed to browsing pressure and this may have increased the phenolics relative to those unbrowsed. The current study, however, indicated no increase in phenolic content with increasing levels of defoliation. Studies comparing wild and cultivated marula plants and their phenolic content have found that wild plants have higher levels of phenolics (Braca *et al.* 2003). The low levels of phenolics in the leaves at 24 months could be due to the leaves being relatively young. Phenolics appear to rise with age since germination and build up after seasonal dormancy to a peak and then drop before dormancy again. Leaves had greater levels of phenolics during the second growing season than the first and third.

5.5.3.4. Allocation to storage

Marula roots in dryland vegetation may be significant storage sites. Marula has a thick taproot, often penetrating up to 2.4 m in depth and subtending extremely widely distributed lateral roots (Hall *et al.* 2002). A tuberous rootstock forms early in development and stores sugars and water. In addition, the ability to exploit soil to a considerable distance from the bole and store reserves may contribute to the ability of marula in the drier parts of its range to attain greater size than most other species (Hall *et al.* 2002). This study found levels of starch of up to 35% in the roots of 1 year old plants. Schutz (2007) found values of up to 32% in *Acacia nigrescens* and 29% in *Acacia gerrardi,* two fine leaved species found in association with marula. Marula appears to have a relatively high starch allocation from a very early age, reaching a peak within 9 months. Significant seasonal fluctuations in starch content have been observed for a variety of woody species (Adams *et al.* 1986).

The carbohydrate economy of a tree can be regarded as an integrated system of sources and sinks (Wareing and Patrick 1975). The sources, consisting of photosynthetic organs, produce photosynthates to supply actively growing sinks; the surpluses are converted to reserve substances. The stored reserves, mainly carbohydrates, can later be hydrolyzed and used for growth and respiration (Ericsson 1979). Since marula seedlings were shown to only grow most during the first half of the growing season, allocation to storage is possible toward the end of the growing season to be available for emergence of new leaves at the beginning of the next growing season.

The functional priorities of growth, resistance, storage and reproduction change as plants age and these changes influence resource allocation. Owing to these differences in resource allocation constraints during plant development, resistance and tolerance of herbivory is likely to change with ontogeny (Boege and Marquis 2005). Given the view that the impacts of herbivory decrease with increasing age, the younger stages should be most defended. Once seedlings deplete the resources stored in cotyledons, the production of above-ground biomass becomes a priority to achieve optimal resource-foraging balance in which all resources are equally limiting. Until this is achieved, allocation to defences may be constrained (Boege and Marquis 2005).

5.5.3.5. Allocation trade-offs

Investment in starch allocation took place at the end of the first growing season when RGR declined (Figure 21). No additional starch allocation occurred during the second growing season, instead a large drop in starch concentration at the end of the second growing season indicated that the consistent growth of above-ground tissue utilised a large proportion of the starch reserves. However, in the third growing season (March, 28 months) both starch concentration and content were again peaking. During the third growing season a gradual increase in starch allocation occurred but was overshadowed by the high allocation into root biomass. Allocation to defence appears to be relatively consistent increasing to a maximum in the middle of each growing season.

No trade-offs between growth and defence allocation were therefore observed, but trade-offs with growth and storage were evident.

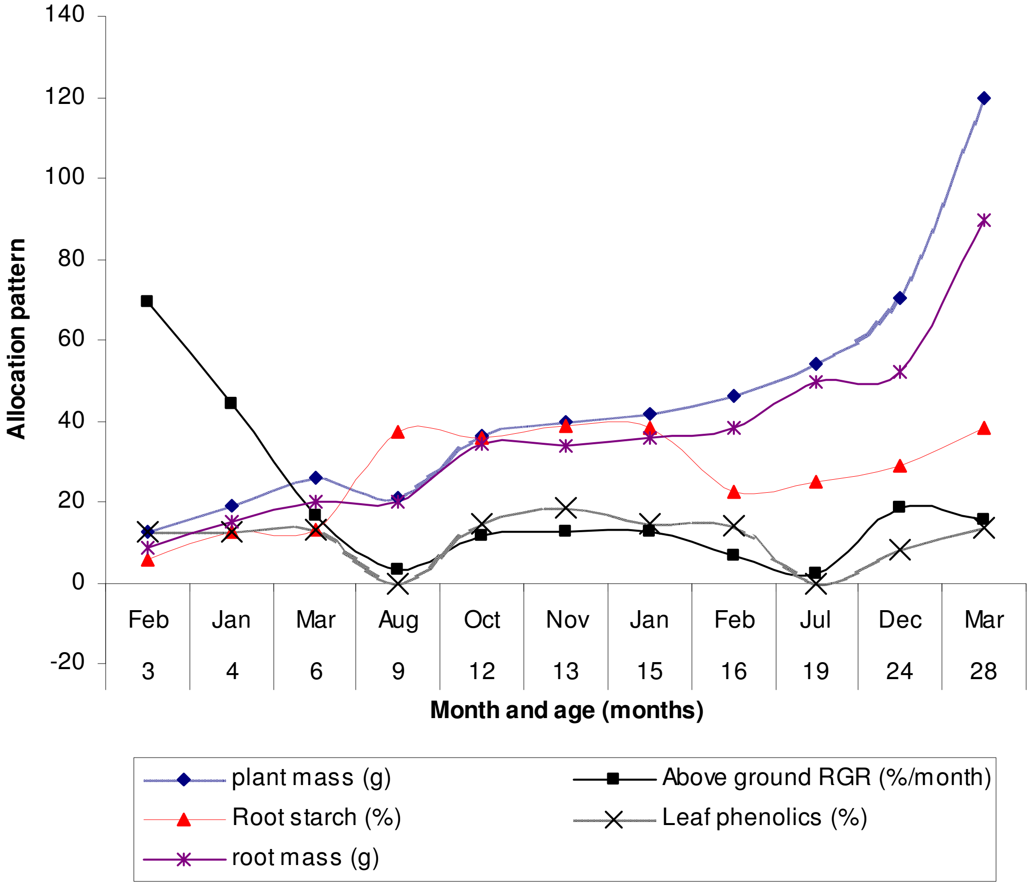

Figure 20: Trade-offs between growth, defence and storage in marula plants of different ages grown under glasshouse conditions between December 2007 and March 2010.

5.5.4. Reliability of the application of results of pot grown plants to naturally grown plants

Finally, the reliability of the application of the functional traits of pot grown plants to naturally grown plants has been questioned (Mokany and Ash 2008). The utility of pot grown plants to quantify traits for field grown plants is highly dependent on the selection of the growing conditions in the controlled environment. Therefore it is extremely important to report the growing conditions of a pot experiment in detail and compare them with the conditions regularly experienced by plants grown in natural communities. In the current study, the temperatures experienced in the glasshouse were slightly warmer during the summer months relative to Skukuza, but well within the range of temperatures experienced in the lowveld of South Africa. Humidity levels were higher than those experienced in the lowveld. The growing medium used was the same as that found in the field. Light conditions were probably slightly less intensive than those experienced in the field and obviously water was not limiting. Cognisance of the effect of pot size on imposing restrictions on root growth and hence plant growth needs to be made. However, given the comparative nature of this study, the restrictions on rooting depth imposed by the pots can be ignored. It is the absolute sizes of the plants that may have been affected and hence field grown plants may be larger. Overall, cognisance of these differences should be taken into account when comparing absolute values. However, the general trends in allocation observed are believed to be sound and can be applied to the natural environment.

5.6. Conclusion

The young plants used in this study represent only a short phase in their potential lifespan. Although early seedling growth is a key ecological factor in natural regeneration, subsequent growth until maturity is also important for the success of the species (Cornilessen *et al.* 1996). Hence comparisons with the growth rates obtained in this study with those obtained in Chapter 4 need to be made. Chapter 4 provided growth rate data under field conditions from 2 cm in diameter, while this study provided growth rate data for the period from germination up to this point. It therefore should take marula seedlings approximately 5 years to reach a stem diameter of 2 cm. Trade-offs in allocation to roots by woody plant seedlings may influence competitive ability with grasses and survival in fire prone ecosystems (Clarke and Knox 2009). Savanna trees allocate large proportions of their reserves to below-ground biomass to ensure against above-ground biomass loss from fire and herbivory and hence the high allocation of biomass to roots in marula is expected. The high allocation to roots regardless of soil type or age indicates a strategy of resistance to fire as well as a well developed ability to outcompete grasses. The lack of trade-off between growth and defence requires further investigation. Marula appears to have constitutive rather than induced defences indicating that marula has evolved in environments with a very high attack probability (Bazzaz 1997). Starch storage is very high from very young and in conjunction with high root allocation, makes seedling roots a potential high quality resource for root herbivores such as monkeys and baboons. Differences in height gain across provenances indicate evolutionary adaptation of provenances to varying rainfall regimes at different sites. Overall, marula is well adapted to fire and low rainfall as well as having a competitive advantage over grasses due to high root allocation.

5.7. References

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Chapter 6: Reproductive potential and seed fate of *Sclerocarya birrea* subsp. *caffra* (marula)

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6.1. Abstract

Even though *Sclerocarya birrea* subsp. *caffra* (marula) is a well-studied, keystone tree species with high ecological, commercial and cultural value, significant gaps in our understanding of its reproductive biology exist, particularly the factors limiting fruit and seed production, seed fate and the persistence of the seed bank. Therefore, a detailed quantitative assessment of these factors was conducted at five sites in the low altitude savannas of South Africa. Sites varied with respect to fire regime, large mammals, geology and rainfall. Most sites showed male-biased secondary sex ratios and the absolute minimum fruiting stem diameter ranged between 7.1 and 15.7 cm across sites. Sites with higher levels of disturbance (fire and large browsers) had trees producing fruit at larger stem diameters than sites with lower levels of disturbance. Fruit production was highly variable between individuals, within and between sites, and from year to year. Variability in fruit production across years at one site was greater than the variability across sites in one year, indicating that drivers such as weather, insect herbivory, fire and predator numbers that vary annually play a greater role than more constant drivers such as mammalian herbivory, soil types or long-term rainfall. No significant relationship was found between environmental variables (rainfall and temperature) and fruit production, indicating a trade-off between vegetative growth and reproduction between years. Since marula fruits are large and heavy, the species relies primarily on mammalian dispersal agents such as the African elephant, which have also been shown to increase the germination rate. However, rodents also appear to play a significant role in dispersal. Seed predation rates tend to be highest in areas of low disturbance (no fire and no large browsers). While marula has a small persistent seed bank, recruitment appears to be reliant on the current season's fruit crop. This study provides a detailed quantitative assessment of important reproductive and seed fate vital rates for future population modelling.

6.2. Introduction

Changes in land use, fragmentation of tree populations and elephant impacts in African savannas has resulted in a growing need for information on the reproductive responses of tree species to disturbance (Nason and Hamrick 1997). African savanna trees are heavily utilised by both humans and wildlife, resulting in departures from the normal reverse J population structures in some areas, typical of healthy recruiting tree populations. For example, elephants target mid-range tree size classes while fire targets the smaller size classes (Midgley *et al.* 2010) and humans often target large individuals, resulting in unstable population structures for certain species such as *Acacia tortilis* (Prins and van der Jeugd 1993) and *A. xanthophloea* (Botha *et al.* 2002). Few studies have addressed the impacts of these unstable population structures on the reproductive biology of African savanna woody species.

Sclerocarya birrea subsp. *caffra* (marula, Family: Anacardiaceae) is a relatively well-studied, ecologically, commercially and culturally important, keystone, deciduous, tree species (up to 1m in stem diameter), commonly occurring in the north-eastern, low-altitude savannas of South Africa (Hall *et al.* 2002; Jacobs and Biggs 2002; Palmer and Pitman 1972; Shackleton *et al.* 2002; Van Wyk and Van Wyk 2007). It is commonly a community dominant, favoured by wildlife in conservation areas and of significant importance in rural livelihoods for food, medicine, and carving (Shackleton *et al.* 2002). Recent studies have expressed concern over unstable population structures (Jacobs and Biggs 2002), male biased sex ratios (Nghitoolwa *et al.* 2003), lack of regeneration (Walker *et al.* 1986) and high adult mortality rates (Helm *et al.* 2009), and the implications of these for future population persistence in various habitats in southern Africa.

Despite being relatively well-studied, gaps in our understanding of marula reproductive biology remain, hampering efforts to predict the future dynamics of populations under threat (e.g. in the Kruger National Park (KNP) (Helm *et al.* 2009)), and to explain the diverse population structures observed in private reserves, national parks and communal areas in South Africa (Walker *et al.* 1986; Gadd 2002; Jacobs and Biggs 2002; Shackleton *et al.* 2003). Of particular significance is our lack of understanding of: 1) the factors limiting pollination success and fruit production, 2) the fate of the seeds, 3) the importance of a seed bank for future persistence, 4) germination rates and 5) subsequent seedling survival. A comprehensive understanding of the interactions between these factors is essential to define management prescriptions for threatened populations and utilisation quotas for harvesting under communal rangeland systems (Emanuel *et al.* 2005). This paper highlights and addresses the gaps in the knowledge of marula reproductive biology through a detailed quantitative study of five populations with varying management regimes, densities and population structures.

Marula is described as a dioecious species (Palmer and Pitman 1972) and, assuming that no differential use of habitats by, or mortality of, the sexes occurs, sex ratios of dioecious species approach unity in undisturbed populations (Bawa and Opler 1977). However, since non-fruit producing marula individuals are regularly chopped down in communal villages by people for wood (Nghitoolwa *et al.* 2003; Shackleton *et al.* 2003), and there is evidence to indicate that females are targeted by elephants in conservation areas (Hemborg and Bond 2006), marula sex ratios regularly deviate from unity. In addition, morphological evidence and observations suggest, that marula is an entomophilous species that produces sticky pollen and secretes nectar, and that the honeybee is a major pollinator (Hall *et al.* 2002). Pollination success in marula therefore relies not only on sufficient pollen mediated by the sex ratio, but also on sufficient pollinators.

The marula fruit is a highly specialised, large, fleshy drupe (von Teichman *et al.* 1986), mostly produced on trees >13.6 cm in stem diameter (Shackleton *et al.* 2003). Significant variability in the size of first reproduction has been reported, which has been largely attributed to rainfall variability (Emanuel *et al*. 2005). However, minimum size of reproduction has been linked to the escape from disturbances such as fire in other savannas species (Wilson and Witkowski 2003). This remains to be investigated for marula. Fruit production on a specific tree is known to vary annually with a potential relationship with rainfall (Shackleton 2002a; Todd 2002) or temperature (Todd 2002), specifically from the year prior to fruit production. However, comparisons across sites varying only in rainfall have indicated that fruit crops are unrelated to rainfall (Shackleton 2002a). Such discrepancies require clarification.

Marula seeds do not germinate readily when dropped from the canopy, but may remain quiescent for more than six months in a transient seed bank in the soil (von Teichman *et al.* 1986; Shone 1979), only germinating after sufficient rain the following growing season. The enforced quiescent period is not due to embryonic dormancy, but rather mechanical dormancy (von Teichman *et al*. 1986; Baskin and Baskin 2001). This dormancy can be broken through prior seed treatment, such as passing through the acidic digestive tract of mammals or manual removal of the opercula covering the seeds (von Teichman *et al*. 1986; Lewis 1987). Elephants (*Loxodonta africana* subsp. *africana*) appear to be the main dispersal agents of marula seeds; however, vervet monkeys (*Cercopithecus aethiops*) and chacma baboons (*Papio cynocephalus ursinus*) have also been observed eating the fermenting fruit and transporting them to new locations (Palmer and Pitman 1972). Remains of marula fruit have also been found in kudu dung (*Tragelaphus strepsiceros*) (Shone 1979; Estes 1991). Parrots, rodents and other small mammals, such as ground squirrels (*Xerus inauris*), also target the nutritious seeds (Palmer and Pitman 1972; Symes and Perrin 2003), and caching of uneaten endocarps is common (pers. obs). Finally, people also disperse the seed as the fruit are highly favoured (Shackleton *et al.* 2002). Further investigation into the effects of mammal dispersers on germination rates as well as the dispersal and predation rates in different habitats is required.

Marula seeds remain viable for up to several years in the laboratory (von Teichman *et al.* 1986; pers. obs.), indicating the potential for a persistent soil seed bank. Little is known about natural germination percentages or variability across populations. Previous studies report, unhelpfully, that germination percentages of treated endocarps can vary between 2 and 100% (Hall *et al.* 2002). Little is known about natural germination percentages or variability across populations.

In an attempt to improve our knowledge of marula reproductive biology the objectives of this study were to: 1) assess fruit and seed production and subsequent seed fate; 2) investigate the drivers of these aspects of its biology; 3) describe the variability in these factors through comparisons across spatial and temporal scales; and 4) provide detailed quantitative information for the parameterisation of future marula population models.

In doing so, the following specific questions were addressed: 1) How do the sex ratios of different marula populations vary, what are the possible ecological drivers and how does this affect pollination success? 2) How is marula reproduction limited by disturbance agents and can the size at which marula becomes reproductive be linked to its size of resistance to fire? 3) How variable is fruit production across sites and years, and is weather, as suggested by previous studies, the most important driving force? 4) Could mammal dispersers potentially increase the germination rate to allow for germination in the same growing season as fruit production? 5) What are the dispersal and predation rates for marula populations under different disturbance regimes? 6) How do germination percentages vary between populations and do marulas rely on persistent seed banks for regeneration?

6.3. Methodology

6.3.1. Study sites

Fruit production and seed bank dynamics of marula were assessed at five sites in the north-eastern low altitude savannas of South Africa (Figure 1): (a) the 254 ha N'washitsumbe enclosure (NE) north of Shingwedzi (northern part of the 18 998 km^2 KNP); (b) the 220 ha Hlangwine enclosure (HE) east of Pretoriuskop (southern KNP), (c) the area outside the Hlangwine enclosure (OHE); (d) the 350 ha Wits Rural Facility (WRF) near Klaserie on the western boundary of the KNP, and (e) Jejane Private Nature Reserve (JPNR) in the 4 500 ha Mohlabetsi Conservancy (western boundary of the KNP). The N'washitsumbe enclosure was established in 1968 (Levick and Rogers 2008), while the Hlangwine enclosure was established in 1972 (Goodall 2006), both to exclude all animals larger than hares apart from rare antelope grazers such as roan (*Hippotragus equinus*) and sable (*Hippotragus niger*). The area outside the N'washitsumbe enclosure is now devoid of adult marula trees (Jacobs and Biggs 2002) and therefore could not be used as a comparison site, as was done for the Hlangwine enclosure and surrounds. These sites were chosen to cover a wide variety of possible drivers responsible for marula tree population structure including elephant and other browsers, fire, rainfall and geology (soil fertility) (Table 1).

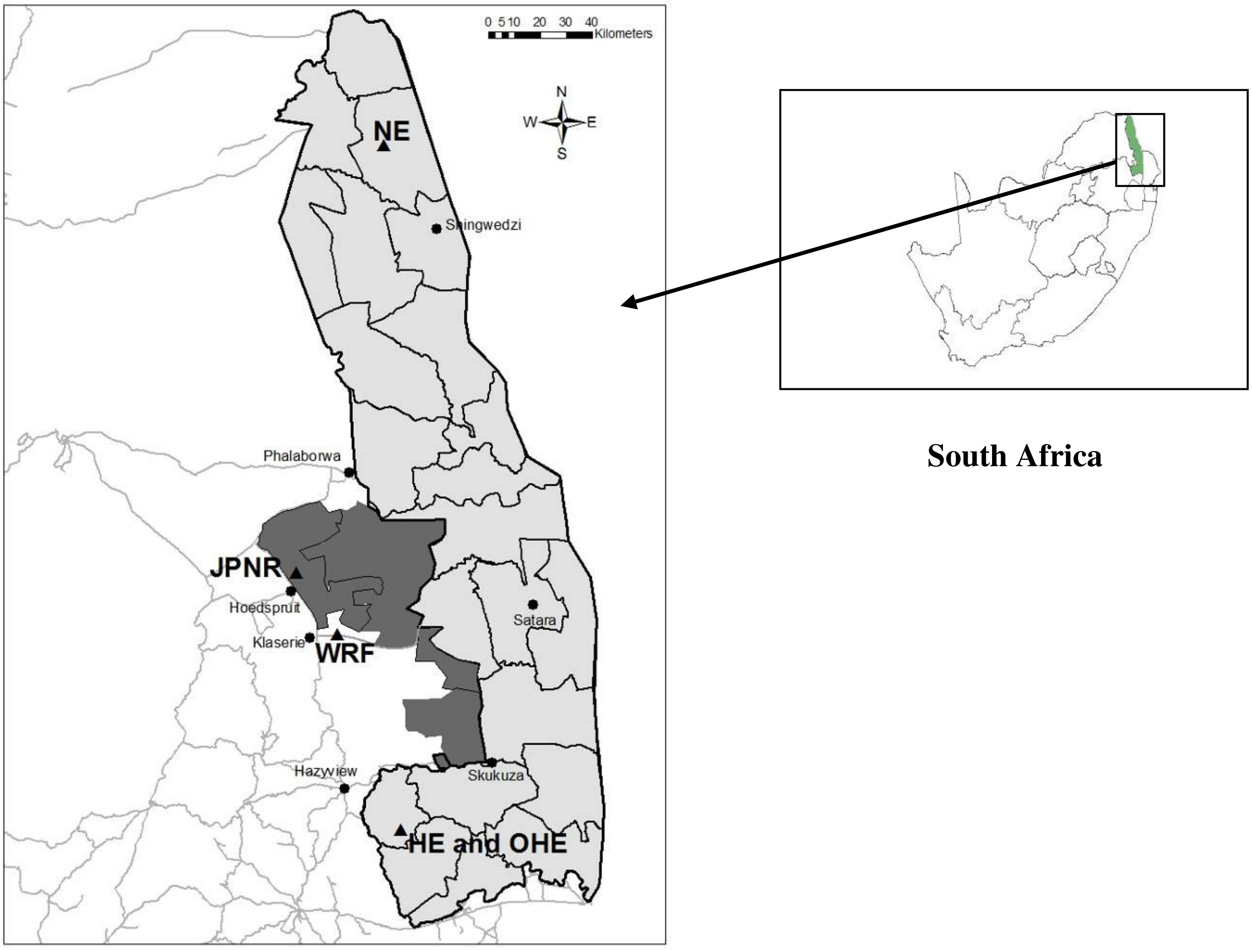
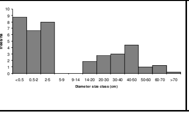
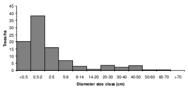
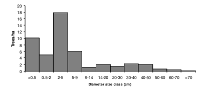
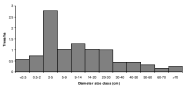
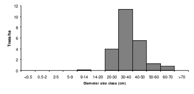


Figure 1: Location of the five study sites (black triangles) in or near the Kruger National Park (KNP), South Africa. Dark grey indicates the private reserves outside of the lighter grey KNP. NE=N'washitsumbe enclosure, HE=Hlangwine enclosure, OHE=Outside the Hlangwine enclosure, WRF=Wits Rural Facility, JPNR=Jejane Private Nature Reserve.

Table 1: Site characteristics and disturbance history of five sites in the low altitude savannas of South Africa (information from Grant *et al.* 2002; Levick and Rogers 2008; Frandsen 2008, G. Thompson pers. comm. (Manager of JPNR)). Methods for population structure determination and density estimates described in text. Values with different superscript letters denote significant differences between sites (P<0.05, LSD).

Site	**N'washitsumbe enclosure (NE)**	**Hlangwine enclosure (HE)**	**Outside Hlangwine enclosure (OHE)**	**Wits Rural Facility (WRF)**	**Jejane Private Nature Reserve (JPNR)**
Location	S22.78049 E31.28185 Northern KNP	S25.20162 E31.29076 Southern KNP	S25.20162 E31.29076 Southern KNP	S24.56386 E31.10331 Western boundary of KNP	S24.29045 S30.97664 Western boundary of KNP
Management	Rare antelope breeding	Rare antelope breeding	National park	Research	Ecotourism
Long-term mean annual rainfall (mm)	520	750	750	680	450
Geology and soil type	Basalt Clay soil	Granite Sandy soil	Granite Sandy soil	Granite Sandy soil	Granite Sandy soil
Year of last fire	2002	2006	2007	2002	>10 years ago
Elephant	No	No	Yes	No	No
Browsers	None	None	Full compliment of African browsers at medium densities	Few impala and kudu	Many impala and other African browsers at high density
Marula tree density (mean trees/ ha±SE) and sample sizes	37.73±5.60^{bc} n_{trees}=236 $n_{transects}$=2	88.88±27.29^{a} n_{trees}=229 $n_{transects}$=4	49.44±10.52^{b} n_{trees}=244 $n_{transects}$=6	8.72±2.73^{c} n_{trees}=218 $n_{transects}$=8	23.63±2.76^{bc} n_{trees}=203 $n_{transects}$=8
Stem diameter size class distributions					

6.3.2. Tree and fruit sampling

Where complete population structure data for marula were not available at a study site, individuals ≥1 m in height were assessed in transects of between 300 and 1000 m in length and 40 m wide, while individuals <1 m in height were assessed in 2 m wide transects nested within the larger transects. The number and length of transects per site were influenced by site factors such as roads but ensuring that at least 200 individuals per site were sampled (range: 4-8 transects per site). A total area of 17.4 ha was surveyed for NE by Jacobs and Biggs (2002). In our study, 32.0 ha, 10.0 ha, 19.9 ha and 8.8 ha were surveyed for WRF (in August 2008), HE, OHE and JPNR (all in January 2009), respectively. Thus an overall total of 88.1 ha was assessed.

To determine sex ratios and minimum size of reproduction, all trees in the HE, OHE and JPNR population transects surveyed in January 2009 were assessed for presence or absence of fruit. For NE and WRF, where population transects were surveyed outside of the fruiting season, wandering transects were used to locate a minimum of 50 marula trees of all sizes per site in January 2009 and 2010 respectively. Where fruiting trees were sparse, additional transects were surveyed until 30 fruit producing trees were found in each site; specifically ten females in each of three basal diameter classes (<30 cm, 30 – 40 cm and >40 cm) in order to cover the full size range of fruiting trees.

The position of each tree was recorded using a GPS and tree height and basal diameter (30 cm above the ground or immediately above the basal swelling) were measured. The longest canopy diameter (D1) and the diameter perpendicular to this (D2) were also measured for fruit producing trees only, from which canopy area was then calculated using the equation for an ellipse.

Adults (male and female) were defined as being >14 cm in basal diameter following Shackleton *et al.* (2003). Male and non-fruiting female adult trees are morphologically similar, therefore female trees were only recognized when fruit was present in the canopy. The rest were classified as males. Since not all females produce fruit each year this method of classification could result in an underestimate of the number of females. Therefore trees with endocarps beneath the canopy were additionally classified as females in a separate analysis. The number of fruit a) in the canopy and b) on the ground (if present) were counted per fruiting tree. Sampling was done early in the fruiting season, just before fruit drop, in January 2009. Where possible, twenty fruit were collected from directly beneath the canopy of each fruiting tree. The fleshy pericarp (pulp and skin) of the fruit was removed and the endocarps were dried in a convection oven for one week at 40°C. The dried endocarps were sanded down to expose all the opercula (usually >1 per endocarp). Each operculum on an endocarp represented a potential seed. The number of seeds per endocarp was established by counting all opercula. In July 2009 (6 months after harvest), 20 endocarps per site were tested for viability. All seeds from each endocarp were removed and the percentage viable (dormant), dead and aborted seeds were recorded. Viability was established by placing the seeds in a 1% tetrazolium salt solution (2, 3, 5-triphenyl-tetrazolium chloride) in the dark for 24 hours (Moore 1985; Wilson and Witkowski 2003). Only seeds that changed to medium or dark pink were considered viable. The remaining endocarps (100 per site, except 80 for WRF) were germinated in seedling trays by watering to saturation every 2 to 3 days. The seedling trays were kept at 30°C for 14 hours of light and 19°C for 10 hours of dark and at 70% humidity in a growth chamber. Newly germinated seeds were counted every 2 to 3 days. After 3 months (9 months after harvest), a sample of the endocarps (10-15 per site) were removed from the trays and the percentage germination, dead, aborted and viable dormant seeds were assessed as above. The process was repeated after 6 months (15-20 endocarps per site) (12 months after harvest). After 9 months (15 months after harvest), all remaining endocarps were removed and assessed as described above.

The fruiting trees sampled in 2009 were resampled in 2010 at all sites except JPNR (due to logistical reasons). Since the number of trees fruiting in 2009 at WRF was very low, all trees marked in January 2009, regardless of fruiting history, were reassessed. In addition, 50% of the trees at the other three other sites classified as “males” in 2009 were revisited in 2010 to ensure that they were not producing fruit in 2010. No “males” in 2009 produced fruit in 2010. Within all sites, all seedlings under the canopy of each fruiting tree, as well as from the outer edge of the canopy to a 15 m radius beyond the canopy, were counted and placed into one of two size classes: a) seedlings <0.25m in height, largely representing new individuals from the current season (directly from seed bank), and b) seedlings >0.25 to 1 m in height, representing older individuals that presumably germinated in previous years.

6.3.3. Seed bank sampling

Data were collected between April and May 2009 after all fruit had dropped from the canopy. The seed banks of ten female trees from each basal diameter size class (<30 cm, 30 – 40 cm and >40 cm) at each site were assessed. Four overlapping microsites were sampled using a total of eight 0.5 x 0.5 m quadrats per tree with some quadrats representing more than one microsite. The microsites were: a) the sub-canopy (area underneath the entire canopy); b) the inner canopy (trunk to half radius); c) the outer canopy (half radius to canopy edge); and d) the outside canopy (area beyond the canopy edge). Four quadrats were sampled in a North-South direction and two quadrats were sampled in an East-West direction underneath the canopy. Two quadrats were randomly sampled at least 10 m from the canopy, and 5 m or more from each other (Figure 2).

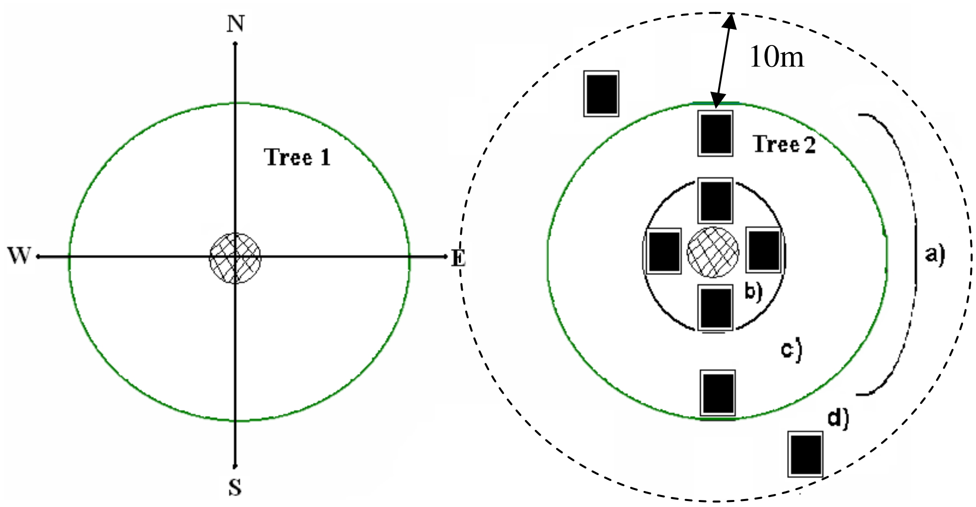


Figure 2: A birds' eye view of the canopy of two trees with the trunk inserted (shaded centre circle); showing bearing (Tree 1), as well as, the position of the eight quadrats (Tree 2) underneath and around the canopy within which the seed banks were sampled. The four microsites are also shown, a) sub-canopy (area between trunk and canopy edge), b) inner canopy (trunk to ½ radius), c) outer canopy (½ radius to canopy edge), and d) outside of the canopy to a radius of 10 m (canopy edge to dotted line). The dark squares represent the 0.5 m x 0.5 m quadrats sampled.

In each quadrat, all endocarps within the litter and the top 5 cm of the soil were collected and separated from the soil and debris using a 1 m^2 sieve (with 12 mm diameter holes). The age of each endocarp was recorded in two categories: 1) “new” endocarps that dropped from the canopy in 2009 which were still surrounded by flesh, dried skin or pulp; and 2) “old” endocarps produced in previous years which had deteriorated with no surrounding pulp. The number of seeds already removed from the locule (operculum open) per endocarp, representing predation (in “new” seeds), or both predation and germination (in “old” seeds) was recorded. Germination was assumed to only take place the following growing season and hence would not form part of the predation estimate from “new” seeds. A closed locule (intact operculum) represented a potential seed still present. Seeds still present in the collected endocarps were tested for viability in tetrazolium as described above.

6.3.4. Data analysis

Statistica v 8.0 or R v. 2.9 (R foundation for statistical computing, Vienna, Austria) were used for all analyses. Data were tested for normality prior to analysis using the Shapiro-Wilks test. Percentage values were arcsine transformed before analysis. All data were assessed for significance at $\alpha = 0.05$. A one-way ANOVA and Fisher LSD posthoc tests were used to compare variables across sites and size classes within years and microsites within sites. A repeated measures ANOVA was used to compare fruit production/tree between 2009 and 2010. A χ^2 contingency table was used to compare sex ratios between sites and percentage aborted, germinated and stored viable seeds between sites 15 months after harvest. A paired t-test was used to compare seed density between the litter and soil, as well as, between “new” and “old” in the seed bank and to compare the number of seedlings/tree under the canopy with those outside the canopy. Differences in predation and dispersal rates between sites were tested using a Kruskal-Wallis test. To compare the population structures of the five sites, the following diameter size classes were used, representing biologically relevant sizes: <0.5 cm, 0.5-2 cm, 2-5 cm, 5-9 cm, 9-14 cm, 14-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, 50-60 cm, 60-70 cm, >70cm. Diameter size class distributions (SCDs) were compared using the Kolmogorov-Smirnov two sample test. Allometric relationships between basal diameter and fruit production, as well as the relationship between fruit production and a) seasonal rainfall and b) mean maximum temperature was investigated using regression analysis.

Sex ratios and absolute minimum size of reproduction was determined in 2009 for JPNR, OHE, HE and NE and in 2010 for WRF. In addition, all trees on which fruit was found were ranked and the mean stem diameter for the smallest 5% of these trees was calculated to provide a more representative value for the minimum diameter of fruit production (Shackleton *et al.* 2003).

Fruit and seed characteristics of fruit collected in January 2009 from at least 20 trees from each of five sites in the lowveld of South Africa were assessed including fruit length, fruit mass, pulp mass, moisture content of endocarp, endocarp dry mass and seed mass.

The average percentage of endocarps with 1, 2, 3 and 4 seeds was calculated from the collected fruit for all assessed sites in 2009 and 2010. These values were used as a proxy to calculate the number of seeds produced in those trees where fruit were counted but not collected. The viability assessments of the fruits collected in 2009 were used to estimate the number of potential new viable seeds added to the seed bank that could potentially germinate in the following season.

Fruit production values for WRF from previous studies (1994, 1995, 2002 from Shackleton (2002a, *et al.* 2003), and 2000 and 2001 from Todd (2002)) were combined with the data from this study (2009 and 2010) to provide seven years of data. Annual rainfall for a particular year (growing season) was calculated by adding monthly rainfall from July of the previous year until June of that year. The CV within sites between individual trees was calculated to determine within site variability. The population CV of fruit/tree was calculated across years for WRF where data was available. The rainfall CV for the same years was matched with the population CV to assess whether rainfall variability explained fruiting variability. The CV for rainfall and fruit production was also compared across the five sites for 2009. Since the rainfall in the previous season may be more biologically meaningful in affecting the current season's fruit crop, the rainfall CV of the rainfall of the previous year was also calculated.

The percentage of seeds produced that remained viable in the short-term/transient seed bank (approximately 4 months old) was calculated. Where values exceeded 100% (due to either overestimating seed production or seeds in the seed bank) these were adjusted back to 100% (this only occurred at NE). All calculations were done on the 30 trees selected per site. The total number of seeds in the seed bank per tree was calculated using the areas for the inner and outer canopies, where the inner area was calculated using half the radius and the outer canopy area was calculated using the outer half of the radius (total-inner). Seeds/m^2 were initially calculated using the quadrat area ($0.25m^2$) and number of quadrats per microsite (4 quadrats for inner and 2 quadrats for outer) and then multiplied by the area for the inner and outer canopies.

Dispersal rates for each tree in 2009 were calculated from the number of "new" endocarps remaining under the canopy as a proportion of the total fruit produced in January 2009. If calculated values were negative due to underestimation these were adjusted back to 0%. Predation rates for each tree four months post fruit production were calculated from the percentage of potential seeds removed from the "new" endocarps still present in the seed bank in May 2009, and hence represent the percentage of undispersed seeds rather than the total seeds produced.

The ratio of "new" seedlings in 2010 to seeds produced in 2009 was calculated to relate seedling establishment to seed production. The ratio of "new" to "old" seedlings associated with each tree was calculated from the numbers of seedlings under the canopy and within 15 m of the canopy of both size classes.

6.4. Results

6.4.1. Population dynamics

Only SCD's between HE and OHE (p=0.53), HE and JPNR (p=0.10) and HE and NE (p=0.10) did not differ (Table 1). Tree density, adult and female tree density differed between sites with the highest density occurring at HE, which was double that of OHE and NE. Adult tree density at JPNR and NE was highest (Table 1 and 2).

Table 2: Summary of the reproductive population densities (trees/ha), sex ratios, minimum stem diameter for reproduction (cm) and allometric relationships between fruits/tree and basal diameter for five sites in the low altitude savannas of South Africa. Different superscript letters indicate significant differences between sites ($p<0.05$, LSD). WRF was assessed in 2010, the others in 2009.

Site	**Adults/ ha (mean±SE) ($F_{4, 22}$=16.13; $p<0.0001$)**	**Females/ ha (mean±SE) ($F_{2, 12}$=4.21; $p=0.041$)**	**Sex ratio** Females (fruit only): males **(N)**	**Sex ratio** Females (fruit and endocarps): males **(N)**	**Absolute minimum fruiting diameter (cm) (N)**	**Lower 5% mean minimum fruiting diameter (cm)**	**Allometric relationships between fruit/tree (y) and basal diameter (x) (N)**	**F**	**df**	**p**	**r^2**
N'washitsumbe enclosure (NE)	14.4±0^{ab}	2.02±0	0.27 (206)	0.32 (206)	14.5 (27)	14.5	Not significant	-	-	0.137	-
Hlangwine enclosure (HE)	9.87±2.63^{b}	4.29±1.41^{ab}	0.75 (93)	0.78 (93)	7.1 (39)	12.7	y=13.41x-245.93	21.08;	1,36	<0.0001	0.37
Outside Hlangwine enclosure (OHE)	7.84±1.44^{b}	1.77±0.95^{b}	0.43 (50)	0.43 (50)	15.7 (37)	16.4	y=10.89x-201.44	15.44;	1,35	0.0004	0.31
Wits Rural Facility (WRF)	3.69±0.73^{b}	1.75±0	0.98 (79)	0.98 (79)	9.5 (70)	11.4	y=52.62x-802.07	23.17	1,68	<0.0001	0.25
Jejane Private Nature Reserve (JPNR)	23.51±2.73^{a}	8.65±1.60^{a}	0.59 (262)	1.00 (262)	19.1 (96)	23.6	y=2.59x-37.447	14.28	1,92	0.0002	0.13
All sites combined	11.79±1.82	5.61±1.10	0.52 (690)	0.73 (690)	7.1 (269)	14.1	y=38.43x-1014.3	70.45	1,281	<0.0001	0.20

6.4.2. Sex ratio and minimum size of reproduction

There were a greater number of males than females at all sites (Table 2) and site affected the sex ratio (χ^2_4=27.75, p<0.0001). Particularly noticeable was the low ratio of females to males in the NE (0.27) and OHE (0.43) and the almost equal ratio at WRF in 2010 (0.98) (Table 2). Including trees with endocarps below the canopy as females, the sex ratio at JPNR was at unity, while the other sites did not change much.

The overall lowest minimum fruiting diameter was 7.1 cm at HE, yet outside the enclosure it was 15.7 cm (OHE, Table 2). Because JPNR had a very low proportion of small trees, its minimum fruiting diameter of 19.1 cm represents an overestimate. The 5% lowest minimum fruiting diameter overall was 14.1 cm (Table 2).

6.4.3. Fruit and seed production

Fruit production/tree in 2009 and 2010 differed between sites (Table 3). One tree, next to a waterhole in NE in 2010, produced 6 398 fruit in 2010 but was excluded from analysis as an obvious outlier. Between-individual variability was highest at WRF in 2009 and HE in 2010 (Table 3).

Fruit production/tree was positively but weakly related to stem basal diameter at all sites (although not significant (p=0.14) at NE) (Table 2). The low r^2 values indicate that a large proportion of the variation in fruits/tree was not explained by stem diameter. Fitting alternative curves did not improve the variation explained. WRF had the steepest slope and JPNR the shallowest. There was also a weak positive relationship between canopy area and fruit production in 2009 ($F_{1,211}$=33.15, p<0.0001, r^2=0.13).

The majority of marula endocarps (>50%) contained two seeds in both 2009 and 2010 (Table 4); but seeds/endocarp varied between sites. Few endocarps had one or four seeds, while almost 50% of endocarps from HE and OHE had three seeds (Table 4). Similar results were obtained for 2010 (Table 4), except that fewer trees at OHE had endocarps with three seeds.

Table 3: Marula fruit and seed production per tree (mean±SE) across all size classes and sites in the low altitude savannas of South Africa during the 2009 and 2010 fruiting seasons. Annual rainfall is from July to June. Different superscript letters indicate significant differences between sites ($p<0.05$, LSD), while different subscript letters indicate significant differences between size classes within a site. WRF 2010 includes females from additional transects. N=number of trees in fruit. Fruit/tree excludes females with zero fruit. * JPNR not assessed in 2010.

Site		**Annual rainfall (mm) 2008,2009, 2010**	**2009**			**2010**		
			N	**Fruit/tree (CV)**	**Seeds/tree**	**N (% 2009 females fruiting)**	**Fruit/tree (CV)**	**Seeds/tree**
N'washitsumbe enclosure (NE)		637,322, 481	44	$66.5 \pm 17.8^{b}(178)$	150.0±40.2	9 (20)	$11.1 \pm 5.9^{b}(160)$	24.0±12.8
	<30 cm		13	$39.2 \pm 15.9^{a}_{a}$	88.5±36.0	3 (23)	$18.7 \pm 17.7^{b}_{a}$	40.3±38.1
	30-40 cm		10	$36.9 \pm 47.2^{a}_{a}$	83.2±33.7	0 (0)	-	-
	>40 cm		21	$97.5 \pm 34.6^{b}_{a}$	220.0±77.9	6 (29)	$7.3 \pm 3.7^{b}_{a}$	15.8±7.9
Hlangwine enclosure (HE)		530,957, 668	39	$204.2 \pm 46.9^{a}(137)$	497.5±114.4	37 (95)	$36.3 \pm 13.2^{b}(221)$	88.4±32.1
	<30 cm		18	$59.0 \pm 12.2^{a}_{b}$	143.6±29.8	17 (94)	$28.9 \pm 11.8^{b}_{a}$	70.4±28.9
	30-40 cm		10	$153.0 \pm 41.4^{a}_{b}$	372.8±100.9	9 (90)	$14.2 \pm 5.3^{b}_{a}$	34.7±12.9
	>40 cm		11	$508.0 \pm 117.7^{a}_{a}$	1237.7±286.7	11 (100)	$65.7 \pm 40.1^{b}_{a}$	160.1±97.6
Outside Hlangwine enclosure (OHE)		530,957, 668	37	$188.5 \pm 35.8^{a}(115)$	457.8±86.9	33 (89)	$96.3 \pm 19.9^{b}(118)$	233.9±48.2
	<30 cm		12	$100.5 \pm 35.6^{a}_{b}$	244.1±88.8	10 (83)	$29.7 \pm 9.1^{b}_{a}$	72.1±22.2
	30-40 cm		15	$118.4 \pm 29.7^{a}_{b}$	287.5±72.1	13 (87)	$63.7 \pm 11.4^{b}_{a}$	154.7±27.7
	>40 cm		10	$399.3 \pm 90.2^{a}_{a}$	969.7±219.0	10 (100)	$205.3 \pm 48.4^{b}_{a}$	498.6±117.6
Wits Rural Facility (WRF)		570,987, 750	16	$84.9 \pm 47.8^{b}(225)$	183.1±103.0	70 (100)	$1502.1 \pm 264.1^{a}(147)$	3238.8±569.5
	<30 cm		3	$14.3 \pm 6.3^{a}_{a}$	30.9±13.7	22 (100)	$311.3 \pm 82.6^{a}_{c}$	671.3±178.2
	30-40 cm		1	4.0_{a}	8.6	8 (100)	$663.6 \pm 223.4^{a}_{b}$	1430.9±481.6
	>40 cm		12	$109.3 \pm 62.7^{b}_{a}$	235.8±135.1	40 (100)	$2324.7 \pm 413.8^{a}_{a}$	5102.6±892.2
Jejane Private Nature Reserve (JPNR)*		437,116, 520	94	$53.7 \pm 5.3^{b}(97)$	114.2±11.4	-	-	-
	<30 cm		23	$36.8 \pm 6.9^{a}_{a}$	178.7±42.9	-	-	-
	30-40 cm		55	$51.9 \pm 6.0^{b}_{a}$	110.4±12.8	-	-	-
	>40 cm		16	$84.0 \pm 20.2^{b}_{a}$	178.7±42.9	-	-	-
Overall			230	106.5±11.7(167)	248.3±28.2	149 (76)	736.7±137.2 (227)	1596.8±295.6
	<30 cm		69	53.1±8.4	124.0±20.1	52 (75)	147.9±39.9	323.2±85.9
	30-40 cm		91	71.8±8.6	164.3±20.7	30 (69)	208.8±76.5	459.0±164.4
	>40 cm		70	204.1±33.3	480.3±80.2	67 (82)	1430.0±280.2	3094.7±603.4

Table 4: Comparison of the seeds/endocarp between the five study sites from a sample of N_{fruit} from N_{trees} during the 2009 and 2010 fruiting seasons in the low altitude savannas of South Africa. JPNR was not assessed in 2010.

Year and site	**N (trees; fruit)**	**Endocarps with 1 seed (%)**	**Endocarps with 2 seeds (%)**	**Endocarps with 3 seeds (%)**	**Endocarps with 4 seeds (%)**	**Mean seeds/endocarp**
2009						
N'washitsumbe enclosure (NE)	15; 175	1	72	27	0	2.3
Hlangwine enclosure (HE)	15; 161	3	51	46	0	2.4
Outside Hlangwine enclosure (OHE)	16; 196	3	51	45	1	2.5
Wits Rural Facility (WRF)	5; 65	4	76	20	0	2.1
Jejane Private Nature Reserve (JPNR)	30; 332	7	74	19	0	2.1
2010						
N'watshitshumbe enclosure (NE)	1; 20	0	75	25	0	2.3
Hlangwine enclosure (HE)	2; 4	0	50	50	0	2.5
Outside Hlangwine enclosure (OHE)	14; 87	4	68	28	0	2.2
Wits Rural Facility (WRF)	26; 144	3	70	26	1	2.3

Initial percentage seed viability (60.3%) was higher than the percentage of seeds that actually germinated (32.4%) (Figure 3a). Up to 25.5% (WRF) of opercula investigated contained aborted seeds, but no significant differences between sites were found (χ^2_4=3.6, p=0.46). Most viable seeds germinated within two months of planting (Figure 3b), leaving <2% of the 15 month old seeds viable but dormant (range 0 (WRF and NE) to 4.5% (OHE)) (Figure 3c). There was also no difference in stored viable seeds between sites (χ^2_4=8.71, p=0.069), but there was a significant association between site and percentage germination (χ^2_4=23.03, p=0.0001). Only 16.2% of the NE seeds germinated, compared to 44.4% at JPNR. The low number of NE germinants could be due to collection of immature fruits.

Most trees produced less fruit in 2010 than in 2009 ($p<0.05$), except at WRF, where more fruit was produced in 2010 ($p<0.001$) (Table 3). Site ($F_{3,184}$=13.19, $p<0.0001$), time ($F_{1,184}$=11.20, p=0.0009) and the interaction between site and time ($F_{3,184}$=20.20, $p<0.0001$) had significant effects on fruit production.

Orders of magnitude fluctuations in fruit production were observed over the seven years analysed (1994-2010) at WRF (Figure 4). There was higher variability in fruit/tree across years (CV=97%) than in rainfall (CV=38%). The rainfall in the previous season was even less variable (CV=31%). Rainfall variability across sites of the previous season (CV=13%) was lower than the variability in the current season (CV=62%) and the fruit/tree variability across sites (CV=59%) for 2009. No significant relationships between fruit production and a) previous season rainfall ($F_{1,5}$=0.02; p=0.90) (Figure 4); b) average maximum temperature ($F_{1,3}$ =0.78; p=0.44), or c) the combination of previous season rainfall and current season average maximum temperature (multiple regression $F_{1,3}$=0.27, p=0.79) were found.

6.4.4. Seed bank dynamics

Endocarp density (soil and litter) under the canopy differed across sites (Figure 5). Endocarp density also differed between the microsites. Endocarps were concentrated near the base of the stem and very few were found outside the canopy of fruiting trees (Figure 5).

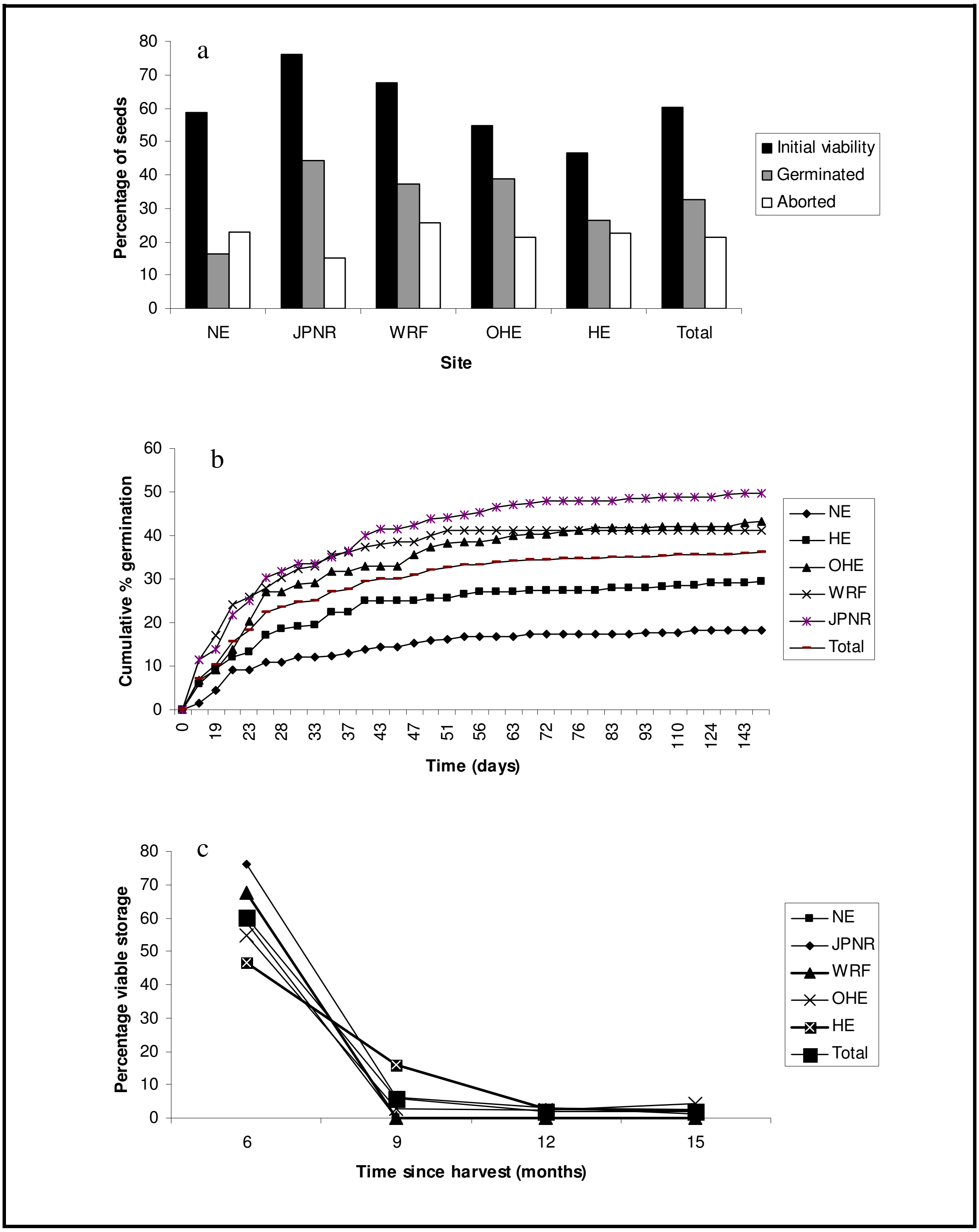


Figure 3: a) Seed abortion, initial percentage viability and germinability from five sites and in total (N=1058) after 15 months in the soil, b) Cumulative percentage germination over time and c) Percentage of viable seeds stored over time. Seeds were collected in January 2009 in the low altitude savannas of South Africa and germinated in July 2009 after 6 months storage in the laboratory.

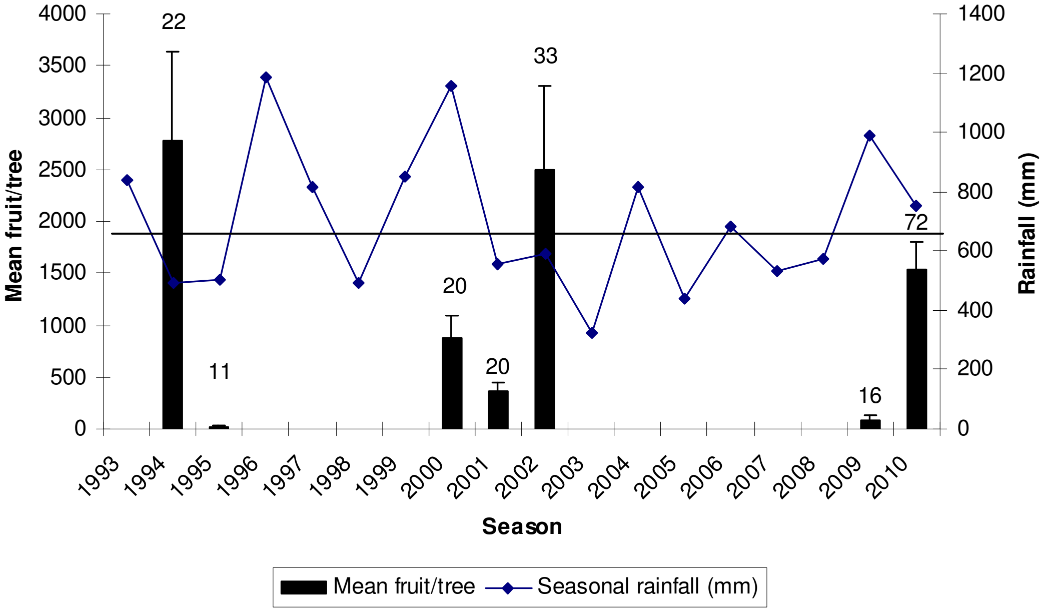


Figure 4: Annual variation in fruit/tree in relation to rainfall at Wits Rural Facility (WRF) in the low altitude savannas of South Africa. The horizontal line indicates the long-term mean seasonal (July to June) rainfall. Numbers above the bars represent the number of trees assessed.

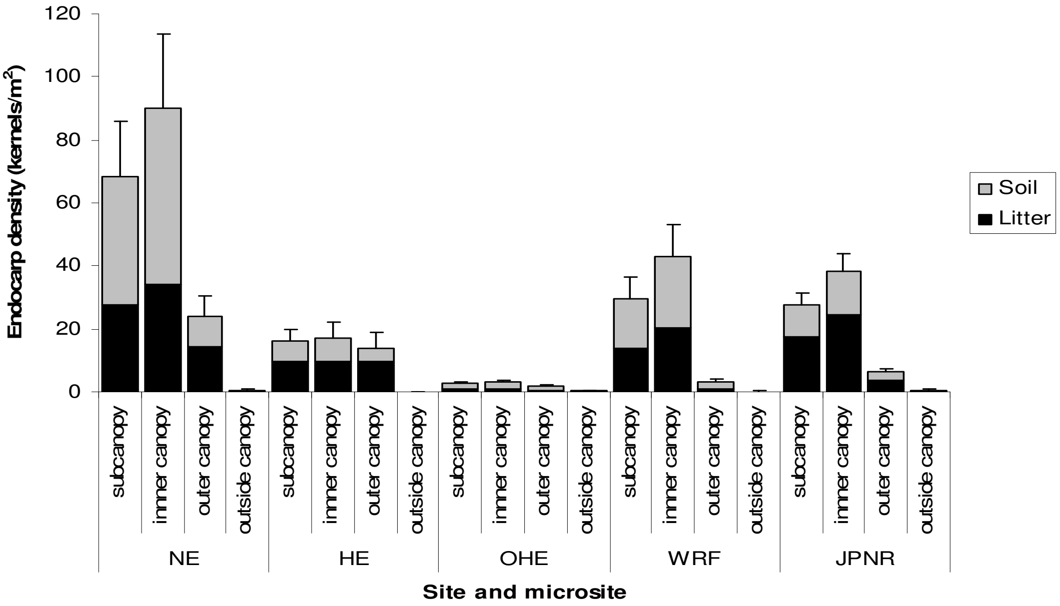


Figure 5: A comparison between endocarp density (endocarps/m^2; mean±SE) within the seed bank (litter and soil) between four microsites for five sites in the low altitude savannas of South Africa.

In OHE and JPNR, <4% of the initial seeds produced in January 2009 remained viable in the sub-canopy seed bank when examined in May 2009 (Table 5). Five trees in HE had more seeds in the seed bank than were produced, indicating possibly either an underestimate of fruit production in January 2009 or an overestimate of seed bank density in May 2009. An alternative explanation could be that rodents or other animals were caching the endocarps under the big trees where seed banks were being sampled, and hence partially uncoupling the seed bank from the current seed production.

There were more "new" than "old" seeds in the litter in NE (p=0.0244), WRF (p=0.0412) and HE (p=0.0026); but not JPNR and OHE (Table 5). However, while all sites had old seeds in the soil, neither WRF nor OHE had old seeds in the litter. There was a significant difference in seed bank density (viable seeds/m^2) between sites ($F_{4,145}$=3.18, p=0.016), specifically between NE and JPNR, and WRF and OHE ($p<0.05$). Both enclosures (NE and HE) had the highest viable seed bank density whilst OHE had the lowest (Table 5). The density of viable "new" seeds differed significantly between sites ($F_{4,145}$=2.61; p=0.038). NE and HE showed no difference (p=0.29), while NE was higher than JPNR (p=0.01), WRF (p=0.02) and OHE (p=0.01). No "new" viable seeds were found in the outside canopy microsite for any site. On average, only 23% of the trees assessed had a transient seed bank, with the enclosures (NE and HE) having the highest (40%; Table 5).

Although the numbers are low, all populations of marula had viable "old" seeds in the sub-canopy seed bank, indicating that marula did have a persistent seed bank (Table 5). There was, however, no significant difference in the density of viable old seeds between the sites ($F_{4,145}$=1.68; p=0.16). Only trees in JPNR had viable "old" seeds in the outside canopy microsite, indicating short distance dispersal. Only 9% of the assessed trees had a persistent seed bank, with trees in JPNR and NE having the highest (17%).

Table 5: Numbers of viable seeds per tree (mean and range) in the transient ("new" seeds produced in January 2009) and persistent ("old" seeds produced in January 2008 or earlier) seed banks in May 2009, between five sites in the low altitude savannas of South Africa. N=30 trees per site. Different superscripts letters indicate significant differences between sites ($p<0.05$, LSD) and * indicates significant differences between new and old seeds within sites.

Site	**Seeds/tree January 2009 (range)**	**"New" seeds/tree in the sub-canopy litter in May 2009 (range) N= number of trees with transient seed bank**	**% of seeds produced in Jan 2009 that remained viable in the sub-canopy seed bank by May 2009**	**"Old" seeds/ tree in the sub-canopy soil and litter in May 2009 (range) N= number of trees with persistent seed bank**
N'washitsumbe enclosure (NE)	194 (6-1198)	145.8 (0-1980) ^{a*} N=12	27.35	10.3 (0-208)^{a*} N=5
Hlangwine enclosure (HE)	511 (17-2541)	94.5 (0-1590) ^{a*} N=12	11.5	2.8 (0-158) ^{a*} N=1
Outside Hlangwine enclosure (OHE)	462 (6- 2244)	1.2 (0-18) ^{b} N=3	0.4	1.2 (0-40) ^{a} N=2
Wits Rural Facility (WRF)	92 (0-1616)	20.2 (0-493) ^{b*} N=5	18.7	1.2 (0-37) ^{a*} N=1
Jejane Private Nature Reserve (JPNR)	141 (13-572)	3.1 (0-85) ^{b} N=2	3.5	7.8 (0-166) ^{a} N=5
Overall **N=150 trees**	280 (0-2541)	53 (0-1980) N=34	11.2	6 (0-208) N=14

Dispersal and predation rates varied between sites, and JPNR had the highest predation rate, probably due to a high number of rodents and OHE the highest dispersal rates most probably due to elephants (Table 6). There was a significant difference in dispersal rates between sites ($H_{4,150}$=29.96, p<0.0001), with WRF having a significantly lower rate than all other sites (p<0.02), except NE (Table 6). There was also a significant difference in predation rates between sites ($H_{4,65}$=18.02, p=0.0012), with WRF showing a significantly higher rate than all other sites, except JPNR (Table 6).

Table 6: Dispersal and seed predation rates (after 4 months) (mean (range)) for the five sites assessed in the low altitude savannas of South Africa from seed release starting in January until May 2009. N=30 trees per site for dispersal rate calculations. N= number of trees that had endocarps within the seed bank from which to calculate predation rates. Different superscript letters indicate significant differences (p<0.05; LSD).

	Fruit dispersed away from tree (%) (range)	**N**	**Seeds predated (%) (range)**
N'washitsumbe enclosure (NE)	49 (0-100)^{ab}	18	30 (0-100)^{b}
Hlangwine enclosure (HE)	68 (0-100)^{a}	14	19 (0-66)^{b}
Outside Hlangwine enclosure (OHE)	92 (55-100)^{a}	13	28 (0-100)^{b}
Wits Rural Facility (WRF)	27 (0-100)^{b}	11	74 (0-100)^{a}
Jejane Private Nature (Reserve (JPNR)	80 (0-100)^{a}	9	70 (0-100)^{a}
Overall	63 (0-100)	65	40 (0-100)

6.4.5. Throughput of seeds into seedlings

The number of newly germinated seedlings associated with each fruit-producing tree varied between sites ($F_{3,187}$=3.52, p=0.016), as did the number of older ($F_{3,187}$=9.86, p<0.0001), and total seedlings/tree ($F_{3,187}$=5.24, p=0.017). Similarly the number of seedlings/tree both under ($F_{3,187}$=3.10, p=0.028) and outside ($F_{3,187}$=5.01, p=0.0023) the canopy differed significantly between sites. NE consistently had the least number of seedlings (Table 7).

Table 7: The number of seedlings per tree (under the canopy and within a 15 m radius of the canopy combined) (mean±SE) for four sites in the low altitude savannas of South Africa in January 2010. Different letter superscripts indicate significant differences between sites.

Site	**N (trees assessed)**	**Seeds/tree (January 2009)**	**<1 year old seedlings/ tree (January 2010)**	**% of seeds produced in 2009 that become seedlings in 2010**	**>1 year old seedlings/ tree (January 2010)**	**Ratio of new to old seedlings**	**Seedlings/tree under canopy**	**Seedlings/tree outside canopy**	**Total seedlings/tree**
N'washitsumbe enclosure (NE)	43	149.8±39.7	0.4±0.2^{b}	1.1	0.3±0.07^{b}	1.4	0.4±0.3^{b}	0.3±0.1^{b}	0.7±0.2^{b}
Hlangwine enclosure (HE)	39	413.1±96.7	3.5±1.1^{a}	5.3	2.1±0.4^{a}	1.7	3.3±1.3^{a}	2.2±0.4^{a}	5.6±1.4^{a}
Outside Hlangwine enclosure (OHE)	35	399.7±79.1	2.0±0.7^{ab}	0.7	1.0±0.3^{b}	2.1	1.3±0.5^{ab}	1.7±0.4^{a}	3.0±0.8^{ab}
Wits Rural Facility (WRF)	74	31.9±22.1	2.9±0.6^{a}	13.7	0.8±0.2^{b}	3.8	2.2±0.5^{a}	1.5±0.3^{a}	3.7±0.7^{a}
Overall	191	299.2±40.9	2.3±0.4	3.5	1.0±0.1	2.4	1.9±0.3	1.4±0.2	3.3±0.4

Overall, 3.5% of the seeds/tree in 2009 germinated and established as new seedlings associated with the parent plant in 2010 (Table 7). Female marula trees were associated with an average of three seedlings. The number of seedlings under the canopy compared to those outside the canopy tended to be higher for NE, HE and WRF and lower for OHE, but this was not significantly different ($p>0.1$).

6.4.6. Landscape analysis

At the landscape scale, there were 2.4 times more seeds/ha in the HE compared to the OHE in 2009. In addition, HE produced 6.6 times more seeds than NE in 2009 (Table 8). At WRF there was an 18 fold increase in seed production between 2009 and 2010 (Table 8). HE had the highest density of viable seeds available for germination in association with the parent plant, while OHE had almost none. The fate of all the seeds dispersed/predated away from the plant remains unknown.

6.5. Discussion

6.5.1. Sex ratio

Our study found a male-biased sex ratio for marula at all sites, except WRF where the ratio was 0.91 and at JPNR if trees with endocarps below the canopy were classified as females. Todd (2002) observed a similar ratio of 0.84 at WRF in 2000. WRF has no elephants, few browsers, infrequent fire, moderate rainfall and well-drained granitic sandy soils (Shackleton 1999), and therefore should represent a relatively undisturbed savanna. Such a savanna should support marula populations representative of the undisturbed state i.e. secondary sex ratio of unity. Any deviations from such a sex ratio, could therefore be attributed to ecological drivers, assuming there is no genetic predisposition for biased primary sex ratios (Opler and Bawa 1978; de Jong and van der Meijden 2004). Both HE and OHE had male-skewed sex ratios, but the skewness was two-fold greater outside, relative to inside the 38 year old enclosure, showing the long-term differential effects of browsers and elephants on the sexes anticipated by Hemborg and Bond (2006). The extremely male-skewed sex ratio at NE is, however, an anomaly. Male-skewed sex ratios indicate that pollen availability should be high, but since marula is thought to be insect pollinated (Hall *et al.* 2002), pollinator limitation may have resulted in the observed sex ratios. Pollination success affects the number of seeds per endocarp (Leakey *et al.* 2005) and the high proportion of endocarps with only two seeds at NE in both 2009 and 2010, indicates that pollination success was low. A greater diversity and number of pollinators would be expected in areas with high plant species diversity (Kearns and Inouye 1997).

NE falls within a homogenous mopane shrubveld landscape with low plant diversity (Gertenbach 1983, Jacobs and Biggs 2002), and since mopane is thought to be wind pollinated (Jordaan *et al*. 2002), a lack of suitable pollinators may have resulted in the observed sex ratio at NE. Following this logic, pollination success is probably low at NE and hence many females could have been classified as males at this site due to lack of fruit production, underestimating the number of females. This highlights the pitfalls of using the presence or absence of fruit to determine sex ratio at sites where pollination success influences fruit set.

Table 8: Landscape level analysis of the reproductive output and seed banks of marula at five sites in the low altitude savannas of South Africa.

	Fruit produced/ha		**Seeds produced/ha**		**Seed banks: viable seeds/ha**		
	2009	**2010**	**2009**	**2010**	**Transient (‘new”)**	**Persistent (“old”)**	**Total (“new”+ “old”)**
N’washitsumbe enclosure (NE)	134	10	303	23	216	21	237
Hlangwine enclosure (HE)	817	139	1990	337	323	11	334
Outside Hlangwine enclosure (OHE)	332	170	806	412	2	2	4
Wits Rural Facility (WRF)	128	2316	277	4993	26	2	28
Jejane Private Nature Reserve (JPNR)	443	-	943	-	14	64	78
Overall	371	659	864	1441	116	20	136

6.5.2. Minimum size of reproduction

Our study estimated that marula could reach reproductive maturity from as small as 7.1 cm stem diameter, but most plants became mature between 11.4 and 16.4 cm. This is comparable with the findings of Shackleton *et al*. (2003) (13.6 cm). OHE, the only site with elephants present, had the highest minimum diameter for fruiting (16.4 cm). In contrast, HE, a low disturbance area, had one of the lowest minimum fruiting diameters (12.7 cm). Less damage from disturbance agents, such as elephants, browsers and fire, allows for greater investment of available resources into growth and hence earlier reproduction (Clark 1991). When comparing between the two enclosures (HE and NE), where disturbance levels are similar, the site with the lower rainfall (NE), had the higher minimum diameter of fruiting (14.5 cm). However, this could also have been influenced by the different soil types, namely nutrient-rich basalt at NE and nutrient-poor granite at HE. WRF has a 9% lower mean annual rainfall than HE, but has had no fire in the last five years. HE, in contrast, has had more recent and more frequent fires. The slightly lower minimum diameter of fruiting at WRF (11.4 cm) compared with HE (12.7 cm), suggests that the lower fire frequency and intensity allowed for a smaller size at first reproduction. However, this may be over interpreting the differences as statistical analyses could not be performed. Marula reaches reproductive maturity at relatively larger stem diameters than other common South African savanna species. For example, *Burkea africana* (Fabaceae) reached reproductive maturity between 6.3 and 12.7 cm (Wilson and Witkowski 2003) and *Pterocarpus angolensis* (Fabaceae), produced fruit from as small as 8.0 cm (Shackleton 2002b). At similar localities, Shackleton *et al*. (2005) reported minimum fruiting diameters of 6.4 cm for *Combretum collinum* subsp. *suluens* and *Diospyros mespiliformis.* Reproduction in most perennial plants must be delayed until the plant is sufficiently fire-resistant (Crawley 2003), and resistant to other natural disturbances (Silverton 1991; Wilson and Witkowski 2003). Size at reproductive maturity has been linked to the stem diameter (specifically bark thickness) at which the tree becomes resistant to fire in *B. africana* (Wilson and Witkowski 2003). Marula has been shown to become completely resistant to fire from only 7 cm in stem diameter (Helm *et al.* in press), which is similar to the absolute minimum diameter of reproduction, indicating a link between size of reproduction and fire resistance.

6.5.3. Fruit and seed production

In our study, the maximum fruit crop observed was 46.8 kg/tree (2 324 fruit) at WRF in 2010, with significant variability between sites and years. At sites near WRF, Shackleton (2002a) estimated a mean marula fruit crop of 36.8 kg/tree (1 786 fruit) with a maximum of 416.6 kg/tree (20 223 fruit) in communal areas, while other studies have reported fruit crops of up to 1 ton/tree (50 000 fruit) (Holtzhausen *et al.* 1990). These studies indicate that marula has a much greater potential for fruit production than what our study would suggest.

Shackleton (2002a) also noted considerable inter-annual differences in fruit production within and between sites for savanna tree species in South Africa. Our study has shown that variability in fruit production across years at one site was greater than the variability across sites in one year, indicating that drivers such as weather, periodic insect outbreaks, and rodent predator numbers play a greater role than fire, mammalian herbivory, soil types or long-term rainfall in marula fruit production. However, no relationship between annual fruit production and rainfall or temperature could be discerned. This is a commonly reported phenomenon (Herrera *et al.* 1998; Yasumura *et al.* 2006). For example, neither yearly rainfall and temperature patterns, nor the previous year's reproductive history were able to explain annual variation in fruit production in 22 bird-dispersed species in Costa Rica (Wheelwright 1986). Instead, annual variation in fruit production could be explained using the resource trade-off hypothesis, where the tree will trade reproductive output with vegetative growth over successive years depending on resource availability (Kozlowski 1992; Koenig and Knopps 1998). This will be particularly important in resource limited habitats (Biere 1995), such as the low nutrient granite soils at four of the sites studied here. In addition, females of dioecious species, such as marula, are expected to show greater trade-offs than males, because of higher investments made to reproduction (Crawley 2003). Trees invest a substantial amount of carbohydrates, nitrogen and phosphorus into reproductive growth (Witkowski and Lamont 1996). Since marula fruit are large and energetically costly to produce, and many fruit are produced per tree in high yield years, the resource trade-off hypothesis could explain the episodic nature of fruit production observed. However, some studies have indicated that there is no difference in the growth of trees between masting and non-masting years (Yasumura *et al.* 2006). Instead the resources used for mast fruiting were hypothesised to come from resources stored in perennial tissues. Hence, long-term monitoring of marula growth rates in relation to rainfall and fruiting behaviour, are a priority to determine whether or not marula trades growth for reproduction.

6.5.4. Seed fate

Primary dispersal occurs when the fruit (diaspore) is dropped directly from the canopy to the ground. Once the fruit has reached the ground, the fruit and seeds face several fates including, secondary dispersal, predation, germination or death. Dispersal away from the tree was highest at OHE (92%) followed closely by JPNR (80%), suggesting that elephants and other browsers are important dispersers of fruit. The high level of dispersal seen at HE (68%) could only be attributed to rodents and some other species present in low numbers, such as white rhino (*Ceratherium simum*), reedbuck (*Redunca* spp.) and warthog (*Phacochoerus aethiopicus*). Rodents can, also be good dispersal agents (vander Wall *et al.* 2005), albeit of relatively short distances, 20m on average (Takahashi *et al.* 2007), and their dispersal contributions should not be overlooked. The levels of dispersal, observed at the study sites (49 to 92%), indicate that the recruitment dynamics of marula are strongly influenced by the movement of seeds away from parent plants. Up to 25% of marula seedlings surveyed in communal lands and private reserves in a low-altitude, South African savanna, occurred beneath reproductively mature parent plants (Neke 2005). These findings indicate that the reproductive contribution of secondary dispersed seeds to the seedling bank is three times that of primary dispersal. In our study, however, slightly more seedlings were found under tree canopies than in inter-canopy spaces for most sites, except for OHE where elephants were present.

Although marula attracts mammals such as elephants and frugivorous birds, and hence is adapted to long distance secondary dispersal, marula seeds still tend to show some level of dormancy when dispersed from the canopy. Dormancy is usually associated with plants with little adaptation to long distance dispersal (Crawley 2003). Hence, the quiescent period, often observed by germination studies of marula seeds collected below the canopy of fruiting trees, probably does not occur in fruit that has passed through the digestive tract of an animal. Thus, elephants and other mammals, already important dispersers of marula seeds to new localities, also potentially increase the rate of germination through acid digestion, which has indeed been shown by Lewis (1987). This allows seeds to germinate in the same growing season in which the fruit was produced. It remains to be investigated whether the seedlings of such a germination event, are also able to establish within the same growing season, and hence survive the dry season, through to the subsequent growing season.

In our study, predation rates ranged from 19 to 74% within 4 months of primary dispersal. Preliminary cafeteria experiments have indicated that rodents accounted for most of the predation observed (pers. obs.). The high levels of seed predation at JPNR (70%) and WRF (74%) suggest high rodent numbers, which could be due to low frequency of fire at these sites. The lack of fire increases the herbaceous biomass, and hence cover for rodents (Anderson 1986). Some studies on seed predation indicate that 92% of seeds can be predated within 3.5 months of seed fall (Wassie *et al.* 2009), while studies on *Acacia* seeds have shown levels of up to 90% (Miller 1994). In Japan, 77% of experimentally labelled acorns were dispersed by rodents (Takahashi *et al.* 2007), whereby 97.4% of the acorns had been preyed upon post-dispersal. The proportion of seeds lost through pre-dispersal predation also varies from year to year (Chidumayo 1997). The high levels of predation of marula seeds in some sites (90% in JPNR; 80% at WRF) represent a severe bottleneck and the likelihood of seed limitation. The impact of seed limitation on the population dynamics of marula would ideally be tested within a modelling framework.

It has been reported that up to 92% of marula fruit can be harvested, without impacting the current population profile (Emanuel *et al.* 2005). If this value is similar to seed removal and all seeds modelled were viable, then the removal rates at JPNR and OHE exceed this threshold. However, a large proportion of this removal could be active (secondary) dispersal of the seeds away from the parent tree and the percentage of dispersed seeds that end up viable (even if transient) in the soil seed bank is unknown. Post-dispersal predation therefore requires further investigation at such sites.

Marula seeds collected at Klaserie Private Nature Reserve were shown by this study to still be viable after ten years in storage (Chapter 5), which is much longer than previous reports of only up to a year (von Teichman *et al*. 1986).

6.5.5. Seed bank dynamics

Marula has a viable transient seed bank associated with the parent tree of 136 seeds/ha but a persistent seed bank density of only 20 seeds/ha. These values are far overshadowed by other South African savanna tree species: for example, at Nylsvley, both fine-leaved species, such as *Acacia nilotica* (3675 seeds/ha in transient and 9667 seeds/ha in persistent), *A. tortilis* (859004 and 608856 seeds/ha) and *Dichrostachys cinerea* (10131 and 7942 seeds/ha) (Witkowski and Garner 2000) and broad-leaved species, such as *B. africana* (333 725 seeds/ha in persistent and transient combined) (Wilson and Witkowski 2003) had much greater seed densities. Marula thus seems largely reliant on the current season's fruit crop for recruitment. Given that marula fruit are large and the seed bank is small and relatively short-lived, it is more likely that seed limitation, rather than microsite limitation, influences its recruitment dynamics (Clark *et al.* 2007). Both JPNR and OHE had a low seed density of viable seeds (<2 seeds/m^2) in the seed bank (both litter and soil) under the canopy. However, providing the seed bank is replenished with viable seeds, regeneration of, and recruitment into, the population is still possible.

The large endocarps of marula do not enter the soil easily and this lack of burial suggests that the seed bank would be mostly transient in nature (Thompson *et al.* 1993). This is supported by our finding of a small persistent seed bank. Large endocarp size and lack of burial also have implications for seed survival in the seed bank after a surface fire (Auld and Denham 2006; Shackleton 2007). Recent studies on marula dispersal, however, indicate that a large number of endocarps are buried by rodents (Gallaher 2010; L. Kruger pers. comm.), affording these seeds protection from fire. Burial in sub-canopy microsites may also provide greater protection from fire relative to the open, because grass production and standing biomass are significantly lower in such sites due to trampling and shading (Grossman *et al.* 1980).

As significantly more seeds are stored under the canopy of fruiting marula, the felling of large fruiting trees may be particularly important for creating regeneration opportunities through the opening of gaps (Wilson and Witkowski 2003). While, seedlings survive under the canopy of large fruiting trees in enclosure environments, trampling under trees outside the enclosure by herbivores seeking shade and fruit, as well as consumption of seedlings by impala (Haig 1999), may be sufficient to prevent seedlings from establishing under the canopy of large fruiting trees in these habitats. More detailed mapping of marula seedlings in relation to adults is required to determine whether the incidence of seedlings increases away from the canopy of large fruiting trees. Genetic marker studies would also be useful in identifying the parent plants of seedlings and their distance from such parent plants (He *et al.* 2004).

6.5.6. Population dynamics

Does reproductive output and seed fate explain the different population structures and densities of marula assessed at the five sites in this study? Higher rainfall at OHE and HE contributes to higher tree density due to greater seedling establishment, which is, in turn, due to greater pollination success and smaller initial reproductive size. This is modified by elephant impact and fire, both of which increases the size for reproduction and reduces the number of seedlings associated with parent trees. The high levels of dispersal could indicate that seedlings are establishing away from the parent plant, which is usually advantageous for a resprouting species with long-lived adults (Lamont and Wiens 2003). However, high levels of adult mortality currently observed in the KNP in some favourable marula habitats (Helm *et al.* 2009), require the presence of a seedling bank to replace the lost adults. Such a seedling bank is absent from these sites (Helm *et al.* 2009). Short distance dispersal would mediate such an effect, whereas long distance dispersal could take seeds away from the favourable habitats and deposit them into unfavourable habitats such as in valleys and along watercourses. In such cases the removal of seeds could be equated to death. Under lower rainfall conditions this could result in the presence of an adult biased unstable population structure. Seedling predators such as impalas would further influence the population structure in a similar direction (Kauffman and Maron 2006).

The JPNR population structure can possibly be explained through low rainfall and hence a lack of seedling establishment. While recruitment at this site is not seed limited, it is predator limited (Crawley 2000), as suggested by high seed predation levels. Small mammal consumers do not usually feature strongly in the theory of drivers of population change. However, population dynamics of tree species can be strongly influenced by seed predators (Kauffman and Maron 2006). Hence episodic recruitment could explain the population structure at JPNR.

The NE structure may have resulted through low pollinator diversity and/or numbers causing low fruit yield and low recruitment, while the WRF population structure represents that obtained under a low disturbance regime with highly variable inter-annual fruit production.

6.6. Conclusions

Sex ratios of marula populations in protected areas appear to be male biased through female removal at some sites and pollinator limitation at others. Minimum size at reproduction is linked to size of resistance to fire, but is further mediated by herbivory and rainfall. Some level of disturbance may be required to ensure dispersal (i.e. elephants and other mammals) and fire possibly controls rodent populations, which then influences seed and seedling survival. Fruit production in marula is highly variable between individuals, both within and between sites, and between years. The variability is not directly attributable to weather patterns but could rather be related to growth and vegetative trade-offs. Dispersal and rodent seed predation rates are high and therefore influence marula population structure substantially. Germination rates are improved through mammal consumption of the fruits allowing for germination of seeds in the same growing season in which they were produced. Marula is unlikely to rely substantially on a persistent seed bank for recruitment and hence the observed variability in fruit production across years will ensure that episodic recruitment is a common phenomenon. The results of this study can be used for parameterisation of the reproductive phase of the marula lifecycle in population models.

6.7. References

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Chapter 7: Seed imbibition, germination and early seedling root growth in *Sclerocarya birrea* subsp. *caffra* (marula)

7.1. Abstract

Marula endocarps collected from a single locality were subjected to various treatments to determine the water requirements for seed imbibition and germination, environmental influences on germination and the growth rate of the roots of young seedlings. Scarified dried endocarps took up to 12 hours to completely imbibe, although 80% imbibition had already taken place after 2 hours. The endocarps took much longer to dry out (>28 hours) after imbibition, indicating that once fully imbibed the woody endocarp holds onto the water for an extended period. At least 16 ml of water every 4 days was required for 10 days, for marula seeds to germinate, indicating that 3 mm every 4 days in the field for 2 weeks would be sufficient for marula seed germination. Overall germination percentages were low both in terms of total seeds (<28%) and in terms of endocarps (<51%). Fire and smoke treatments did not improve marula seed germination percentages, indicating that marula seeds do not require fire for germination. However, in agreement with many other studies, acid digestion increased the germination percentage (over the monitoring period), by up to 50%, indicating that mammal ingestion is important for germination by greatly increasing the rate of germination. Higher temperatures also increased the germination percentage. Prior soaking of the seeds increased the germination rate but not percentage. Marula roots grew on average 28 mm per day which was shown to be much higher than the rates reported for other species. Marula is therefore probably highly adapted to summer drought as the fast root growth rate allows for quick penetration of the soil profile to the water reserves. In conclusion, marula do not appear to be hindered by water availability both in terms of seed germination and seedling survival. The low germination rates need to be considered in modelling exercises as only a small proportion of the fruit produced are potential germinants.

7.2. Introduction

Marula reproductive potential and seed fate in the field were investigated in Chapter 6 together with germination of seeds collected from five sites. However, for a complete understanding of seed fate, water requirements for seed imbibition and germination as well as the environmental influences on germination success, still need to be investigated. In addition, adaptations to water stress such as root penetration rates especially in African savannas also require investigation.

Water availability has a major influence on determining the onset of germination (Wilson and Witkowski 1998), but has only been studied in very few savanna tree species. For non-dormant seeds, germination occurs once the seed imbibes sufficient moisture from the substrate (Baskin and Baskin 2001). The water requirements for germination are an essential life history attribute determining when and how often seeds will germinate. Water requirements for germination have not been reported for marula.

Various other factors also influence seed germination success, some of which are internal, others environmental (Oliker *et al.* 1978). Such factors include temperature, pre-treatment such as scarification through acid digestion in the stomach of a mammal disperser or fire heat shock (Gardiner *et al*. 1993; Bradstock and Auld 1995; Mbalo and Witkowski 1997). Overall, it is the prevailing microclimate in the vicinity of the seed that will be the main determinant of germination (Oliker *et al*. 1978).

Marula endocarps (the dispersal unit of marula seeds) have an operculum that restricts germination until it is removed (von Teichman *et al.* 1986). Von Teichman *et al.* (1986) found that moisture uptake for marula endocarps that had been stored desiccated for 6 months was 23% and that the endocarp did not impede seed water uptake. Were and Munjuga (1998) (in Hall *et al.* 2002) found that the moisture content associated with 3 month storage of marula endocarps had no effect on germination, indicating that marula seeds may not require complete desiccation over the dry season for germination to occur.

Marula seeds do not germinate readily in the same season in which they were produced, possibly due to either physical restriction presented by the hard endocarp or chemical barriers present in the fleshy mesocarp surrounding the endocarp (von Teichman *et. al.* 1986). Germination trials to test the effect of removal of the opercula, temperature, oxygen, light exposure and age of endocarp, were conducted by von Teichman *et al.* (1986). It was found that the operculum covering the seed was the main inhibitor of germination, followed by temperature. Maximum germination success was observed at temperatures of 32°C (range tested: 21-37°C) and the response was unimodal. The endocarp age (range tested: 0.5-23 months) also affected germination percentage, whereby older endocarps had greater levels of germination than younger endocarps, indicating that the operculum is not the only factor inhibiting germination. The unfortunate part of this study was that very few of the intact endocarps (where the operculum was not mechanically removed) germinated regardless of treatment. This was probably due to the short period of observation (6 days) and this study therefore only represents immediate germination and not longer term natural germination. Since mechanical removal of the endocarp is unlikely to occur naturally, the results of this study do not provide insight into the natural germination requirements of marula. A study of intact endocarps over longer periods and under more natural germination scenarios, exposed to different treatments, may be more ecologically relevant.

Marula has a mostly transient seed bank lasting approximately six months during the dry cool season (March-October) (Chapter 6). At the onset of the rains at the beginning of the next growing season (Oct/Nov), marula seeds are likely to germinate (pers. obs.). Since the seeds are large, passive burial is unlikely and hence endocarps often lie on the soil surface where very hot temperatures can be experienced. Soil surface temperatures can exceed 60°C after fire has removed all vegetation and blackened the soil (Bradstock and Auld 1995). Hot temperatures increase the drying rate of seeds as well as the soil layer after a rainfall event. However, cafeteria experiments have shown that rodents such as squirrels, bury 46% of the supplied marula endocarps up to 1 cm below the soil surface (Gallahar 2010). This may mitigate the effect of high temperatures at the soil surface (Shackleton 2007), reducing the drying rate of the seeds and the soil surrounding the seeds. Once the seed has acquired sufficient moisture to germinate, rapid downward growth of the roots is required to track retreating water supplies. Since South African savannas are characterised by high rainfall variability and frequent summer drought, savanna tree species would be expected to have rapid root penetration to ensure survival of the seedling (Wilson and Witkowski 1998). The root depth penetration rate of marula seedlings is unknown.

Overall, we have a very poor understanding of marula seed imbibition, germination and early seedling root growth under natural conditions. Soil type is likely to affect root penetration rate with clay soils being more compacted than sandy soils and may restrict growth.

In order to understand the transitions from seed to seedling, this study was undertaken to determine: a) the imbibition characteristics of marula endocarps; b) the water and other environmental requirements for germination of intact endocarps; and c) the root penetration rate directly following germination.

7.3. Methodology

Experiments for this study were conducted in either a Phytotron growth chamber or under glasshouse conditions at the University of the Witwatersrand, Johannesburg. The Phytotron chamber was set to 14 hours daylight and 10 hours darkness. Temperatures were kept at 30 °C during the day and 19 °C at night and relative humidity of 70%, unless otherwise stated. The temperature and humidity regime in the growth chambers were set to mimic those in the southern KNP during the summer months. The environmental conditions in the glasshouse were described in Chapter 5.

The endocarps used for the experiments in the study were purchased from Lifestyle Seeds and had been collected from the Potgietersrus vicinity in January 2008 from below wild trees. The fruit pulp was removed and the endocarps were allowed to dry in the sun. Once dry, the outer mesocarp was scarified and the endocarps were stored in a cardboard box in a cool dry place until the start of the experiments.

All watering was done by hand so that the exact quantity of water dispensed was known. All other methodology in the glasshouse followed those outlined in Chapter 5 unless otherwise explicitly stated.

7.3.1. Imbibition experiment

In February 2009, ninety desiccated, scarified endocarps were weighed (to 0.01g accuracy) and then placed into marked 250 ml polystyrene cups, covered with water and placed in a Phytotron growth chamber. After 2 hours the endocarps were reweighed, and thereafter at 4, 6, 8, 12, 16, 24 and 32 hours until full imbibition had been achieved, and no further weight gain occurred. The endocarps were then placed into a convection oven at 40 °C (simulating midday summer temperatures). As the seeds dried, endocarp mass was determined after 2, 5 and 28 hours.

7.3.2. Water requirements for germination and initial seedling survival

A total of 100 endocarps were planted in 250 ml polystyrene cups filled with nutrient-poor granite derived soil collected at Wits Rural Facility (WRF) in 2007 and a further 100 in nutrient-rich basalt derived soil collected at N'washitsumbe enclosure (NE) near Shingwedzi KNP in 2007. Soil type characteristics have been described in Chapter 5. The cups were watered with 0, 2, 8 or 16 ml of water every 2, 4 or 7 days (10 cups in each treatment) and kept in a Phytotron growth chamber. Germination was scored by monitoring seedling emergence above the soil surface every three days for 30 days. Emerged seedlings were assessed for survival after 30 days.

7.3.3. Environmental influences on germination

In February 2009, 700 endocarps containing 1 to 4 seeds, were used to test the effect of temperature (25, 30 and 35 ºC), acid digestion, fire, and smoke on germinability of marula endocarps (Figure 6). All endocarps were soaked for 48 hours in water before germination trials began, unless the treatment involved prior soaking or was explicitly not soaked. For each treatment, three endocarps were placed in each of 33 Petri dishes and covered with cottonwool. All endocarps were treated once off with 1% sodium hypochlorite (jik), and then a Funginex fungicide of 3 g to 1.5 l of water once a month. Germination, or protrusion of the radicle by 2 mm, was recorded three times a week and water was replenished to saturation every second day.

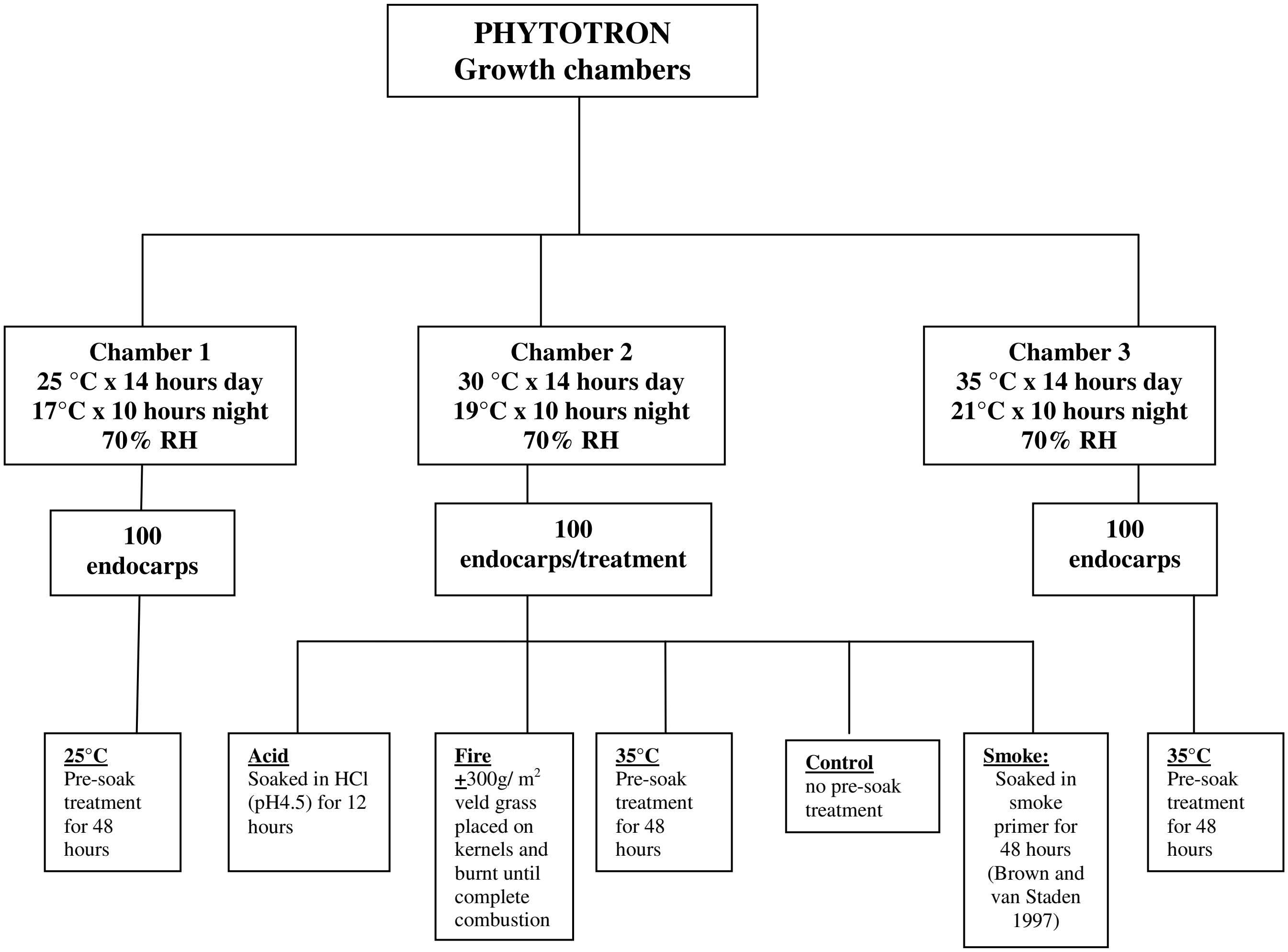

Figure 1: Description of the seven treatments used to assess the effect of environmental factors on marula germination in the Phytotron growth chambers.

The total number of seeds was determined by counting the number of opercula present and the germination percentages calculated as a percentage of the total potential seeds as well as the percentage of total endocarps. The trials were run for 87 days at which time watering of the seeds was stopped since no more germination was taking place.

7.3.4. Early seedling root growth

In December 2009, 100 endocarps were planted into ten rooting chambers each containing 5 compartments (40 cm deep, 270 cm^2 cross sectional area at the surface) and with a single sloping glass front and kept under glasshouse conditions. The compartments were filled with nutrient-poor granite derived soil collected at WRF in 2007. Two seeds were planted against the front glass panel of each chamber approximately 1 cm below the soil surface. The glass was covered with black card board as well as two layers of black plastic to exclude light. Root growth could thus be observed against the glass. Two watering treatments were applied to test seedling growth and root depth penetration: a) Five rooting chambers received 15 ml of water every 3 days which is equivalent to the mean rainfall received in a typical rainstorm at WRF (20 year mean); b) Another five rooting chambers only received 7.5 ml of rainfall every 3 days.

Seedling emergence was recorded and then root length was monitored every 3 days and the time taken to reach the bottom of the rooting chamber was calculated as was growth rate per day. The experiment was terminated when all of the seedlings had grown sufficiently for the taproots to reach the bottom of the rooting chamber. Comparisons between treatments were made using an independent t-test.

7.4. Results

7.4.1. Imbibition and drying curves

Complete endocarp imbibition occurred by 12 hours, although 80% imbibition had already occurred by 2 hours (Figure 2a). Marula endocarps were able to imbibe up to 40% of their initial mass by 32 hours. Water was lost much more slowly than the rate of water uptake during imbibition, as only 5% of the mass when fully imbibed was lost by 2 hours and by 28 hours only 26% of the mass was lost (Figure 2b). This indicates that marula endocarps hold onto the water gained after a rain storm and may be able to make several small cumulative gains from separate rain storms.

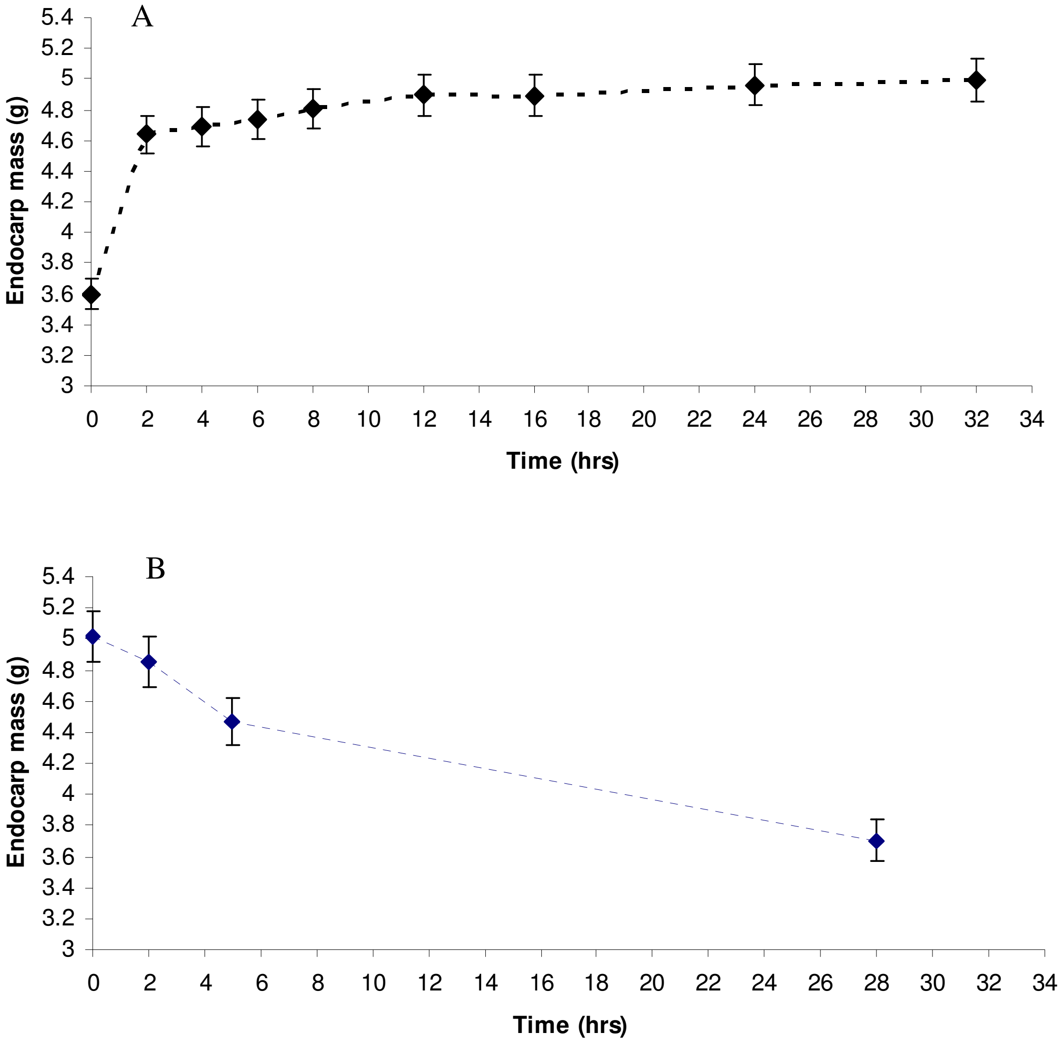

Figure 2: a) Wetting and b) drying curves (mean±SE) of marula endocarps. The starting and ending masses were the same indicating that no further weight loss could occur by 28 hours.

7.4.2. Water requirements for germination

Seedlings emerged nine days after planting, with 70% of the marula endocarps in the sandy soils and 50% in the clay soils acquiring sufficient moisture for germination when watered with the largest quantity of water (16 ml) at the highest frequency (every 2 days) (Table 1). Only 10% of the endocarps in the clay soil and 20% in the sandy soil watered with 16 ml every 4 days managed to germinate. Germination percentage was not associated with soil type (χ^2_1=0.39, p=0.532). Even after two months, all other watering combinations resulted in no germination and hence it is clear they were receiving insufficient water to germinate. All germinated seedlings established. Viability of the remaining seeds was not assessed, but in all likelihood the viability percentages were equal across treatments.

Table 1: Percentages of marula endocarps that germinated after exposure to various watering regimes for two months.

Soil type	**Watering periodicity (days)**	**Water additions (ml)**			
		0	**2**	**8**	**16**
Basalt derived clay soil	**2**	0	0	0	50
	4	0	0	0	10
	7	0	0	0	0
Granite derived sandy soil	**2**	0	0	0	70
	4	0	0	0	20
	7	0	0	0	0

7.4.3. Environmental influences on germination

Mean germination percentages of all seeds ranged between 10 and 55%. The highest levels of germination were recorded in the 35°C and the acid digestion treatments (Figure 3; Table 2). The fire treatment resulted in the destruction of the endocarps due to heat damage and hence significantly reduced the germination percentages. Temperature had a significant influence on germination percentage. The pre-soaking treatment increased the rate of germination but not the overall percentage. Exposure of the endocarps to smoke primer increased the germination percentage only slightly. In terms of endocarps, 51% of the 35 °C, 50% of the acid, 35% of the smoke, 34% of the soaked 30 °C, 33% of the unsoaked 30 °C, 17% of the 25 °C and 7% of the fire treatment endocarps had at least one seed that germinated. Endocarp germination percentage was significantly associated with seed treatment (χ^2_6=70.63, p<0.0001).

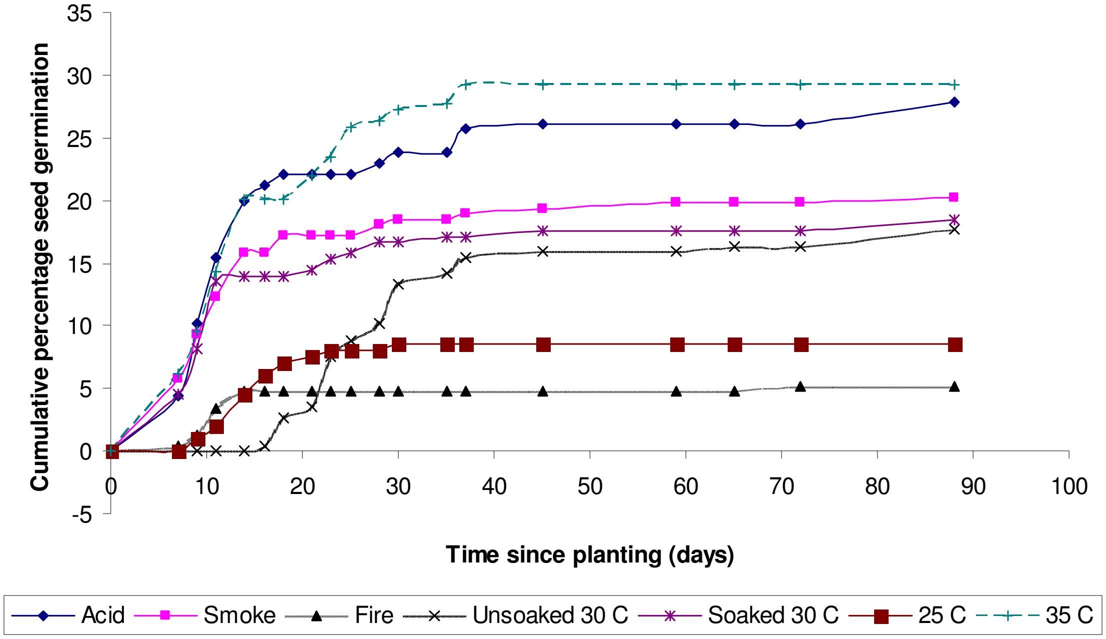

Figure 3: Comparison of cumulative percentage germination curves of marula seeds between seven different treatments.

7.4.4. *Early seedling root growth*

In total 52 seedlings emerged, 28 in the 15 ml treatment and 24 in the 7.5 ml treatment. After 29 days all the 15 ml and 7.5 ml seedling roots had reached the bottom of the rooting chambers (approximately 400 mm) (Figure 4). More seedlings reached the bottom after 15 days in the 15 ml treatment than in the 7.5 ml treatment. The mean time taken to reach the bottom in the 15 ml treatment was 16.2±1.2 days while in the 7.5 ml treatment it was 18.6±1.6. Taproots grew downwards on average, 29.9±2.5 mm per day in the 15 ml treatment and only slightly less at 27.7±3.1 mm per day in the 7.5 ml treatment. These differences were not significant (t_{51}=-0.55, p=0.584).

Table 2: Seed and endocarp final germination percentages and mean days to germinate of marula endocarps between seven different treatments.

Treatment	**Final seed germination (%)**	**Final endocarp germination (%)**	**Mean days to germinate (mean±SE)**
Acid	28	50	19.±2.54
Smoke	20	35	16±2.25
Fire	5	7	16±5.11
Unsoaked 30°C	18	33	32±2.91
Soaked 30°C	18	34	20±3.38
25°C	9	17	16±1.28
35°C	29	51	15±1.09

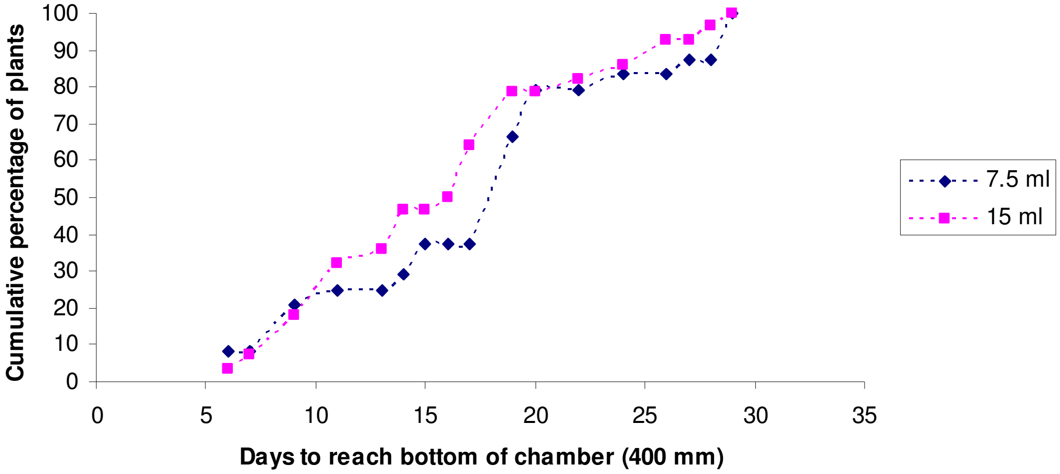

Figure 4: Time taken for marula seedling taproots grown under two watering regimes to reach the bottom of a 400 mm deep rooting chamber.

7.5. Discussion

7.5.1. Imbibition and drying curves

Wilson and Witkowski (1998) identified an inverse relationship between imbibition rate and dry seed mass for *Acacia* spp. seeds with a range of 0.04-0.12 g, with larger seeds taking longer to fully imbibe. Since marula seeds are enclosed in an endocarp and since each endocarp contains up to four seeds, it is difficult to compare imbibition rates with these *Acacia* species that fully imbibed from 4 to 28 hours for the different species. However, it can be determined that marula endocarps appear to imbibe relatively faster than *Acacia* seeds even though marula seeds are much larger (0.2-0.3 g). This is probably due to the woody structure of the endocarp, which is full of airspaces and hence probably very water absorbent and probably a store for ‘extra water’. Marula seeds take twice as long to dry out than to imbibe, indicating that once the endocarp is fully imbibed, there is sufficient moisture for germination for an extended period of time. Botha (2006) found that marula seedlings could survive for more than 170 days after only receiving sufficient water for germination. In addition to the extraordinary ability to resist drought that this finding suggests, the endocarp probably also acts like a sponge, absorbing all available water quickly and holding onto the water for an extended period of time. This then cushions the seeds within the endocarp from external stresses and allows for germination after sequestered absorption of water from a series of small rainfall events. Natural scarification of marula endocarps is probably much less intense (a slow process in the field) than laboratory scarification and hence the rate of water absorption may be slower (Wilson and Witkowski 1998). Under field conditions water uptake occurs after a rainfall event but loss of water may be much more rapid as soil surface temperatures can exceed 70 °C after fire has removed all vegetation and blackened the soil (Bradstock and Auld 1995). Seeds have been shown to lose viability after drying at 40 °C (Wilson and Witkowski 1998) and thus the loss of water after full imbibition can result in death of seeds. Whether marula seeds can sustain repeated wetting and drying cycles was not tested in this study, but observations indicate that this is possible but with loss of viability.

7.5.2. Water requirements for germination

Acacia karroo, *A. nilotica* and *A. tortilis* and *Mundulea sericea* (another leguminous shrub), only acquired sufficient water for germination when watered with 16 ml of water every two days (Wilson and Witkowski 1998). Marula seeds germinated under the same regime, however a small proportion did germinate with 16 ml every four days. Germination was only evident after the ninth day. Marula seeds therefore require a critical level of soil moisture for a period of ten days before germination. Soil moisture stress commences when the soil moisture drops below 40% of the water available to plants in the soil profile (Schulze 1997). Schulze (1997) also reported that in most places in the lowveld of South Africa, where humidity is generally high, 50-60% of the days in January experience moisture stress. This indicates that a large proportion of the wet period in a year is not suitable for germination. The minimum water requirement of 16 ml every four days for two weeks equates to 3 mm rainfall every four days in the field. The Phytotron growth chamber conditions mimicked the hot humid conditions in the lowveld, where marula is common and hence this level of rainfall would be a relatively accurate assessment of minimum water requirements for marula germination.

7.5.3. Germination

Marula seeds are orthodox in that they are tolerant of desiccation (Pritchard *et al.* 2004). Orthodox seeds usually show some form of dormancy (Mng'omba *et al.* 2007). Mechanical weathering of the endocarp by acid digestion or through scarification can result in germination of the seeds within the same season as fruit production (pers. obs.). Hence the embryos must be mature at fruit drop. However, previous reports suggest that marula seeds are dormant for at least 6 months after fruit drop (Shone 1979; von Teichman *et al.* 1986; Lewis 1987). Therefore scarification of the endocarp appears to be a prerequisite for germination and the seeds have mechanical dormancy. Further experimentation with unscarified endocarps is still required to test this hypothesis.

Pritchard *et al.* (2004) showed that marula seeds germinated best at low seed moistures, indicating that in addition to scarification, a drying period is required before germination takes place. The post release seed maturation period often involves drying in temperate environments (Baskin and Baskin 2001). Fruit pulp may contain germination inhibitors and its removal could allow for germination (Samuels and Levey 2005). However, marula fruit in the dung of elephant during the fruiting season were often still covered in fruit pulp (pers. obs.). This could indicate that marula fruit passing through the digestive tract of a mammal may not be able to germinate in the same season as fruit production, as the seeds would still be covered in some mesocarp tissue and drying out of the seed would not yet have occurred. This may depend on the mammal species, with ruminants more likely to scarify than non-ruminants like elephant.

Fire can be used to rupture the tough seed coat but can also cause high seed mortality if too hot (Mng'omba *et al.* 2007). Indeed many of the marula endocarps subjected to the fire treatment in this study ruptured, killing the seeds. Smoke has also been reported to enhance seed germination in some species (Brown and van Staden 1997). In marula, smoke had little effect on the germination percentage, indicating a lack of adaptation of marula plants to germination after surface fires. Lewis (1987) found that marula seed germination was enhanced when the fruit had passed through the digestive tract of an elephant, as well as when placed inside the dung bolus of an elephant. Germination, however, was only observed in the following rainy season. In the current study, digestion of the scarified endocarps in acid increased the germination percentage by 50%, supporting the findings of Lewis (1987).

Overall, germination percentages for marula were low, both in terms of total seeds (<28%) and endocarps (<51%). Under natural conditions, if any single one of the seeds within an endocarp germinate then that can be counted as germination success. Additional seeds in an endocarp could perform the function of backup in case the first seedling dies due to dessication, predation or if the other seeds are predated. The germination trials showed that most seeds in an endocarp germinated concurrently, and hence the additional seeds per endocarp may not play a role in delayed germination but may rather be a backup for predation, as not all seeds in the endocarp may be eaten. However, Gallahar (2010) has shown that predation of endocarps resulted in all the seeds in an endocarp being eaten. Therefore the adaptive advantage of more than one seed per dispersal unit remains unclear.

One fruit therefore most likely represents only one possible seedling, especially since if two seeds do germinate, it would be unlikely for both seedlings to establish in such close proximity to each other, and ultimately one would outcompete the other resulting in its death.

7.5.4. Root penetration

Once germination has taken place, the seedling still needs to successfully establish and withstand possible summer drought conditions, as well as the dry season often lasting more than six months. Frost (1987) defines summer drought as one or more months during December to February receiving <50 mm of rain. Botha (2006) found marula seedlings to be extremely resistant to drought. This will be further investigated in Chapter 10. Drought avoidance/tolerance can, however, be achieved through rapid root penetration.

Witkowski (1991) found that *Acacia saligna* (an alien invasive tree in South Africa) seedlings grown in rooting chambers reached 200 mm in depth after 30 days (7 mm per day) while *Protea repens* roots only reached 180 mm after 80 days (2 mm per day). Sugar maple seedlings were found to have root elongation rates of 12 mm/day, indicating that the roots would take 32 days to reach the base of the rooting chambers had they been grown there (Webb 1976). Elongation rates for unimpeded roots of tree seedlings have been shown to be 5 mm/day for *Pinus radiata* and 3 mm/day for *Eucalyptus nitens* (Misra and Gibbons 1996). Root growth rate in marula was on average 28 mm/day, indicating a root penetration rate four fold faster than *A. saligna* and 14 fold faster than *P. repens*. Watering regime had little effect on the root penetration rate of marula. The rapid penetration of the soil by marula roots allows the seedlings to exploit soil moisture from deep in the profile very early during development of the seedlings. This will allow for considerable drought avoidance in marula seedlings.

Soil mechanical impedence is one of the greatest factors affecting root penetration rates. A non-linear inverse relationship between root elongation rates and soil mechanical impedence has been reported (Misra and Gibbons 1996). Soil impedence has been shown not to impact the production and distribution of dry matter due to compensation in root growth and variable root thickening (Misra and Gibbons 1996). Hence root penetration in clay soils will most likely be slower than in sandy soils.

7.6. Conclusions

Marula seeds are large and the endocarp surrounding the seed does not hinder germination, but rather absorbs moisture relatively quickly and retains this moisture for an extended period of time. This dampens the variability in moisture availability surrounding the seed. A minimum of 3 mm of rain every 4 days for two weeks is required for germination of marula seeds in the field. When that minimum water requirement has been met, seed germination percentages range between 7 and 28% and endocarp germination between 7 and 51% for a period of 87 days. Success depends on temperature and leaching to remove germination inhibiting chemicals. Marula roots require approximately one month to penetrate 0.5 m into the soil profile and rainfall seems to have little effect on this growth rate (at least of the order of treatments tested here). Therefore once sufficient moisture has been achieved for germination, and temperatures are high enough and inhibiting chemicals preventing germination have been diluted via passing through the digestive tract of a mammal or through leaching (possible over the rainy season), seeds will germinate and the resulting seedlings will establish.

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PART C: ADAPTIVE RESPONSES TO FIRE, HERBIVORY AND RAINFALL VARIABILITY

Chapter 8: Investigating the vulnerability of *Sclerocarya birrea* subsp. *caffra* (marula) to fire and herbivory

Parts of this chapter are now in press in the journal Austral Ecology

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8.1. Abstract

Marula is considered vulnerable to the effects of fire, herbivory and their combination, which have been attributed to high adult mortality, low recruitment and the “missing size class” observed in some populations in South Africa. This study investigated the relative importance of these agents of disturbance, at the level of the individual stem, by specifically focussing on the following questions: (1) What is the greatest cause of mortality in adult marula stems exposed to fire and herbivory?; (2) How vulnerable are damaged adult trees to subsequent fire?; (3) Does bark stripping increase the vulnerability of adult marula stems to fire and if so, what is the dominant mechanism?; (4) At what stem diameter are marulas resistant to fire?; (5) How fast do topkilled marula stems recover their prefire height after a fire?; (6) What effect does the combination of fire and herbivory have on the survival and growth of marula saplings? Field surveys quantified the extent of damage in marula individuals in the southern KNP, highlighting the high levels of extreme herbivory such as toppling (7%) and pollarding (8%), and bark stripping (6% with >50% of the circumference stripped). After a controlled fire, up to 30% of pollarded and 21% of toppled trees were killed, indicating the vulnerability of adult marula trees that have experienced extreme herbivory to subsequent fire. In addition to extreme herbivory, the progression from bark stripping through to invasion of the soft, exposed heartwood by wood borers, often facilitated by fire, through to toppling of the weakened stem after successive fires, appears to be an important mechanism by which fire interacts with herbivory to cause adult stem death. Bark stripping and fire manipulation experiments indicated that bark stripping failed to increase the vulnerability of stems to fire directly, through transport tissue damage. However, the combination of bark stripping and fire reduced the ability of the stem to regrow bark, increasing the vulnerability of the exposed stem to boring insects and future fires. Fire manipulation experiments showed that the minimum marula stem basal diameter of resistance to fire was >3.4 cm. However, after a controlled fire in the field, marulas >1.2 cm resisted topkill from fire. These diameters of resistance are lower than that found for other common savanna species. This superior ability was attributed to the relatively high allocation to bark from very small diameters and the high water content in the stems. Topkilled marula stems protected from intense mammalian herbivory, resprouted basally and were able to regain there prefire height

within one growing season. However, in the KNP, 11% of 46 saplings tagged, were dead one year after a natural fire and only 51% were able to regain their prefire height, due to the combination of fire and herbivory. Under glasshouse conditions, all topkilled marula saplings regained their prefire height within 5 months, regardless of the frequency of complete defoliation following the burning treatment. These results indicate that the rainfall patterns following fire could have an overriding effect on the fire-herbivory interaction. In conclusion, marula saplings within the fire trap are highly resistant to the effects of fire and therefore the "missing size class" phenomenon observed cannot solely be ascribed to fire alone.

8.2. Introduction

No single factor determines woody plant dynamics in savannas (Hoffman and Solbrig 2003), but rather complex interactions between fire, climate, nutrient availability and herbivory (Scholes 1997; Eckhardt *et al*. 2000; Sankaran *et al.* 2008). The effect of fire on woody plant dynamics has received much attention (Bond and van Wilgen 1996) and the general consensus is that whole tree mortality due to fire alone, specifically surface fire, is low. Individuals in the smaller size classes are subjected to topkill (death of aerial biomass) (Trollope 1984), but survive by resprouting from the rootstock (Trollope 1984; Hoffman and Solbrig 2003; Wilson and Witkowski 2003). Escape from topkill arises through the insulating effect of thicker bark, which smaller individuals lack ("escape diameter hypothesis") (Vines, 1968; Gignoux *et al.* 1997; Hoffman and Solbrig 2003; Wilson and Witkowski 2003), or alternatively through the escape of vulnerable terminal buds from the flames ("escape height hypothesis") (Higgins *et al.* 2000; Balfour and Midgley 2006). Bark thickness and tree size could act sequentially in protecting the tree from fire (Jackson *et al.* 2007). The size of escape from topkill influences the population structure of a species. Fire is therefore, generally accepted to only affect tree recruitment and not adult survival (Gignoux *et al.* 1997).

However, structural damage by elephants and other herbivores may induce vulnerability in adult stems. Non-senescent post-fire adult stem mortality in savannas regularly occurs due to prior damage (Yeaton 1988, Wilson and Witkowski 2003). Possible mechanisms through which prior damage increases the mortality of stems after a fire include: 1) Heat damage to exposed transport tissues of bark stripped stems, resulting in moisture stress and eventual stem death (Balfour and Midgley 2006; Moncrieff *et al.* 2008); (2) Invasion of damaged stems by wood boring insects which structurally weaken the stem, thereafter allowing aeration and passage for fires into the deeper tissues of the stem, eventually resulting in stem snapping and usually stem death; (3) Exacerbation of the original damage through reduced ability to recover (fire reduces the growth rate of trees (Staver *et al.* 2009)), and hence subsequent pathogen and insect attack occurring under circumstances where it may have been avoided, if the fire had not occurred. Mortality after additional fire would occur as in 2 above; (4) Toppling or pollarding (stem snapping) by elephants, exposing vulnerable apical buds to the flames (Jacobs and Biggs 2002a) ultimately killing the stem (Holdo 2005).

Due to increasing elephant densities in the Kruger National Park (KNP), South Africa (Shannon *et al.* 2008) and many other African savanna areas (eg. Skarpe *et al.* 2004), and decreasing numbers of large trees (Pellew 1983; Ben-Shahar 1993; Eckhardt *et al.* 2000, Edkins *et al*. 2007, Moncrieff *et al.* 2008, Helm *et al*. 2009), more information is needed on how elephants and other agents of disturbance result in the death of individual stems (Trollope *et al.* 1998; Holdo 2005; Moncrieff *et al*. 2008). Previous studies in the southern regions of the KNP have shown that the relative use of *Sclerocarya birrea* subsp. *caffra* (marula) by elephants, is greater than other common species such as *Acacia nigrescens* (knobthorn), when measured against availability (Shannon *et al*. 2008). In addition, a number of unusual population phenomena within marula populations in the KNP have been identified, including: 1) high adult mortality rates of up to 7.1% per annum for certain size classes (Helm *et al.* 2009), 2) the "missing size class" (between 2 and 8 m in height (Jacobs and Biggs 2002 a and b)) and 3) low recruitment rates (Helm *et al*. 2009). These phenomena have been broadly ascribed to the effects of herbivory, fire and their combination, but the relative importance of each is unknown.

This study therefore aimed to determine the current levels of utilisation of marulas in terms of toppling, pollarding and bark stripping in the southern regions of the KNP as well as to determine the response of saplings in the fire trap to fire and herbivory, through field surveys and to investigate which mechanisms induce vulnerability of adult marula stems to fire through manipulation experiments and observations after a controlled fire. In addition the resprouting response of topkilled saplings in response to repeated defoliation was investigated to provide some understanding of the resilience of this life history stage to fire and herbivory.

This translated into a number of key questions:

1) What is the greatest cause of mortality in adult marula stems in conservation areas with both fire and elephants?
2) How vulnerable are damaged adult trees to subsequent fire?
3) Does bark stripping increase the vulnerability of adult marula stems to fires, and if so what is the dominant mechanism?
4) At what stem diameter are marula stems resistant to fire?
5) How fast do topkilled marula stems recover their prefire height after a fire?
6) What effect does the combination of fire and herbivory have on the survival and growth of marula saplings in the field and under glasshouse conditions?

8.3. Methodology

8.3.1. Study area

Marula population surveys and field observations after controlled and natural fires of marked marulas were conducted in the southern regions of the KNP (Figure 1). Manipulation experiments were conducted in the communal land adjacent to Justicia Village approximately 15 km west of the Paul Kruger Gate of the KNP (Figure 1). Experimental work in the KNP was not possible due to its protected area status.

8.3.2. Data collection

8.3.2.1. Field surveys

Impact survey of adult marula populations in the southern KNP

To quantify the response of adult marula populations (>3 m in height and/or >10 cm in basal diameter (Higgins *et al.* 2000; Jacobs and Biggs 2002a)) to the fire and herbivory regime in the southern KNP, 23 transects perpendicular to the access roads were assessed in the Skukuza (7 transects), Pretoriuskop (6 transects), Lower Sabie (7 transects) and Tshokwane (3 transects) sections (Figure 1) within the Granite Lowveld (9 transects), Pretoriuskop Sour Bushveld (6 transects) and Gabbro Grassy Bushveld (8 transects) vegetation types (Mucina and Rutherford 2006) between June and October 2008. Each transect was 40 m wide and approximately 1 km long and occurred in areas that had been burnt within the previous two years (except for the transects in the Gabbro Grassy Bushveld which have not had fire for at least the last four years). An approximate area of 92 ha was surveyed. For each marula >3 m in height encountered in the transects, the following were recorded: GPS (global positioning device) co-ordinates; basal stem diameter; height; maximum percentage of bark removed around the circumference, total percentage of bark area removed up to 3 m, height of the lowest point of removal, percentage of bark recovery, presence or absence of exposed sapwood charring (judged by blackening of the sapwood), presence or absence of wood boring insect infestation into the sapwood; and the agent of bark removal (porcupine: assumed to be bark removal at the base of the trunk or elephant: assumed to be any bark removal not at the base of the trunk) (following Moncrieff *et al.* 2008). The percentage of sapwood still exposed was calculated by subtracting the percentage bark recovered from the percentage originally stripped. Each tree was classified as either dead, pollarded, toppled or standing and damage scores were assigned (following Helm *et al.* 2009) to the standing category.

Impact survey of a juvenile marula population near Pretoriuskop in the KNP

To quantify the response of juvenile marula stems (<10 cm in basal diameter) to the fire and herbivory regime in the southern KNP, 46 saplings in the Pretoriuskop section of the southern KNP were tagged and their position marked with a GPS in November 2007. This was done because this size class was relatively rare in occurrence and widely scattered spatially in the transects surveyed above (Chapter 2). The sampled saplings ranged in height from 0.9 to 2.6 m in height and 2.0 to 7.2 cm in basal diameter before an intense accidental fire in September 2007. Stem height (live and dead), basal diameter of the dominant stem and resprouting vigour (summed length of all resprouts) were assessed for each plant. In November 2008 these stems were revisited and reassessed for survival, stem height and resprouting vigour.

Before and after a controlled fire in the KNP experimental burn plots (EBPs)

The effect of a controlled fire on marula stems was examined in the annual and biennial August burn plots of the Kambeni, Fayi, Numbi and Shabeni replicate "strings" in the Pretoriuskop KNP experimental burn plots (EBPs) by assessing marulas before and after the controlled fire was implemented. The Pretoriuskop EBPs are part of a fire experiment started in 1954 with the aim of providing a scientific basis for fire management policies within the KNP (O'Regan 2005, Higgins *et al.* 2007). The experimental design was a pseudo-randomised block design in which 12 fire treatments were replicated four times each, within each of four major vegetation types defined in the KNP by Van Der Schijff (1958). The Pretoriuskop EBPs occur in the *Terminalia- Dischrostachys* sourveld land type (van der Schijff 1958) which occurs within the Pretoriuskop Sour Bushveld vegetation type (Mucina and Rutherford 2006). Each plot is approximately 6.5 ha in size. The August (late dry season) Pretoriuskop EBPs were chosen for their high fire intensities, where marulas were in sufficient numbers to assess the effects of high impact fires on marula stem mortality.

All marulas seen within 50 m wide transects, approximately 50 m apart, in each selected plot were marked with a GPS in July 2008 and their basal diameter and height measured. The same parameters recorded for the field survey trees described above were recorded for these trees. In October 2008, after the fire, these individuals were relocated and percentage canopy dieback, and post-fire fate recorded. Long-term mean fire intensities for the Pretoriuskop EBPs are 2 567 kJ/s/m and 3 518 kJ/s/m for the annual and biennial August burns respectively (Trollope and Potgieter 1985). The mean intensities for the fires in 2008 were 48% and 44% lower than the long-term mean at 1 342 kJ/s/m and 1 961 kJ/s/m for the annual and biennial August burns respectively (N. Govender, SANparks fire ecologist, Kruger National Park, unpublished data).

In total 89 marula stems were sampled including: 20 pollarded, 14 toppled, 26 >7 cm (standing, fire resistant) and 29 <7 cm (gullivers) in basal stem diameter. The thickest stem was assessed in the case of multi-stemmed individuals.

8.3.2.2. Experimental manipulations

Two manipulation experiments were conducted on marula saplings growing under natural conditions in the communal lands of Justicia Village. Given the limitations of working on a protected tree species, as few stems as possible were used and experimentally manipulated trees were not larger than 10 cm in basal diameter. It was assumed that mechanisms responsible for mortality in this size class would also operate on larger trees (following Moncrieff *et al*. 2008). All field experimental work was conducted under permit issued in 2008 by the Department of Water Affairs and Forestry, South Africa. An additional manipulation experiment was conducted on saplings grown from seed under glasshouse conditions at the University of the Witwatersrand.

Fire simulations

The stem diameter at which marula becomes resistant to fire was investigated through fire simulation experiments in July 2008, while the trees were dormant (without leaves). Different dynamics might be expected for trees burnt during the early spring. However, until 1996, 80% of the fires in the KNP occurred during the dry season (June-Nov) (van Wilgen *et al*. 2000). Following Moncrieff *et al.* (2008), the modified wick technique (first designed by Hare (1965) and Uhl and Kaufmann (1990)) was used to experimentally burn marula stems for a fixed period of time. Intermediate intensity (2 min burn) and high intensity (3 min burn) savanna fires were simulated following regressions calculated by Moncrieff *et al.* (2008). Intermediate intensity fires in the KNP are often in the range of 1001 to 2000 KJ/s/m, while a high intensity fire falls within the range of 2001 to 3000 KJ/s/m (Trollope and Potgieter 1985).

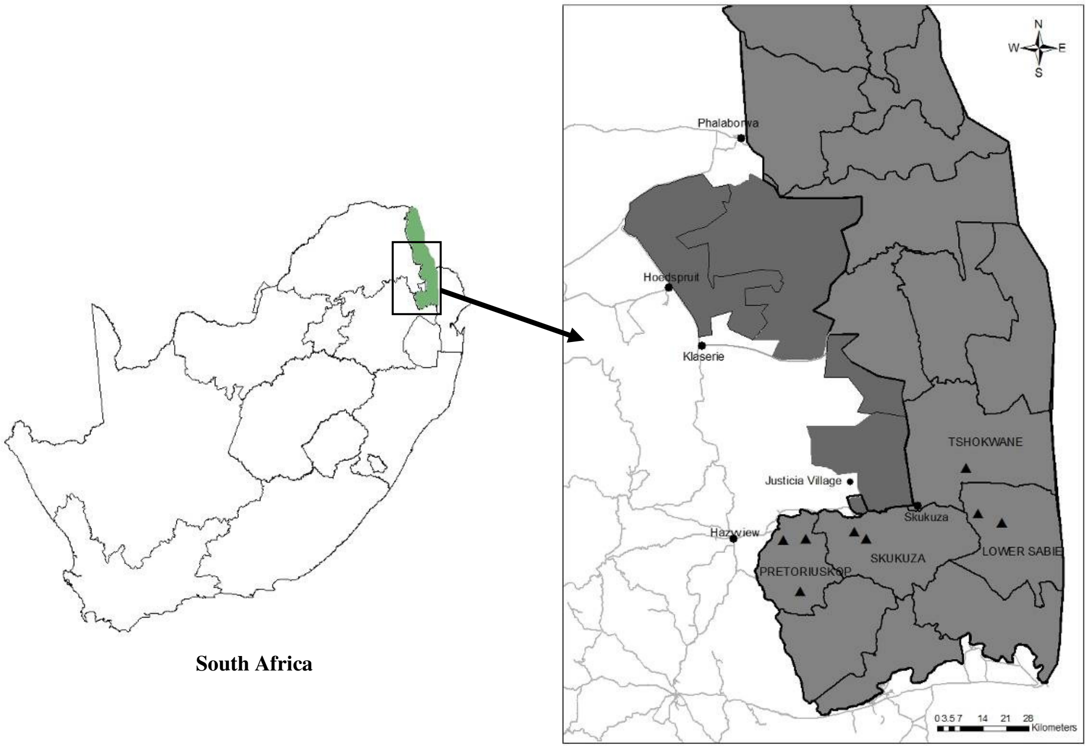

Figure 1: Map indicating the presence of the survey sites (black triangles) within the Kruger National Park (KNP) and Justicia Village communal land outside the KNP, South Africa.

Sixty marula trees ranging in size from 1.5-10 cm in basal diameter were selected in the communal land, marked with plastic tags and their position recorded with a GPS. The height, basal diameter and bark thickness (30 cm from the ground or above the basal swelling), and percentage canopy dieback were measured for each stem. Forty marula stems were burnt using the wick burn technique (20 each at each burn intensity), while the other 20 trees were used as unburnt controls. Each treatment consisted of five individuals in each of four basal diameter size classes: 1.5-3 cm; 3-5 cm, 5-7 cm and 7-10 cm.

The stems were reassessed for survivorship in October 2008, November 2008 and January 2009 (3-6 months post-fire). These 3 monitoring times were chosen to assess immediate stem death before the growing season, the subsequent stem survival post leaf production (marula shoot growth commences in late October (C. Helm, pers. obs.) before rains fall), and subsequent stem survival mid-growing season once insect and ungulate herbivory had taken place. During the reassessments, percentage canopy dieback; percentage of canopy in leaf; and basal (below burn scar) and epicormic (above burn scar) resprouting vigour (summed length of all resprouts) were recorded.

Fire and bark stripping simulations

Bark removal and fire simulation experiments were also conducted in July 2008 to investigate whether fire increases the vulnerability of bark stripped stems to stem death and if so, by what mechanism. Stems were subjected to four bark removal intensities: 0%, 30%, 60% and 100% of the circumference. The bark was removed by cutting away 5 cm vertical bark strips from around the stem for each of the bark removal intensities. Forty six stems between 7 and 10 cm in basal diameter from multi-stemmed trees (the largest stem in each case) were used for this experiment. Half of the trees selected were only stripped (0%, 30%, 60% or 100%), whereas the other half were both stripped and burnt at an intermediate fire intensity (2 mins). All stems were reassessed for survivorship in October 2008, November 2008 and January 2009 as described for the fire simulation experiments. Bark regrowth percentage was also assessed at each monitoring period. In November 2009, these stems were relocated where possible (a large portion of the communal land had been ploughed and many experimental marulas had been removed) (16 months post fire) to determine stem survival and bark regrowth percentage only.

Simulated fire and herbivory glasshouse experiment

Forty marula seedlings germinated from randomly selected seed batches were grown for 1.5 years under glasshouse conditions in the University of the Witwatersrand glasshouse from December 2007 to July 2009. Initial heights ranged from 185-600 mm and stem diameters ranged from 3.52 to 10.64 mm. Temperatures and relative humidity in the glasshouse are presented in Chapter 5 and the watering schedule also followed that described in Chapter 5. The plants were grown in 12 litre black nursery bags containing granite derived nutrient-poor soil collected from Wits Rural Facility in 2007 (fertility and textural analysis: Chapter 4). The plants were rotated at monthly intervals throughout the experiment to prevent positional effects. In July 2009 the stem height and stem diameter at the cotyledon node were measured. The plants had lost their leaves and were dormant at this time. Plants were randomly allocated to four treatments with ten replicates per treatment. The first treatment was the control where the plants were allowed to continue to grow (unburnt). The second treatment involved using the wick method described above to burn the stems for 2 minutes, thus simulating a medium intensity dormant season burn (burnt). The third treatment involved burning the stem followed by 100% simulated herbivory after 90 days of watering (burnt with single defoliation). Herbivory was simulated by cutting off all resprouting stems at the base. The fourth treatment involved burning the stem followed by 100% simulated herbivory after 45 days of watering and again after another 45 days of watering (burnt with double defoliation). The burning of the last 3 treatments was conducted in July 2009, 30 plants were burnt and 10 plants were left unburnt. The herbivory simulations were conducted in October 2009 and again in December 2009. The cut leaf and stem material from each herbivory simulation was dried in a convection oven for 48 hours at 50 °C and then weighed to determine regrowth rate after topkill by fire. All plants were harvested in January 2010, 45 days after the last defoliation event. The plant was divided into root, stem and leaf material and dried in a convection oven for 48 hours or until no further loss in mass was recorded, at 50 °C for the leaves and at 60 °C for the roots, and then weighed for dry mass.

8.3.3. Data analysis

Data were analysed using Statistica V.6 (Statsoft, Inc. 2004) with a significance level of $p<0.05$. All percentage values were arcsin transformed before analysis. All data was tested for normality using the Shapiro Wilks test. The relationship between the number of large trees and the number of trees which had bark utilised in each diameter class was analysed using a linear regression. ANCOVA (comparing the regression slopes for abundance and bark stripping across diameter classes with null hypothesis of equal slopes (Zar 2010)) was used to determine whether bark stripping of large trees was related to abundance in each size class. Chi-squared analyses were used to compare basal diameter classes of bark stripped trees with those that were not bark stripped and to compare the intensity of bark stripping (area bark stripped up to 3 m) between medium (<50 cm) and large (>50 cm) trees. A two-way ANOVA and Fisher's LSD were used to determine the effects of burn treatment and basal diameter size class on the mean percentage canopy dieback and resprouting vigour in January 2009. Canopy dieback was used as a proxy for stem health, with 100% canopy dieback indicating stem death or topkill (not necessarily whole plant death). Canopy dieback due to the fire simulation was calculated by subtracting the percentage canopy dieback present in July 2008 from that estimated during the follow up assessments. The stripping and burn treatment were analysed in the same way as the burn treatments. A repeated measures ANOVA was used to determine whether time influenced the level of canopy dieback.

A multiple logistic regression with logit link using the glm function in R v.2.12 (R Development Core Team 2010) was used to assess the mortality patterns observed in the field surveys and controlled fires in the EBPs using stem survival as the binary response variable (dead=1, alive=0) and presence or absence of bark stripping, damage/prefire state (toppled, pollarded, standing/none), basal stem diameter and presence or absence of borers as predictors. The Akaike's Information Criterion (AIC) was used to choose the most parsimonious model (lowest AIC). The validity of the model was assessed using the log-likelihood ratio test approach (Logan 2010). This test asks whether the model with predictors fits significantly better than a model with just an intercept (i.e. a null model). The test statistic is the difference between the residual deviance for the model with predictors and the null model. Reference levels were used for each categorical variable as follows: damage (pollarded), bark stripping (none), and borers in trunk (none).

For height and diameter change comparisons before and after the treatments, the maximum values were used where there was more than one stem. Since multiple stems resprouted from the base of the plant after burning and defoliation, the heights and diameters of all stems were used to calculate an overall volume for the above-ground tissue of the plant. RGR was then calculated from the change in plant volume since before the application of the treatments using the formula:

RGR = ln (final volume) - ln (initial volume) *100

ANCOVA and Fisher's LSD was used to compare between the treatments in the simulated fire and herbivory experiments in the glasshouse. Since the initial stem diameters were found to be different across treatments, stem diameter was used as a covariate in all comparisons.

Throughout this chapter whole plant death versus stem death/topkill are distinguished by explicitly stating stem death/topkill when the plant was still alive but the entire above-ground canopy was dead and the plant resprouted from the base. When the plant has been described as dead without further qualification, this means that whole plant death has occurred and no resprouting will follow.

8.4. Results

8.4.1. Patterns of mortality and utilisation in adult populations in the southern KNP

A total of 730 trees ranging from 10 to 85 cm in basal diameter were surveyed. Of these, 90 (12.3%) were dead, 51 (7.0%) were toppled but still alive and 55 (7.5%) were pollarded but still alive (Figure 2). Of the remaining individuals (n=534), only 75 (14.0%) had no damage, 363 (68.0%) had moderate levels of damage (damage score 1 and 2) and 96 (18.1%) had extreme levels of damage (damage score 3 and 4) (damage score classification following Jacobs and Biggs (2002)).

Bark stripping affected 261 (48.9%) of the standing live trees (n=534), 186 (71.3%) of which still had a proportion of their circumference sapwood exposed at the time of the survey (Figure 2). Of these, 44 (16.8%) had the exposed sapwood affected by fire. For 82 (31.4%) of the bark stripped trees the causal agent was porcupine. Two of the bark stripped trees (0.8%) had been completely ring-barked. Only 6% of the standing population had >50% of the circumference stripped (Figure 2). The highest circumference stripping intensities were found in the 35-40 cm basal diameter class (Figure 3). Larger trees tended to have a higher area of bark stripping up to 3 m than smaller trees (χ^2_1=3.45; p=0.0631).

For large stems (>30 cm in diameter), there was a negative linear relationship between tree basal diameter and abundance, for all trees and for bark stripped trees (Figure 4). There was no difference in the slopes of the two regression lines, indicating that bark stripping was related to abundance ($F_{1,18}$=1.30, p>0.05). Bark stripped trees overall were not significantly larger in basal diameter than trees with no bark stripping (χ^2_1=1.23; p=0.27).

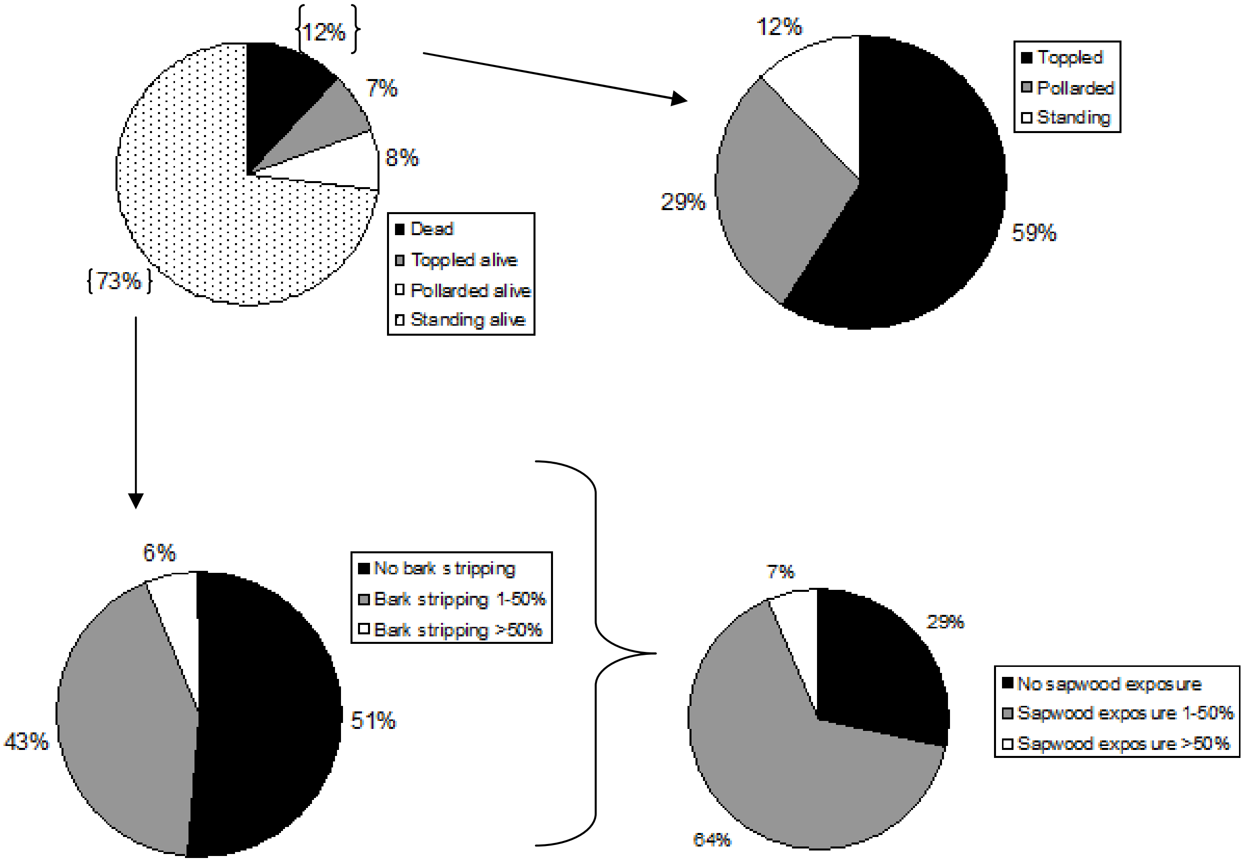

Figure 2: Categorisation of the adult trees from the field surveys in the southern regions of the Kruger National Park, South Africa (n_{total}=730).

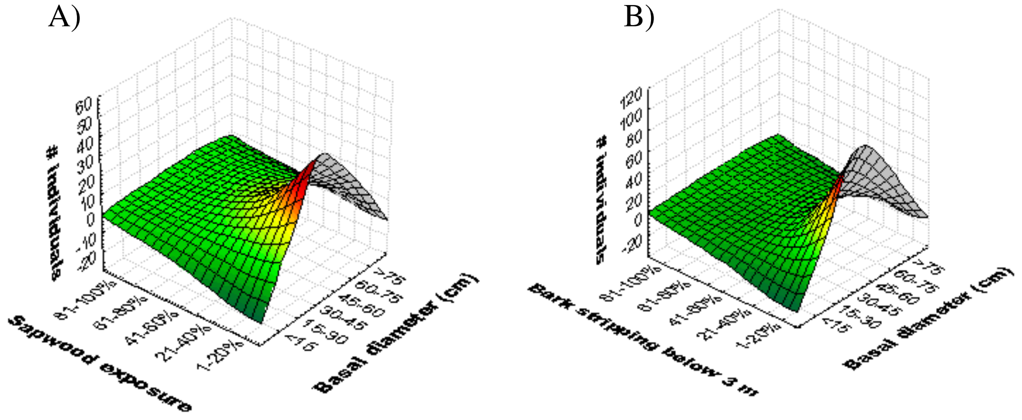

Figure 3: Number of marula stems (in each basal diameter size class) with varying degrees of a) sapwood circumference and b) total stem area below 3 m, bark stripped in the Kruger National Park, South Africa in 2008 (n=730). The number of individuals axis has been modified to fit the distance weighted least squares model.

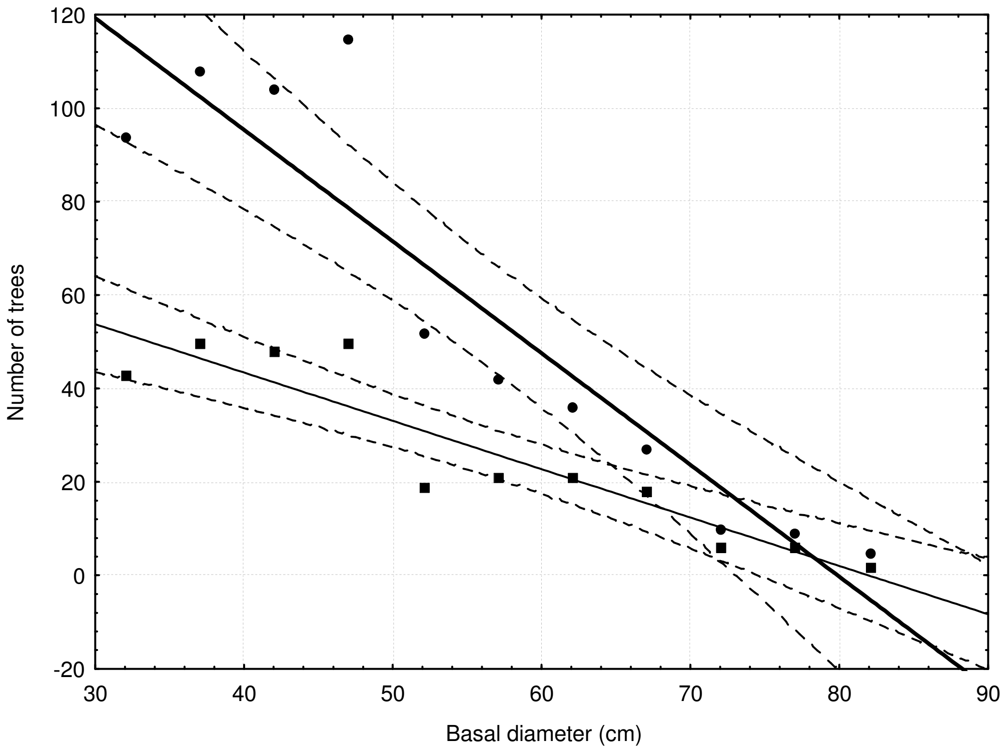

Figure 4: Regression analyses of the number of trees >30 cm in basal diameter (closed circles) (y= -2.390909x + 191.009, R^2=0.8593, p=0.00004) and the number of trees bark stripped (closed squares) in relation to basal diameter class (y= -1.0345x+ 84.7873, R^2=0.8498, p=0.00005). Dotted lined indicate the 95% confidence intervals for each line.

The best valid generalized linear model indicated that the best predictors of mortality were presence of bark stripping, type of damage (toppled and pollarded), and the presence of borers in the trunk (Table 1). Survival of toppled marula stems was significantly lower than pollarded stems. Toppled stems with bark stripping had substantially lower survival than the references (pollarded and no bark stripping).

Table 1: The most parsimonious model to assess marula stem survival in the KNP in relation to basal diameter (cm); presence of borers (yes or no (reference in model)), bark stripping (yes or no (reference in model)) and damage (none, toppled, or pollarded (reference in model)) (null deviance=544.41, d.f.=726; residual deviance=261.79, d.f=720; Likelihood ratio test: χ^2_6=282.62, p<0.0001).

	Estimate	**SE**	**Z**	**P**
Intercept	-3.59	0.67	-5.39	<0.001
Bark stripping (yes)	-0.13	0.56	-0.23	0.82
Damage (none)	-2.61	0.74	-3.52	<0.001
Damage (toppled)	1.85	0.59	3.11	0.002
Borers in trunk (yes)	3.61	0.54	6.75	<0.001
Bark stripping (yes)*Damage(none)	-0.03	0.90	-0.04	0.97
Bark stripping (yes)*Damage (toppled)	-1.83	0.79	-2.33	0.02

8.4.2. Response of juvenile marula stems to natural fire and herbivory

None of the sampled saplings were topkilled by the relatively intense (all grass was burnt in the fire) accidental fire in September 2007 and all stems that were within the flame zone (~3 m in height), managed to resprout epicormically. Forty one percent of the saplings also resprouted basally even though complete topkill had not occurred. Maximum resprouting vigour (summed length of all resprouts) 2 months post fire was 1152 cm (mean=246.95 cm). In addition the maximum height loss by 2 months after the fire was only 65 cm. Most plants were able to either maintain their prefire height through little canopy damage or regrow between 55-97% of their prefire height within 2 months of the fire through epicormic resprouting. By November 2008 however, 11% (n=5) of the saplings had died and a further 20% (n=9) had been pollarded (Figure 5). Death was attributed to the complete removal of the entire sapling from the ground most probably through elephant feeding (Figure 6). A similar mechanism could have caused the pollarding, but these plants managed to resprout basally after the feeding event. Contrary to expectations, only 51% of the live recovered plants (includes pollarded, bark stripped and undamaged) managed to increase in height between November 2007 and November 2008, and by only a maximum of 40 cm. Forty one percent of the saplings actually decreased in height. Seventy percent of the saplings showed some form of browsing, with browsing levels reaching up to 50% of the canopy foliage. These results indicate that even though none of the saplings were topkilled by the intense initial fire itself and were able regain their prefire height through epicormic resprouting, they were subsequently heavily browsed by herbivores. Hence, despite their vigorous regrowth capacity, many saplings were unable to maintain the height regained directly after the fire.

8.4.3. Before and after a controlled fire in the KNP EBPs

Under the field conditions experienced in August 2008, the threshold for stem survival after fire was between 1.2 cm in stem diameter and complete resistance occurred at 4.2 cm (Figure 7). Three (33.3%) of the topkilled individuals between 1.8 and 4.2 cm had been bark stripped (n=2) or pollarded (n=1) before the fire, thus exposing the sapwood to the fire and confounding the pattern. Only 2 of the topkilled individuals did not resprout from the base, indicating a whole plant mortality rate for individuals <7 cm in stem diameter of 7%.

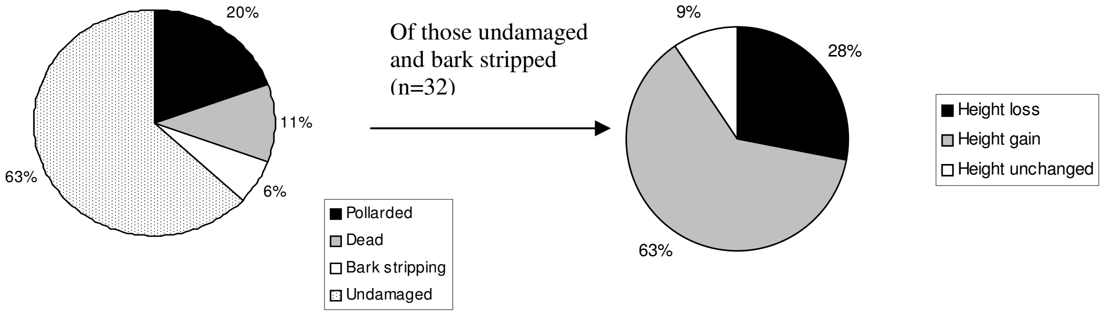

Figure 5: Categorisation of the marula saplings sampled in the Pretoriuskop region of the KNP after an intense accidental fire, in November 2007.

Figure 6: Photograph of a marula sapling pulled completely from the ground, roots and all, most likely due to elephant feeding.

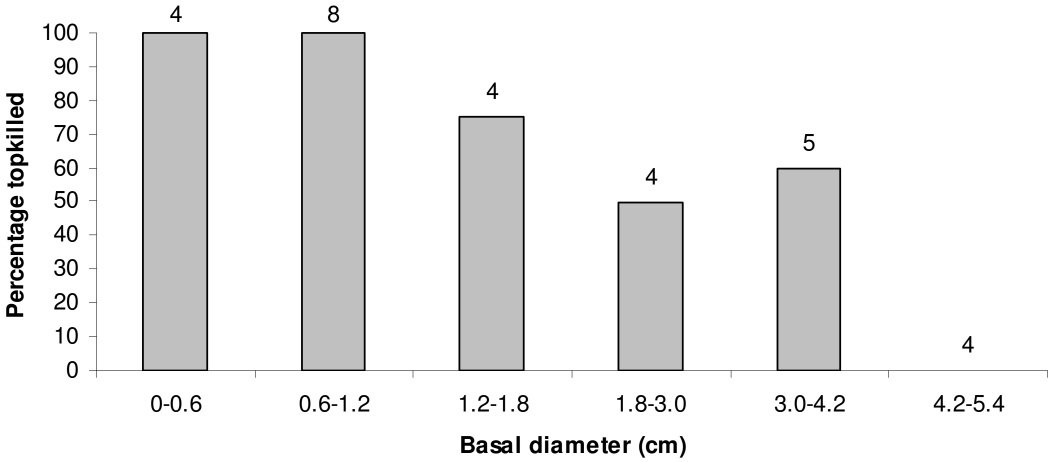

Figure 7: Percentage of marula stems within different size classes (<7 cm in basal diameter), topkilled by a controlled fire in the Pretoriuskop experimental burn plots (EBP) in the Kruger National Park, South Africa (n=29). Values above the bars represent the sample size per size class.

Six of the 20 pollarded and three of the 14 toppled individuals in the burn plots had died due to the fire. The overall mortality rate directly caused by this single fire event for stems >7 cm in diameter was 15% (Figure 8). The most parsimonious significant logistic model (null deviance=54.067, df=59 and residual deviance= 47.460, df=57; Likelihood ratio test: χ^2_6=282.62, p<0.0001) incorporated only prefire state as a predictor of stem mortality, with pollarded stems being more likely to die than undamaged stems (estimate=-2.3716, SE=1.1305, z=-2.098, p=0.036) and no difference found between toppled and pollarded stems (estimate=0.4520, SE=0.8138, z=-0.555, p=0.5786). Since the fire intensity in these plots was relatively low, probably as a result of low fuel loads due to annual burns, these surveys should be conducted in the three, four or six year return period plots for comparison at sites where the fire intensity may be higher.

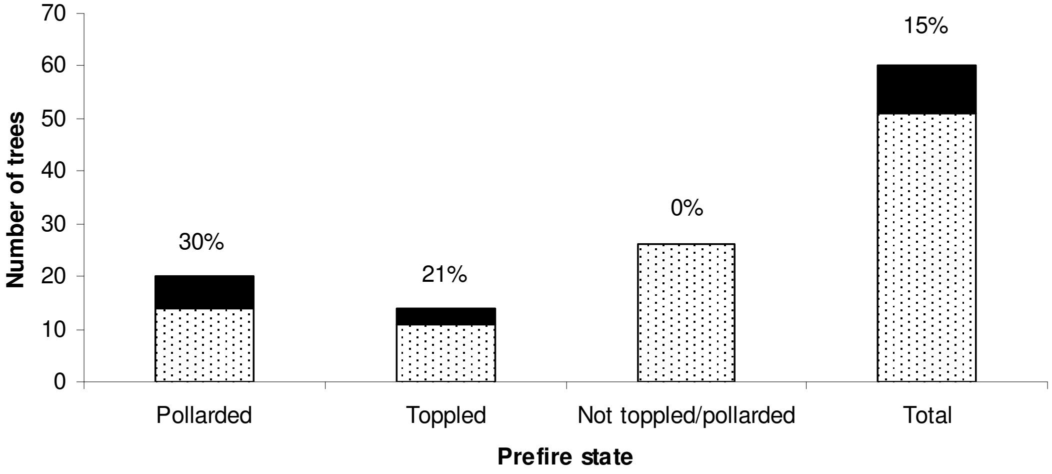

Figure 8: The proportion of total marked marula stems in the Pretoriuskop experimental burn plots (EBP) in the Kruger National Park found dead (black block) after the controlled fire was implemented in August 2008. Values above bars indicate the percentage of dead stems (n=89).

8.4.4. Fire simulations

Of the 40 individual marula stems subjected to burning, 11 (28%) were topkilled by January 2009. Nine of these had a diameter between 1.5 and 3 cm (Table 2). All killed stems resprouted from the base, resulting in no whole plant mortality. The threshold for stem survival of 3 min burns was 3.4 cm (Table 2). No stems above 7 cm were killed and only one stem between 5 and 7 cm (5.5 cm) experiencing the 3 min burn died.

Diameter size and burn treatment both significantly affected canopy dieback and resprouting vigour, but their interactive effect was not significant (Table 3). Stem mortality increased with fire intensity (2 versus 3 min burns) and decreased with increasing stem diameter (Figure 9). Overall, 57% of the burnt stems resprouted basally, while none of the unburnt stems resprouted (Figure 7). Fifty four percent of the individuals that had basal resprouts had not experienced 100% stem death. Individuals experiencing the 3 min burn were more vigorous resprouters than those experiencing the 2 min burn (Figure 10). For individuals <1.5 m in height that were topkilled, the mean maximum basal resprout growth rate was 1.6 m per season (8 months of growth).

When examining the change in percentage canopy dieback over the three monitoring periods (Oct 2008, Nov 2008, Jan 2009), representing 1, 2 and 6 months since treatment respectively, there was an emerging trend of increased canopy dieback over time, however, this failed to reach significance ($F_{2,96}$=2.79; p=0.066) (Figure 11).

Table 2: Numbers (percentage) of marula stems that died (100% canopy dieback/ topkill) from fire simulation experiments, conducted in the Justicia Village communal land, Limpopo Province, South Africa in 2008 ($n_{treatment}$=5; n_{total}=60).

	Diameter (cm)			
Burn Treatment	1.5-3	3-5	5-7	7-10
Unburnt control	0	0	0	0
2 minute burn	3 (60%)	0	0	0
3 minute burn	5 (100%)	2 (40%)	1 (20%)	0

Table 3: Two-way ANOVA results for the fire simulation experiments and the fire and bark stripping simulations, based on percentage canopy dieback, conducted at Justicia Village communal land, Limpopo Province, South Africa in 2008.

Effect	**df**	**F**	**p**
Fire simulations			
Canopy dieback (%) 6 months post treatment			
Diameter	2, 48	8.620	0.0006
Burn treatment	3, 48	7.438	0.0003
Diameter*burning treatment	6, 48	1.985	0.0800
Resprouting vigour (mm) 6 months post treatment			
Diameter	2, 48	6.331	0.0040
Burn treatment	3, 48	2.966	0.0410
Diameter*burning treatment	5, 48	1.188	0.3280
Fire and bark stripping simulations			
Canopy dieback (%) 6 months post treatment			
Bark stripping treatment	3, 38	4.527	0.0083
Burn treatment	1, 38	0.800	0.3765
Bark stripping*burn treatment	3, 38	0.279	0.8400
Resprouting vigour (mm) 6 months post treatment			
Bark stripping treatment	3, 38	6.772	0.0010
Burn treatment	1, 38	2.096	0.1560
Bark stripping*burn treatment	3, 38	0.866	0.8690
Bark recovery (%) 16 months post treatment			
Bark stripping treatment	2, 26	23.80	<0.0001
Burn treatment	1, 26	8.679	0.0067
Bark stripping*burn treatment	3, 26	0.047	0.9538

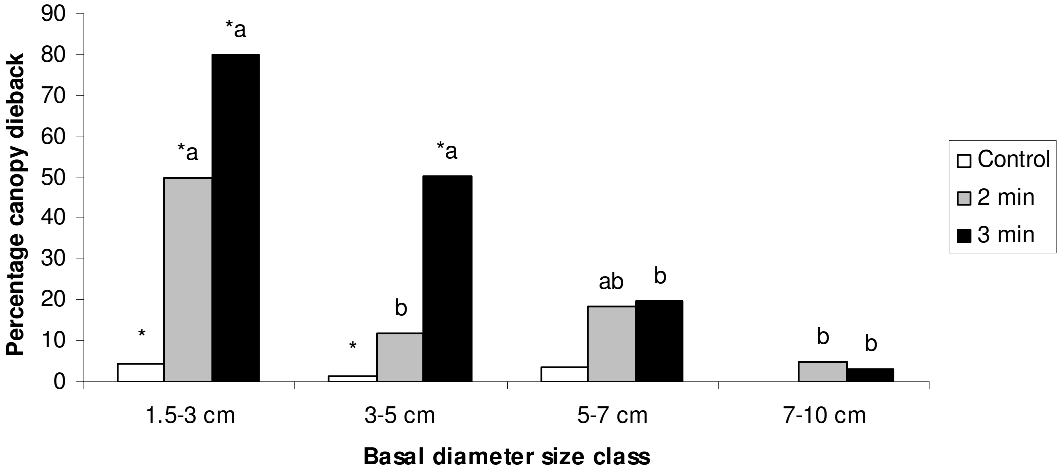

Figure 9: Mean percentage canopy dieback of marula stems (1.5-10 cm in diameter), six months post fire treatment (January 2009) in the Justicia Village communal land, Limpopo Province, South Africa. * indicates significant differences between treatments in the same size class, while different letters indicate differences between size classes.

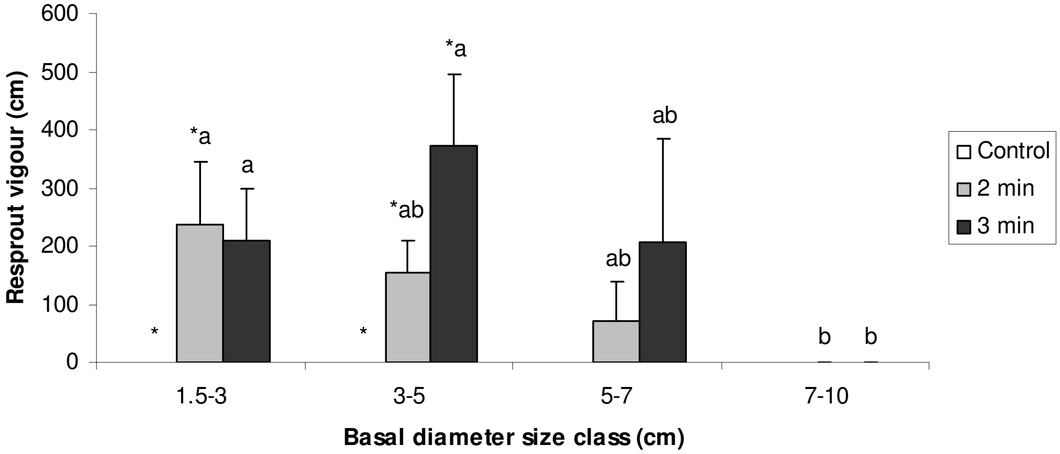

Figure 10: Resprouting vigour (summed length of basal resprouts (mean and SE)) of marula stems (1.5-10 cm in diameter), six months post fire treatment (January 2009), in the Justicia Village communal land, South Africa. * indicates significant differences between treatments in the same size class, while different letters indicate differences between size classes.

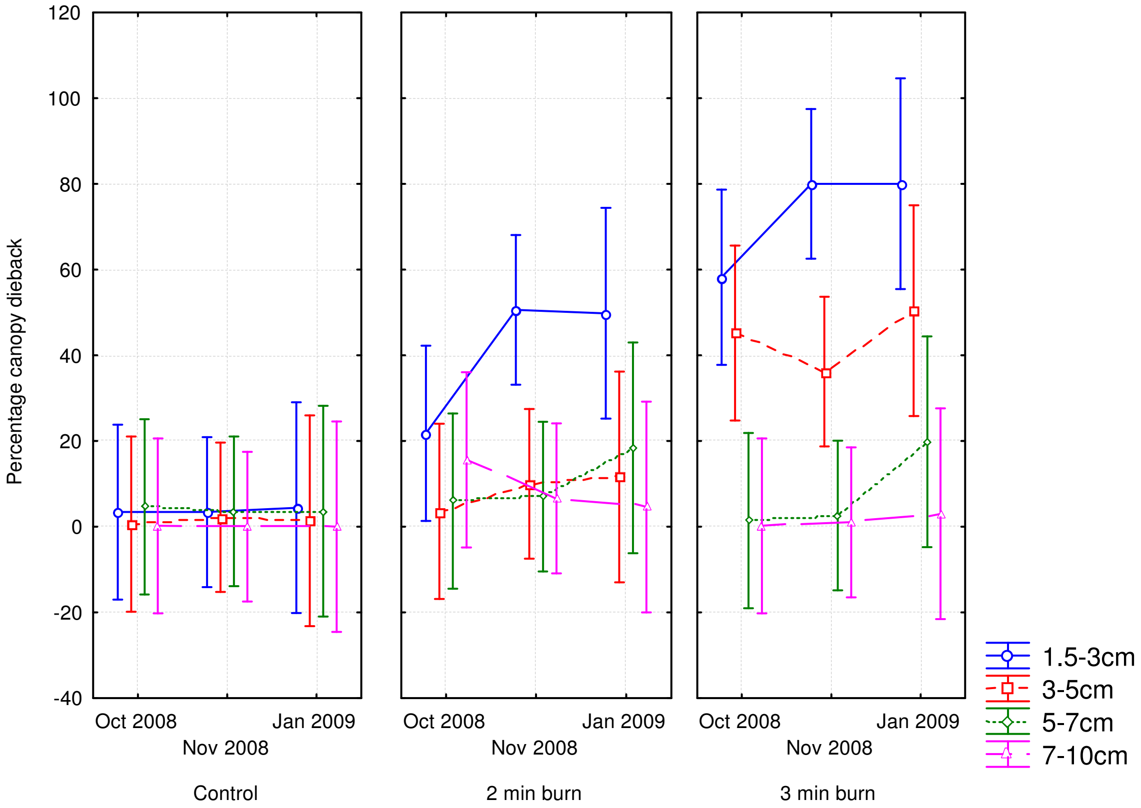

Figure 11: The combined effects of time since fire, burn treatment (control, 2 min burn or 3 min burn) and basal diameter size class on percentage canopy dieback (mean ± SE) of marula stems subjected to fire simulation experiments in 2008 in the Justicia Village communal land, Limpopo Province, South Africa.

8.4.5. Fire and bark stripping simulations

None of the marula stems that received stripping and/or burning treatments had died by January 2009. However, the intensity of stripping had a significant effect on canopy dieback and resprouting vigour in January 2009, but the burn treatment or the combination of stripping and burning had no significant effects (Table 3).

Observations of the stems in November 2009, 16 months post treatment, found two stems dead (one 100% stripped and unburnt and one 100% stripped and burnt), but the effect of the burn treatment on canopy dieback was still not significant (p>0.05). There was, however, a significant effect of stripping and burn treatment on bark recovery but no interaction (Table 3). Stems that had been burnt after stripping were slower to regrow bark than those that had not been burnt (Figure 12).

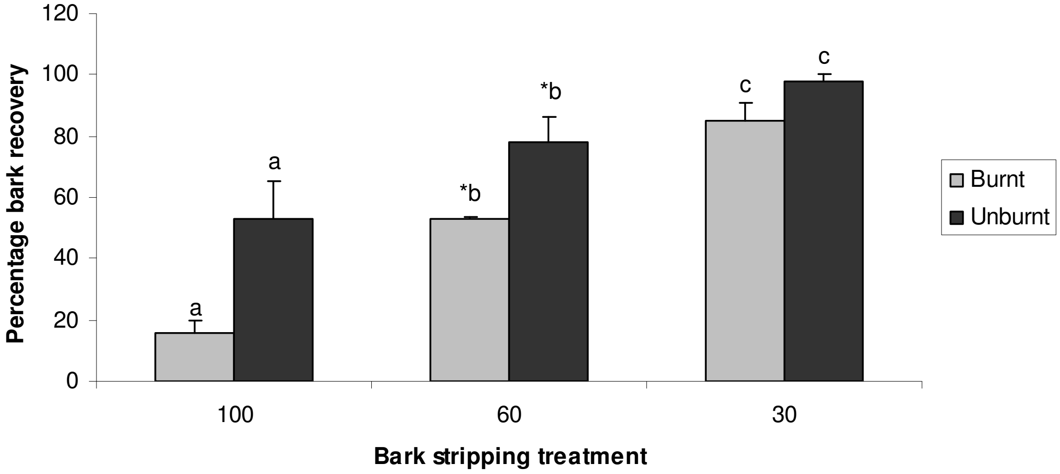

Figure 12: Bark recovery percentage (mean and SE) of stripped and burnt stems in the Justicia Village communal land, Limpopo Province, South Africa, 16 months post treatment. * indicates significant differences between burn treatments in the same stripping class, while different letters indicate significant differences between stripping classes.

8.4.6. Response of saplings to simulated fire and herbivory in the glasshouse

There were no significant differences in height ($F_{3,36}$ =0.83, p=0.49) across treatments before the implementation of the experiment. However, plants assigned to the simulated burning and single defoliation treatment had stem diameters 33% (1.7 mm) larger than the unburnt control plants ($F_{3,36}$ =3.32, p=0.03). Hence stem diameter was used as a covariate in all further analyses.

All topkilled stems resprouted at the beginning of the "rainy season" in the glasshouse. Even after topkill and two 100% defoliation events, almost all marula saplings were able to regain 90% of their prefire height after 5 months of growth (Figure 13). Differences across treatments were observed for percentage change in height ($F_{3,35}$=3.18, p=0.036) and diameter ($F_{3,35}$ =14.12, p<0.001) between July 2009 and January 2010, even after controlling for the effect of initial stem diameter. Unburnt stems and burnt stems did not differ with respect to their height gain, but burnt stems with simulated herbivory decreased slightly in height and diameter after the treatments (Figure 13), indicating that the combination of fire and herbivory has a significant effect on the recovery response of marula stems, whereas fire alone had little effect.

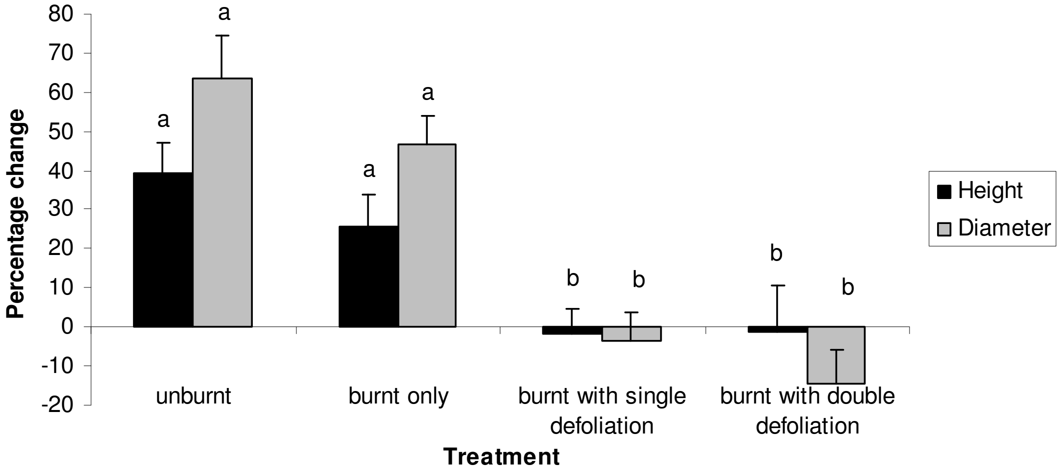

Figure 13: Percentage change in height and diameter five months post treatment, relative to that before the treatments were applied in marula saplings grown in the glasshouse and exposed to simulated fire and herbivory. Different letters indicate significant differences between treatments.

All plants, regardless of treatment regained their pre-treatment sizes. RGR (relative to the size of the plant before any treatment was applied) differed between treatments ($F_{3,35}$=11.05; p<0.0001; Figure 14). Burnt and unburnt stems did not differ in their RGR, even though the burnt individuals had lost all their above-ground biomass after the burning treatment and had to regain it all. Comparing the RGR of the burnt and unburnt plants relative to the size after the burning treatment was applied (essentially zero), indicates that RGR of burnt plants was up to eight fold higher than that of unburnt plants ($F_{3,35}$=900.36; p<0.0001).

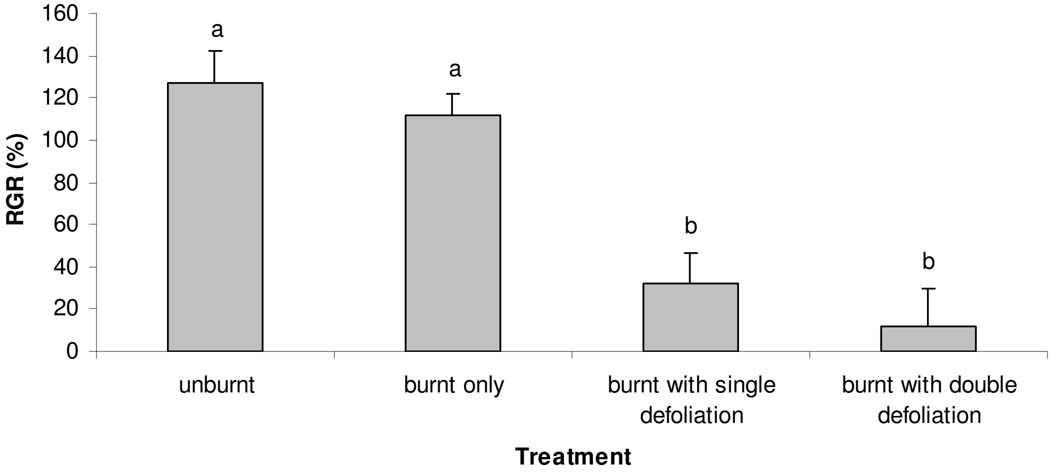

Figure 14: Relative growth rate (RGR, %) (relative to the size before the application of the treatments) (mean+SE) after five months of growth of marula saplings exposed to simulated fire and herbivory. Different letters indicate significant differences between treatments.

After 45 days, 3.4±0.5 g of above-ground biomass per plant had regrown from the base of the burnt stem. After 90 days this had increased to 9.9±1.4 g and after 135 days it weighed 18.1±1.7 g. Final overall biomass was significantly affected by treatment ($F_{3,35}$=3.18, p=0.036), as was above-ground biomass ($F_{3,35}$=3.18, p=0.036) and below-ground biomass ($F_{3,35}$=6.35, p=0.0015) (Figure 15). Above-ground biomass did not differ between the burnt only and unburnt treatments but these two treatments differed with the treatments that had both burning and defoliation (Figure 15). Below-ground biomass only differed between the burnt only and the other three treatments.

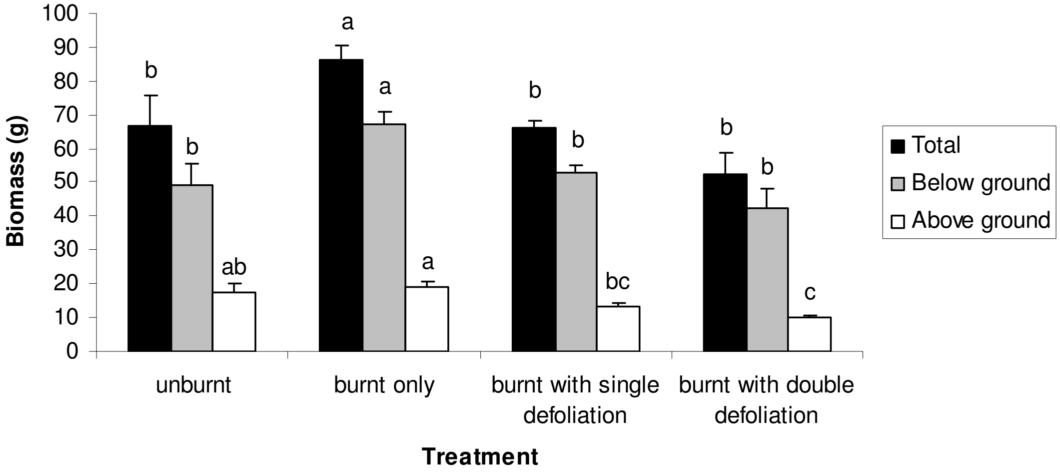

Figure 15: Biomass allocation 5 months post treatment in marula saplings grown in the glasshouse and exposed to simulated fire and herbivory. Different letters indicate significant differences between treatments.

8.5. Discussion

8.5.1. How vulnerable is the marula population in the Kruger National Park to fire?

A large proportion of the bark stripping observed was caused by porcupines. This indicates that outside of the KNP, in reserves with no elephants, marula trees may still be vulnerable to bark stripping and hence increased vulnerability to fire (Yeaton 1988). Additionally the bark of marula is utilised in traditional medicine (Hall *et al.* 2002), and hence bark stripping would be prevalent in unprotected populations as well (eg. Luoga *et al.* 2004). Accumulation of bark stripping on larger stems was also observed by Gadd (2002) for marula in private reserves west of the KNP and by Moncrieff *et al.* (2008) for *A. nigrescens* in the southern regions of the KNP. Although relatively prevalent, severe bark stripping was only evident in 6% of the standing live population. Thus marulas in the KNP do not seem to have large proportions of their sapwood exposed to fire. For complete xylem girdling to occur (and hence stem death) through vessel damage from fire, a large proportion of the stem needs to be exposed through bark stripping within the flame height. These populations, therefore, do not appear to be made vulnerable to mortality through transport tissue damage.

Instead, a large proportion of the adult stem mortality observed could be attributed to stem damage through pollarding and toppling (extreme herbivory). Even without fire these forms of herbivory can result in stem death (Jacobs and Biggs 2002a; pers. obs.). However, the subsequent exposure of vulnerable apical buds to fire through toppling or pollarding increases the vulnerability of adult stems to death. Seven percent of the surveyed population had been toppled, 7.5% had been pollarded by elephants and 26% of the pollarded and toppled trees in the experimental burn plots died after the applied fire. The interaction of bark stripping and toppling also appears to be very important in predicting stem death. The exposure of the sapwood to borers and the subsequent failure of the stem as a result of successive fires, is a feasible mechanism resulting in the mortality of large marula stems in the KNP. Hence bark stripping of large trees, otherwise highly resistant to death, ultimately results in stem death. Porcupine damage at the base of large marula stems is common (27%) and many trees felled by wind or fire, have been found by previous studies (Helm *et al.* 2009). Yeaton (1988) found that four fires would kill a bark stripped *Burkea africana* and fewer than that for *Terminalia sericea*. This is probably a relatively common phenomenon which has been largely overlooked as a mortality agent in large trees. Moncrieff *et al.* (2008), however, concluded that this was not a feasible mechanism for adult stem mortality in *A. nigrescens.*

In terms of juvenile survival in the field, it appears that resprouting marula stems are heavily utilised after a fire. Topkilled plants typically resprout at the base, making the new leaves accessible to a larger proportion of the browsing guild (Midgley and Bond 2001). Epicormically resprouting marulas are highly sensitive to browsing since large amounts of resources are allocated to regaining leaf surface area after the fire, not leaving sufficient reserves for future resprouting following further damage.

8.5.2. Does bark stripping increase the vulnerability of stems to fire, and if so, what is the dominant mechanism?

Surprisingly little mortality of bark stripped and burnt stems occurred. When bark is removed all the phloem and cambium is also removed, in effect girdling the stem, which should result in root death over time due to lack of photosynthate transport to the roots.

Stem death would occur thereafter due to moisture stress (Michaletz and Johnson 2007). Since multi-stemmed individuals were used in these fire simulation experiments, this did not occur. When stems are bark stripped, the xylem is exposed, which should render it susceptible to damage from fire (Moncrieff *et al.* 2008). In the study of fire induced stem mortality in *Acacia nigrescens,* Moncrieff *et al*. (2008) found that with the removal of bark and subsequent burning of stems in the dormant season, rapid topkill and death occurred due to fire induced xylem damage. Balfour and Midgley (2006) showed that fire induced xylem damage resulted in rapid leaf loss and wilting after fire in *Acacia karroo*. These studies concluded that heating and vaporization of the water travelling in xylem vessels by the fire, causes damage to lignified vessels. This results in the reduction of water-conducting capacity and eventual stem mortality. Burning of bark stripped marula stems did not significantly increase the likelihood of mortality, indicating that xylem damage by fire was unlikely.

Possible explanations for the observed lack of stem mortality in the fire simulation bark stripping experiments include 1) Marula is known to store large quantities of water in the sapwood (R. Verweij pers. comm. 2009 PhD Candidate, University of Cape Town). This moisture would reduce the heat exchange capacity of the wood and protect the xylem vessels from fire damage. Therefore, a period of drying between stripping and application of fire (as would occur in the field) may be required; 2) The fire intensity used in the fire simulation experiments was too low to cause damage to the xylem vessels; 3) It may simply be a case of stems taking a significant period of time to die after the exposure of the sapwood to fire (>16 months); 4) Marula stems are extremely well adapted to resist mortality even if completely bark stripped and burnt.

Bark surface area recovery in marula is extremely fast (up to 5.7 cm per annum (Coetzee *et al.* 1979)). If the bark is able to recover before the invasion of boring insects and fire, no detrimental effects of the stripping treatment would be evident. However, given the finding that bark regrowth of a stripped stem after having been burnt is significantly slower than if it had not been burnt, we can deduce that bark stripped stems are made more susceptible to insect and microbial attack by fire, due to the reduction in bark regrowth capacity, and hence will be more vulnerable to future fires.

8.5.3. Fire resistance

Marula stems less than 3.4 cm in diameter (bark thickness of 5.7 mm) were not able to survive an intense simulated savanna fire, while field based observations, indicated a diameter of only 1.2 cm for partial fire resistance. Similar fire simulation experiments have shown that *Acacia karroo* stem diameters of 6.5 cm experienced 90% stem death (Balfour and Midgley 2006), while *Acacia nigrescens* experienced stem death up to stem diameters of 5 cm (Moncrieff *et al.* 2008). This suggests that marula stems are more fire resistant at smaller stem diameters than other common African savanna species. Size classes resistant to fire in the Brazilian cerrado savannas were similar to marula (Hoffman and Solbrig 2003), where topkill from a field fire (for both a high and low intensity burn) was most prevalent in stems <3.2 cm in diameter.

Bark thickness is one of the most important parameters instilling fire resistance in trees (Michaletz and Johnson 2007; Paine *et al.* 2010). The negative allometry of defence (Jackson *et al.* 1999) identified for marula (Appendix 1), where a high investment is made into a defence structure (bark thickness) while the plant is small, could explain its relatively high level of resistance to fire at smaller diameters than other species. Marula does not, however, have a significantly thicker bark than other savanna species at larger sizes (Nikolei 1989). Pinard and Huffman (1997) found that 18 mm bark thickness was required to withstand lethal temperatures from low intensity fires for dry forest trees in Bolivia. Marula has a bark thickness of 4.6 mm at diameters between 1.5 and 3 cm. Insulating capacity per unit of thickness could also influence the protective capacity of the bark over and above the bark thickness (Jackson *et al.* 2007). Nikolai (1989) found that marula stems have a relatively low level of insulation per mm thickness of bark (0.6 C/Joule.cm^{-2}.min^{-1}) compared to other savanna tree species (average= 2.0 C/Joule.cm^{-2}.min^{-1}). Thick bark appears to be compensating for low insulating capacity in marula. The type of bark that a tree has will also influence stem diameter (Williams *et al.* 2007) and hence its resistance to fire. Marula has a scaly bark type characterised by blocks of bark in older trees that are shed intermittently. This is thought to be associated with high fire resistance (Nikolai 1989).

A stem diameter of 3.4 cm corresponds to a height of approximately 1.5 m in marula, which is within the flame height of 2-3 m (Trollope *et al.* 1998). All stems >7 cm in diameter (2.64 m in height) survived fire, even high intensity fires. These results compare well to the estimated fire escape height of 2.75 m (7.35 cm in diameter) for marula found by Jacobs and Biggs (2001). In contrast, all assessed marula stems in the experimental burn plots, within the flame height but greater than 4.2 cm in diameter (1.8 m in height), managed to survive the fire and resprout epicormically. This indicates that fire resistance is more likely to be dependent on stem diameter rather than stem height for marula. It must be noted that these experimental results apply only to burns during the dormant season (May to September) and that fire intensity throughout the burn plots is not constant.

Cognisance must be taken of the fact that the fire simulations were conducted in the dormant season which is when most fires occur in the KNP. Natural fires are more likely to occur later in the year when lightning is more prevalent. This may coincide with a period of leaf flush. Responses of marula stems at this time may differ from the results presented and hence further investigation at different time periods is required.

8.5.4. Resprouting response

Marula stems thicker than 3.4 cm would be able to maintain their prefire height by resprouting epicormically (from the stem rather than the rootstock) and hence have an advantage over other species which would have to resprout basally. Marula should thus be able to escape from the pressures of fire (in the absence of herbivore pressure) after approximately six growing seasons, given an annual diameter growth increment of ~ 0.5 cm (Chapter 4). However, past experimental evidence suggests that resprouts grow 3-40 times faster than an unburnt stem (Hodgkinson 1992). The results from the current study indicate that the resprouts grow eight times faster than an unburnt stem. Hoffman and Solbrig (2003) explain that there is a critical range of pre-burn diameters (approximately 1-2 cm) within which very few or no individuals are capable of regaining their pre-burn size within one year of burning. These individuals are too small for their stems to avoid topkill, yet are too large to be able to regain their original size rapidly, presenting a severe restriction for many species under frequent burning. All topkilled marula stems <3.4 cm in basal diameter were able to regain their prefire height within one growing season, suggesting that marula is not under such constraints at smaller diameters. In addition, Hodgkinson (1992) found a faster resprouting response in Eucalypts in Australia after a more intense fire, similar to that found for marula by this study. A higher fire intensity could cause loss of apical dominance due to an increase in the damage to tissues, increase radiation levels, or increase nutrient status and thus result in a faster resprout response (Hodgkinson 1992). This induced response could aid in a faster recovery of prefire height. Marula stems are hence extremely resilient to damage caused by fire. This suggests that the lack of recruitment to the higher size classes observed, and the "missing size class" are unlikely to solely be due to fire.

The finding that marula saplings grown in the glasshouse are able to at least regain 90% of their prefire height within 5 months regardless of the frequency of subsequent defoliation (one or two), provides further evidence that marula is highly resilient to the effects of fire and herbivory. It appears that the treatments applied during this study were not sufficient to impact the highly vigorous resprouting response of marula saplings to disturbance. Future studies should therefore apply much more frequent disturbance levels in order to induce a significant response. The lack of response observed in root biomass allocation could, however, be ascribed to the seasonal time frame of the experiment. In Chapter 5 it was shown that root biomass allocation occurred between December and March in the glasshouse. Since this experiment was run from July to January, differential increases in root mass across treatments may not be significant at the time of harvest. Hence the lack of difference in root mass observed across treatments is understandable. The higher allocation to roots for the burnt only treatment relative to the others could indicate that plants that have been burnt will start to allocate biomass to roots faster than those that were not burnt, while the burnt and defoliated individuals would not have had sufficient resources to allocate to root material as yet. An assessment of starch content would be instructive in this regard. In *lieu* of this, Chapter 9 indicated that 100% defoliation would result in a 25%

reduction in starch concentration in the roots of marula. Failure to resprout would indicate a depletion of carbon reserves (Bowen and Pate 1993). However, observations of marula stems that were cut repeatedly and continued to resprout after up to six defoliation events, indicate that allocation to starch begins almost immediately post regrowth from topkill (pers. obs.). Such a strategy would make marula highly resilient to the effects of fire and herbivory. The resprouting response may however, be heavily reliant on post fire and/or post herbivory rainfall and by extension, soil moisture content (Cruz *et al.* 2009). Since fire in the savannas where marulas are prevalent, occur mainly in the dormant season (van Wilgen *et al.* 2000), and resprouting in marula is observed to occur before the onset of the rains in the KNP (M. Hofmeyr pers. comm.; pers. obs.), intensive herbivory on the emerging leaves may deplete the resources of the plant. Replacement of these resources may not occur until sufficient rain has fallen. It is therefore, postulated that the strategy of marula is to immediately resprout following a disturbance event, regardless of the prevailing conditions. If moisture is present for growth, carbon allocation to the roots will occur simultaneously. However, if moisture is not present, and further disturbance takes place, the vigorous resprouting response will soon deplete the stored reserves resulting in death of the plant. This would require testing through further multifactorial experimentation, where various levels of watering are combined with different levels and frequencies of defoliation.

In many studies it has been assumed that seedlings have no defence against fires or other large scale disturbances. Marula seedlings start storing starch in the root from 3 months of age (12%; Chapter 4), with maximum values reached by 9 months of age. It is reasonable to assume that resprouting would be possible in seedlings <1 year of age. It has been assumed that only older plants (e.g. >10 months of age- Bowen and Pate (1993); >2 years old- Pate *et al.* (1990)) allocate significant resources to storage. Resprouting requires the use of substantial amounts of stored reserves (Bowen and Pate 1993) and has only been documented in seedlings older than 1 year (Zammit and Westoby 1987; Pate *et al.* 1990; Bowen and Pate 1993). Marula seedlings can resprout directly after germination (pers. obs.), which indicates that previous assumptions on the vulnerability of seedlings to fires do not apply to marula.

8.6. Conclusion

In conclusion marula has a negative allometry of defence in terms of bark thickness making it less vulnerable to fire at smaller stem diameters than other savanna tree species even though the bark has a low insulating capacity. The low recruitment rates and the "missing size class" observed for marula in the KNP therefore cannot be simply attributed to fire alone, but rather to interactions between fire and herbivory or even perhaps herbivory alone. A large proportion of the high adult mortality rates observed are attributable to the high levels of extreme herbivory occurring in the KNP. The quick invasion of the soft wood of marula by borers into the exposed sapwood (Coetzee *et al.* 1979; Guy 1989; Hall *et al.* 2002) after stripping, resulting in aeration for later fires eventually weakening the trunk and ultimately toppling and killing the tree (Yeaton 1988) could encompass a large proportion of the adult mortality observed. Finally fire can directly affect the ability of the bark to recover, exacerbating the original damage through prolonged exposure and probably resulting in a higher probability of stem death in the presence of fire.

Regardless of the remarkable ability of marula to resist mortality from fire, the adult population in the KNP is under threat due to the interaction of fire and utilisation, with 7% possibly dying after the next fire (assuming toppled stems succumb to fire) and another 10% possibly dying after the next 4 successive fires (>50% exposed sapwood following Yeaton's (1988) estimate discussed above). With no recruitment into the adult size classes observed over the last seven years (Helm *et al.* 2009), this does not bode well for the future dominance and perhaps local persistence of marula in some regions of the southern KNP.

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Chapter 9: Responses of *Sclerocarya birrea* subsp. *caffra* (marula) seedlings to simulated herbivory in relation to nutrient availability - resistence or tolerance?

9.1. Abstract

Since marula seedlings are heavily utilised by browsers and due to the lack of seedlings in some populations in the Kruger National Park (KNP), the responses of seedlings to various levels of defoliation and how these differ in the two dominant soil types found in the KNP (ie. nutrient-rich basalt derived clay soil and nutrient-poor granite derived sandy soil) was investigated. A resistant strategy, coupled with compensation only at extreme levels of herbivory, were evident in nutrient-poor granite derived sandy soil, while overcompensation, even at low levels of herbivory, was observed in nutrient-rich basalt derived clay soil. Compensation resulted in decreased allocation to roots and decreased starch reserves. Nutrient-poor environments resulted in higher allocation to stems in undefoliated plants, which could possibly compensate for leaf loss if defoliated. The loss of marulas from nutrient-rich arid environments in the northern regions of the KNP appears to be mediated by overcompensation at low levels of herbivory. This could result in overuse of stored reserves under continuous herbivory prevalent in the KNP, which would be more intense in arid environments. Hence the loss of marula from the northern basalt plains in the KNP appears to be primarily due to herbivory.

9.2. Introduction

Herbivory typically has negative effects on plant fitness. Following a bout of feeding by a herbivore, plants must grow or resprout to recover lost photosynthetic biomass (Holdo 2006). Various intensities and frequencies of herbivory may result in a range of photosynthetic to structural biomass ratios (Childes and Walker 1987), which may affect the future growth of the plant. In order to regrow lost biomass, a plant allocates resources to reserves in the roots, root crowns, stem bases and meristematic tissues (Maze 2001). Following injury, these reserves are mobilized to produce new growth and this could mean that there are fewer resources available for height gain or other functions (Keeley and Zedler 1978). Several factors are thought to affect recovery, including the size of the tree and the intensity, season and frequency of injury (Maze 2001), as well as resource limitations (Craine 2009).

Herbivory can function to increase levels of plant defence (Stevens *et al.* 2008). Plant defence strategies include both resistance and tolerance. A resistant plant deters herbivores via chemical or physical means while a tolerant plant counteracts the negative effects of herbivory by compensating for tissues lost (Stevens *et al.* 2008). Compensatory responses to damage include, reallocation of biomass from below-ground to above-ground structures and activation of dormant meristems that increase branching (Strauss and Agrawal 1999). Plants are thought to be either resistant or tolerant but not both (van der Meijden *et al.* 1988). This prediction assumes that highly resistant plants are not likely to receive damage and thus will not benefit from tolerance. However, several studies have shown no detectable trade-offs between resistance and tolerance (Stevens *et al.* 2008).

Tolerance involves compensatory growth, which is usually measured through differences in relative growth rate (RGR) before and after herbivory, while resistance is measured through assessment of secondary metabolites in the leaves. Tolerance has been shown to be conferred by prior distribution of biomass to storage organs (Hochwender *et al.* 2000) or post-herbivory shifts in allocation from roots to shoots (McNaughton 1983, Trumble *et al.* 1993, Houle and Simard 1996, Mabry and Wayne 1997). Secondary metabolites in the leaves (discussed in detail in Chapter 5), specifically phenolics, are often assessed as indicators of the level of defence in a plant (Makkar 2003). Phenolics are made up of tannins and other compounds, and specifically condensed tannins have been shown to deter herbivores by having anti-feedant properties (Makkar 2003).

Genotype and resource availability may modify the responses of a species to herbivory. With regards to environmental influences on tolerance, the compensatory continuum hypothesis (CCH) predicts that a plant's ability to tolerate herbivore damage is greater in resource-rich environments (Maschinski and Whitham 1989). Although this hypothesis may seem intuitive, the meta-analysis by Hawkes and Sullivan (2001), and further assessment by Wise and Abrahamson (2007), did not generally support the CCH (Stevens *et al.* 2007). Environmental influences on resistance often occur before herbivory takes place, although induced defence is also possible (Feeny 1976).

Does marula defend itself through resistance or tolerance? Herbivory of seedlings by impala and other browsers has been reported to be of high importance by Lewis (1987) and Haig (1999). Since juvenile trees are expected to be more vulnerable to browsing, they should in turn be more defended than mature trees (Rooke *et al.* 2004). Therefore this study focussed on the responses of marula seedlings to herbivory. While many studies on marula chemistry have been conducted, most of these were in the context of its antibacterial or antioxidant properties related to human use (Braca *et al*. 2003; Ojewole *et al.* 2010), and little is known about the chemical responses of marula seedlings to herbivory. With regard to potential resistance traits, undamaged marula seedlings produce large quantities of phenolics and condensed tannins (Chapter 5). They also have relatively fast growth rates, which could facilitate compensatory growth following herbivory (Chapter 4 and 5). Marula has also been shown to store large quantities of starch in its roots (Chapter 5), which could be translocated to allow regrowth after damage. A likely mechanism of tolerance to herbivory in marula would be altered allocation of photoassimilate across plant organs in response to damage (Gassmann 2004) and/or translocation of stored reserves to allow regrowth. The high levels of phenolics found in the leaves indicate that chemical defence could impart resistance to herbivory. Marula could therefore tolerate as well as resist herbivory.

Marula grows on a variety of soil types (Hall *et al*. 2002), but in the KNP there is a significant contrast between nutrient-poor granite derived soils in the west and nutrient-rich basalt derived soils in the east (Venter 1990). The basalt soils are high in clay content and do not drain well, while the granite soils are relatively well drained but relatively nutrient-poor (Venter *et al*. 2003). Chemical and physical analyses indicated that the basalt soils had higher total nitrogen content, total cations and organic carbon than the granite soils, but there was no difference in phosphorus content (Chapter 4). Additionally, pH was more acidic in the granite derived soils, which also had a higher bulk density (Chapter 4). Other reported differences include lower water holding capacity, lower cation exchange capacity, lower organic matter and lower clay content in sandy granitic soils than in clayey basaltic soils (Venter *et al.* 2003). The vegetation on basalt consists mainly of open grassy plains where rainfall is high, and of homogeneous *Colophospermum mopane* shrubveld under lower rainfall conditions further north in the KNP (Gertenbach 1983). The vegetation on granite in the KNP consists of mixed Combretum woodlands, Acacia thickets in the southern and central regions and mopane combretum shrubveld further north (Gertenbach 1983).

Eckhardt *et al.* (2000) found that trees >5 m in height decreased by 38% on basalt and by 15% on granite between 1984 and 1996 in the KNP. Trollope *et al.* (1998) found a negligible decline in large trees on granites and a moderate decline on basalts between 1940 and 1960. However, between 1960 and 1986/1989, a moderate decline was observed in granites while a marked decline was observed on basalts. Viljoen (1988) also reported significant decreases in large trees on the basalt plains surrounding Satara between 1940 and 1981. The differences between the granite and basalt substrates could possibly result from the combination of high grazing pressure due to high herbivore density and the different growth responses of grasses to herbivory on the two substrates (Eckhardt *et al.* 2000). On low nutrient granite substrates, high grazing pressure could result in overgrazing during the dry season and hence reduce the competitive effect of grasses on woody plant growth. On nutrient-rich basalt substrates, overgrazing would not have such a detrimental effect on the grass layer due to the greater recovery potential on fertile soils and therefore would not favour woody plants as much. In addition rainfall could moderate the response of woody plants to herbivory on basalts and granites. Low rainfall could exacerbate the vulnerability of woody plants to herbivory on basalts. Differential responses to disturbance under different resource environments will ultimately affect the long-term dynamics of marula and its distribution.

Marula is abundant within an enclosure (NE) situated in the basalt plains in the northern parts of KNP, but has become all but extinct outside of the enclosure (Jacobs and Biggs 2002; Figure 1). The enclosure was erected in 1968 and by 2001 was made up of more diverse plant assemblages than that found outside (Levick and Rogers 2008). The various woody species also occur at higher densities inside than outside (Levick and Rogers 2008). It could be argued that the current species assemblage and tree density inside the enclosure is an artefact of the protection from browsing, allowing for the regeneration of woody species. In support, anecdotal reports (Joubert 2007) indicate that the area surrounding the enclosure was dominated by mopane as far back as 1940. However, many large marula trees occur within the enclosure, which long predate its construction (Chapter 4), indicating that before 1960 the surrounding mopane shrubveld had a greater frequency of marula trees (and probably other species) than it does today. Where rainfall is higher, marula is a dominant canopy species in the basalt plains surrounding Satara (Gertenbach 1983). Marula occurs at high densities on the granite substrates in the southern regions of the park (Chapter 2). Comparisons of marula density inside and outside an enclosure in the southern KNP on nutrient-poor granite soil in the Pretoriuskop region constructed in 1972 (OHE and HE; Figure 1), do not show large contrasts as observed in the northern enclosure on basalt soils, although marula densities inside are double those outside (Chapter 2). All these results indicate that trees are more vulnerable to herbivory on basalt than on granite substrates. What are the reasons for this difference? Clearly trees prefer sandy granitic to basaltic soils, most likely due to better infiltration of water deeper into the soil profile (Knoop and Walker 1985; Trollope *et al.* 1998). However, this does not explain why a greater large tree decline has occurred on basalts versus the granites. Trollope *et al.* (1998) suggest that clay soils regardless of moisture regime support woody vegetation that is particularly vulnerable to the effects of elephants and fire. The reasons why woody vegetation should be more vulnerable on basalt than on granite remain unclear.

The overall aim of this study was therefore to assess the impact that browsing by impala and other browsers may have on the allocation patterns of marula seedlings by assessing regrowth, starch reserves and chemical defences after simulated defoliation and to determine how soil type interacts with herbivory to influence growth. It was hypothesised that the resistance and/or tolerance response of marula to simulated herbivory depends on complex interactions between the level of damage and soil type.

Specific objectives include:

1) Assess the response of marula seedlings to repeated defoliation at four levels in terms of growth, storage and defence in nutrient-poor granite derived sandy soils
2) Compare the growth response of marula seedlings to a single defoliation event at four levels in nutrient-rich basalt derived clay soil and nutrient-poor granite derived sandy soil.

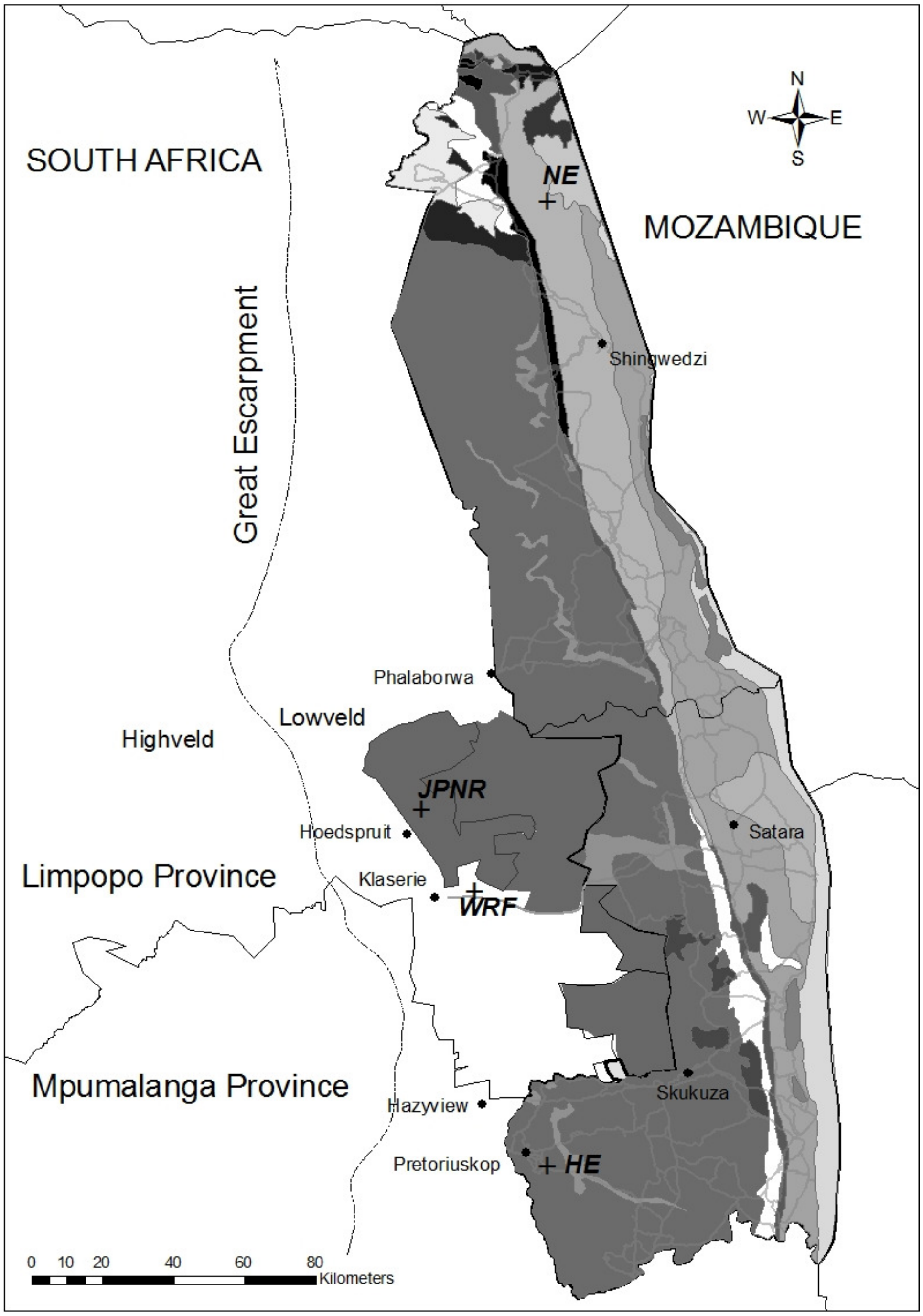

Figure 1: Location of the sites mentioned in the text (crosses) in relation to the Kruger National Park (KNP) and private reserves within South Africa (inset). HE=Hlangwine enclosure in the KNP, NE=N'washitsumbe enclosure in the KNP, WRF=Wits Rural Facility, JPNR=Jejane Private Nature Reserve. The grey scale represents the granitic soils on the left and the basaltic soils on the right.

9.3. Methods

Two defoliation trials in a glasshouse at the University of the Witwatersrand, Johannesburg were conducted: the first in 2007/2008 and the second in 2008/2009. The first trial was used to determine the effects of four defoliation treatments on growth, storage and defence in a single soil type (nutrient-poor granite derived sandy soil collected at Wits Rural Facility (WRF)), while the second trial was used to compare the growth responses of plants grown in two soil types (nutrient-poor granite derived sandy soil and nutrient-rich basalt derived clay soil collected from NE) to four defoliation treatments.

Descriptions of the glasshouse environmental conditions, initial seed preparation and germination, seedling transplant and conditions for growth can be found in Chapter 5. Any differences in methodology are made explicit below.

9.3.1. Defoliation treatments in a nutrient-poor granite derived soil (2007/2008)

9.3.1.1. Experimental design

Forty, one month old seedlings were purchased from the Skukuza Nursery in the KNP (Figure 1) in November 2007. These had been planted from seed that had been collected from trees in and around Skukuza village on granite soils. The seeds were planted into low-nutrient granite derived soil from the vicinity of the village, fertilised with rhino dung, in 5 litre black nursery bags (~170 cm in depth) in October 2007. The soil type used was similar to that found in the NE near Pretoriuskop and has been described in Chapter 4. The seedlings were transported to the glasshouse at the University of the Witwatersrand, Johannesburg and allowed to acclimatise for 3 months, during which time they were regularly rotated to prevent positional effects on intial growth. Spacing between plants was sufficient to ensure that there was no competition for light from adjacent plants. Plants were watered with a sprinkler system for 6 min, providing approximately 250 ml of water per watering session per plant, three times per week. Before treatments were applied the plants were assessed for stem height, number of leaves and stem diameter at the cotyledon node. On the 3rd March 2008 the first defoliation was conducted. A randomised block design was used where the 40 plants were allocated to ten blocks, each consisting of four plants and randomly allocated to four defoliation treatments: i) undefoliated control (ii) removal of

33% (iii) 66% and (iv) 100% leaf area. Since marula has compound leaves with 3 to 13 leaflets per leaf (Palmer and Pitman 1972), the treatments were applied per leaf, removing the correct proportion of leaflets per treatment on all leaves present. Thereafter, monthly defoliation treatments were applied (April, May, June) on the newly emerged leaves only. The plants were harvested in December 2008 at 14 months of age and 9 months after the treatments had commenced.

9.3.1.2. Analysis of total starch in the roots and total phenolics and tannins in the leaves

Total percentage root starch and percentage phenolics in the dried leaf samples were measured as described in Chapter 5 and Appendix 2.

9.3.2. Comparing defoliation effects on nutrient-poor versus nutrient-rich soils (2008/2009)

Marula fruit were collected from Jejane Private Nature Reserve (JPNR) in January 2007 (Figure 1). Seeds were planted in seed trays in October 2008. Germinated seedlings were transplanted into 5 litre pots (and hence contained an equivalent volume of soil to that used in the previous trial (2007/2008)), 5 to 7 days after germination in November 2008. The same soil types used in Chapter 5 were used in this study.

After 3 months of growth, during which time the pots were randomly relocated monthly, a randomised block design with fourteen blocks was used to allocate seedlings to defoliation treatments and to obviate any positional effects in the glasshouse. The same four treatments were used as described above but the defoliation treatments were only applied once. Each defoliation treatment was initially allocated seven plants per soil type, but two seedlings died after transplant due to root damage resulting in only six 0% defoliation replicates in the clay and six 100% defoliation replicates in the sandy soil.

In total, 27 seedlings were grown in each soil type. The plants were assessed for height, stem diameter at the cotyledon node, and number of leaves, before defoliation treatments began. The plants were harvested in February 2009 at 14 months of age.

9.3.3. Data analysis

From the primary data the following variables were derived: percentage biomass allocation to roots (root mass/total plant mass), stems (stem mass/total plant mass), and leaves (leaf mass/total plant mass); absolute leaf stem and root dry mass; leaf area ratio (LAR; leaf area/total plant mass, in m^2/g); leaf area root mass ratio (LARMR; total leaf area/root mass, in m^2/g); root to shoot ratio; mean leaf size (MLS, total leaf area/total leaf number, in mm^2) and specific stem length (SSL; stem length/stem mass, in mm/g). These variables refer to biomass allocation (% roots, leaves and stems), leaf display (LAR), the balance between investment in light intercepting organs versus water and nutrient up-taking organs (LARMR and root: shoot ratio) and the efficiency of biomass investment for height gain (SSL) (Poorter 1999). Whole plant mean above-ground RGR since defoliation was calculated using the formula ln(final stem diameter^2*final stem height)-ln(initial stem diameter^2*initial stem height) (Stevens *et al.* 2008). Height and diameter relative growth rates were calculated using the adjusted formulae. Leaf area (mm^2) was calculated using the regression formula derived in Appendix 1.

Normality was tested using the Shapiro Wilk’s test prior to all analyses. Statistica V.6 and R freeware were used to analyse the data at $p<0.05$. Initial differences between the plants allocated to the four treatments before the treatments were applied were tested using a one-way ANOVA. Since the design for both trials had both a fixed effect (treatments) and a random effect (blocking), a mixed model ANOVA was used to compare between treatments. For the second trial two fixed effects were present (soil type and treatment) and therefore a mixed model factorial ANOVA was used and the interaction between soil type and treatment assessed using Type 1 sum of squares in Statistica due to the unbalance design. Fisher’s LSD post hoc tests were used to determine any specific differences between treatments only if the main effect was found to be significant. Any initial differences between treatments were treated as covariates when the final analysis was done.

9.4. Results

9.4.1. Defoliation treatments in a nutrient-poor granite derived sandy soil (2007/2008)

9.4.1.1. Plant size and biomass allocation

There were no significant initial differences between the plants allocated to the four defoliation treatments in terms of stem height ($F_{3,36}$=0.46, p=0.71), stem diameter ($F_{3,36}$=1.3, p=0.30) and number of leaves ($F_{3,36}$=0.21, p=0.89) (Figure 2).

Treatments did not significantly affect the final stem height, number of leaves or stem diameter of marula seedlings (p>0.20; Figure 2), but there was a significant effect of blocking on stem height ($F_{9,27}$=2.37, p=0.040), with four blocks having plants with stems 30% taller than the remaining six blocks. This indicated the presence of a positional effect, possibly due to differences in light quality or water availability. However, neither stem diameter or number of leaves were affected.

No blocking effect on final leaf, stem or root dry mass was found (Table 1). There was a significant effect of treatment on final dry root mass ($F_{3,36}$=6.4, p=0.001) (Figure 3; Table 1). Root mass for the 100% defoliation treatment was 48% less than the control (0% defoliation).

Irrespective of treatment, marula had a high percentage allocation to roots. Biomass allocation (%) across plant organs was strongly affected by defoliation treatment (Table 1; Figure 4). Defoliation increased allocation to leaves by up to 80% and to stems by 20%. It decreased allocation to roots by 17%. Blocking did not affect biomass allocation. The 33% defoliation treatment had little impact on the allocation patterns of marula seedlings grown in nutrient-poor granite derived sandy soil.

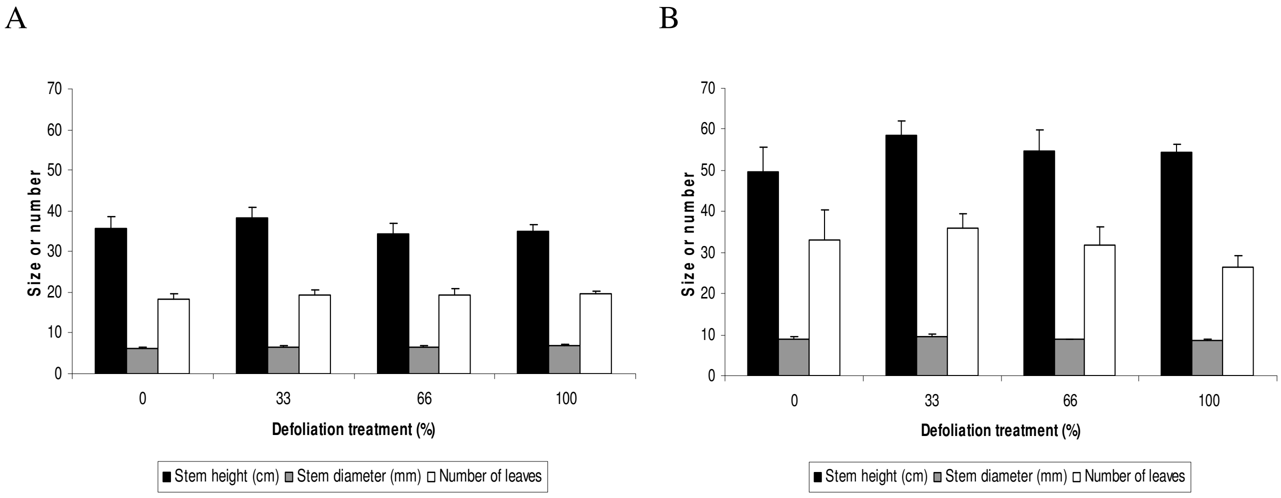

Figure 2: Stem height, diameter and number of leaves (mean ± SE) of marula seedlings grown under glasshouse conditions in nutrient-poor granite derived soils between October 2007 and December 2008 a) immediately before and b) nine months after the application of the first defoliation treatments. No differences across treatments were observed.

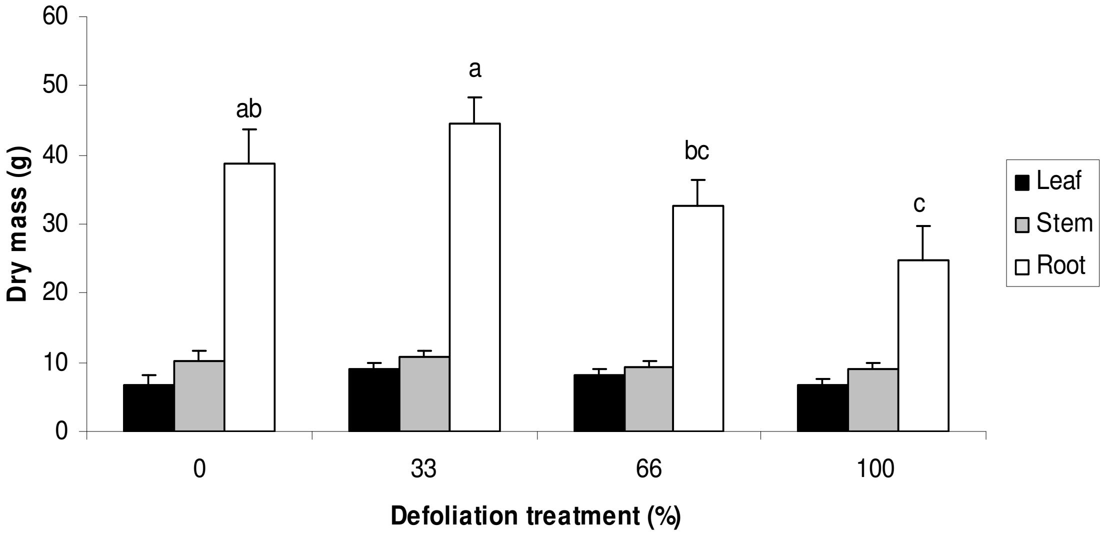

Figure 3: Absolute leaf, stem and root dry mass (mean±SE) of marula seedlings exposed to four defoliation treatments and grown under glasshouse conditions between October 2007 and December 2008. Different letters indicate significant differences across treatments (LSD, $p<0.05$).

Table 1: F ratios and P-values from a mixed model ANOVA assessing the effect of defoliation treatment (df=3) on absolute leaf, stem and root dry mass post defoliation. Corresponding data shown in Figures 3 and 4. N=40 plants. Significant p values in bold.

	Leaves		**Stems**		**Roots**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
	Absolute dry mass					
Defoliation treatment	1.62	0.208	0.56	0.649	3.79	**0.022**
Blocking	1.86	0.104	1.68	0.144	1.05	0.428
	Biomass allocation (%)					
Defoliation treatment	4.99	**0.007**	2.306	0.099	4.04	**0.017**
Blocking	1.592	0.168	0.222	0.989	0.554	0.822

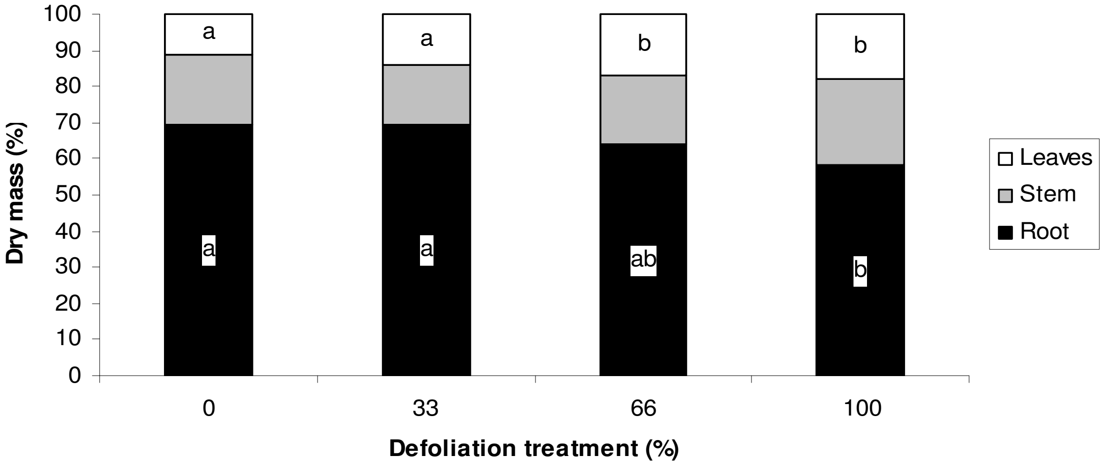

Figure 4: Relative allocation of dry mass to leaves, stem and roots in marula seedlings exposed to four defoliation treatments and grown under glasshouse conditions between October 2007 and December 2008. Different letters indicate significant differences across treatments (LSD, p<0.05).

The defoliation treatments affected the balance between investment in light intercepting organs versus water and nutrient up-take organs (Table 2; Figure 5). LARMR increased with increasing levels of defoliation.

9.4.1.3. Allocation to growth post defoliation

There was no effect of defoliation treatment on whole plant RGR ($F_{3,26}$=1.088, p=0.371) (Figure 6), but there was a significant effect of blocking ($F_{3,27}$=2.38, p=0.039). Height RGR did not differ across treatments ($F_{3,26}$=0.75, p=0.534), however stem diameter was affected by treatment ($F_{3,26}$=3.35, p=0.034) and by blocking ($F_{3,27}$=2.75, p=0.020). A low level of defoliation appeared to be stimulating growth but this was not a significant effect.

Table 2: Results of a mixed model ANOVA assessing the effect of defoliation treatment (df=3) on root/shoot and root/stem ratios as well as LARMR of marula seedlings in response to defoliation treatments. Corresponding data are shown in Figure 5. N=40 plants. Significant p values are in bold.

	Root: shoot		**Root:stem**		**LARMR**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
Defoliation treatment	3.79	**0.022**	2.62	**0.071**	4.42	**0.012**
Blocking	0.93	0.518	0.63	0.764	1.02	0.450

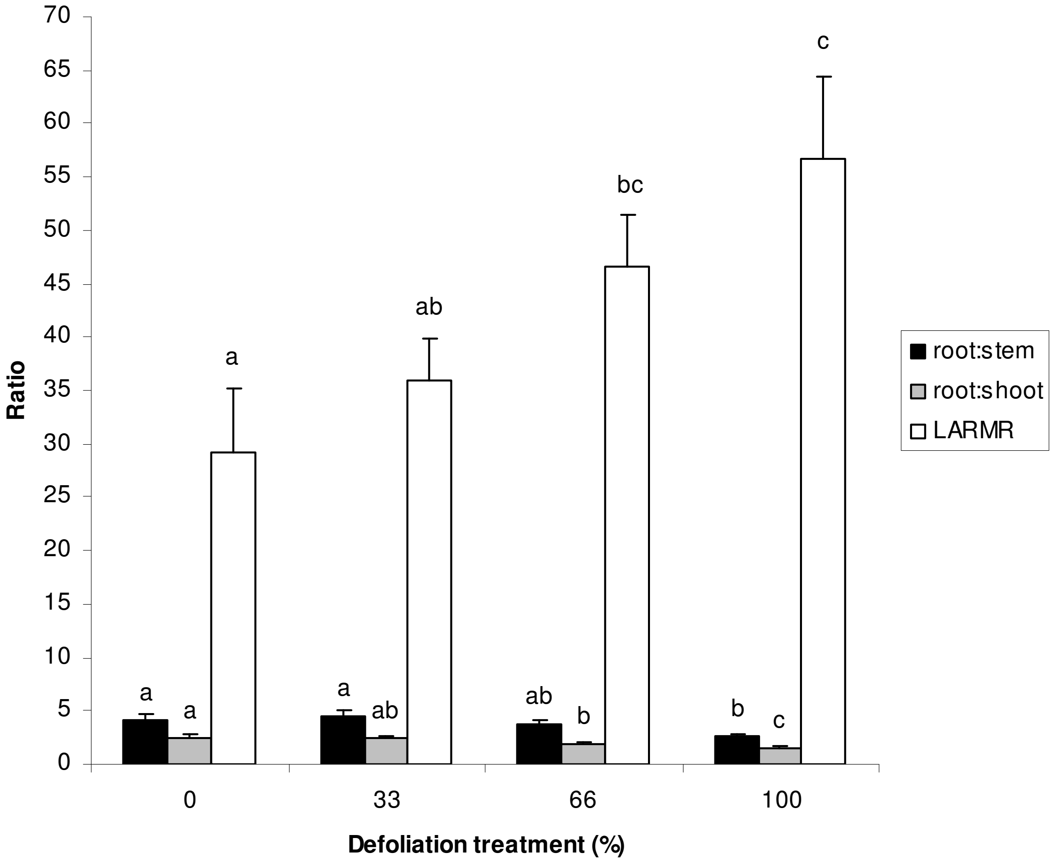

Figure 5: Balance between investment in light intercepting organs versus water and nutrient up-take organs in terms of root/stem, root/shoot ratio and LARMR (cm ^{2}/g) (mean±SE) of marula seedlings exposed to four levels of defoliation under glasshouse conditions between October 2007 and December 2008. Different letters indicate significant differences across treatments (LSD, p<0.05).

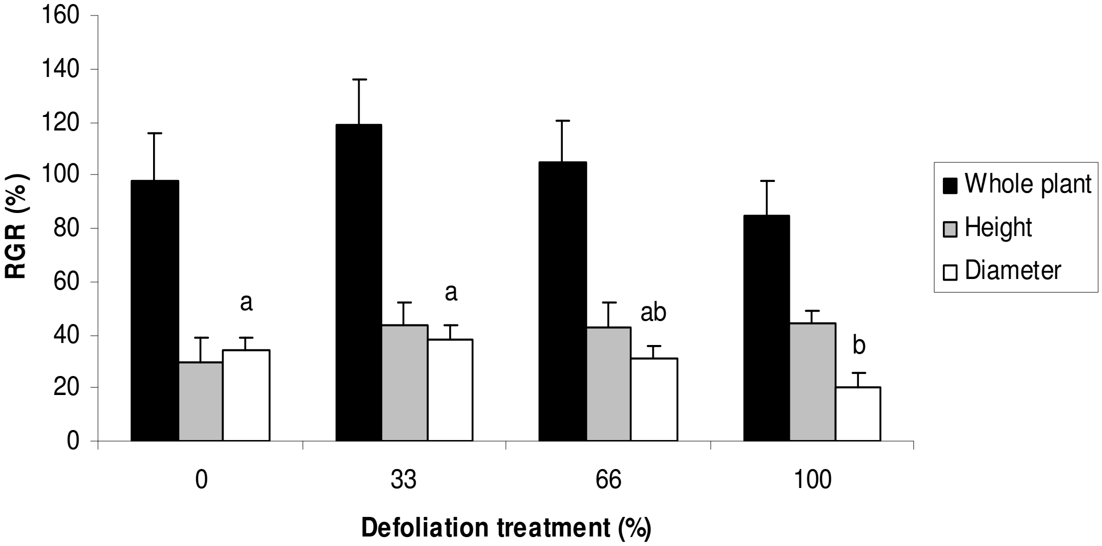

Figure 6: Whole plant, stem height and diameter relative growth rate (%) across defoliation treatments of marula seedlings grown under glasshouse conditions between October 2007 and December 2008. Different letters indicate significant differences between treatments (LSD; p<0.05).

9.4.1.4. Starch allocation

Marula roots had very high starch concentrations (%) irrespective of treatment. No effect of defoliation treatment on starch concentration was found nor of blocking (Table 3; Figure 7). However, there was a significant effect of treatment on starch content, with the 100% treatment having less than half the levels of starch in the roots than the control (Table 3; Figure 8).

Table 3: Results of a mixed model ANOVA assessing the effect of defoliation treatment (df=3) on leaf phenolic and root starch concentrations and contents. N=40 plants for starch and N=39 plants for phenolic analyses due to one plant not having leaves at harvest. Significant p values in bold.

	Starch (%)		**Starch (g)**		**Phenolics (%)**		**Phenolics (g)**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
Defoliation treatment	1.95	0.147	3.16	**0.042**	0.51	0.677	0.93	0.442
Blocking	0.47	0.877	0.75	0.663	0.92	0.525	1.51	0.197

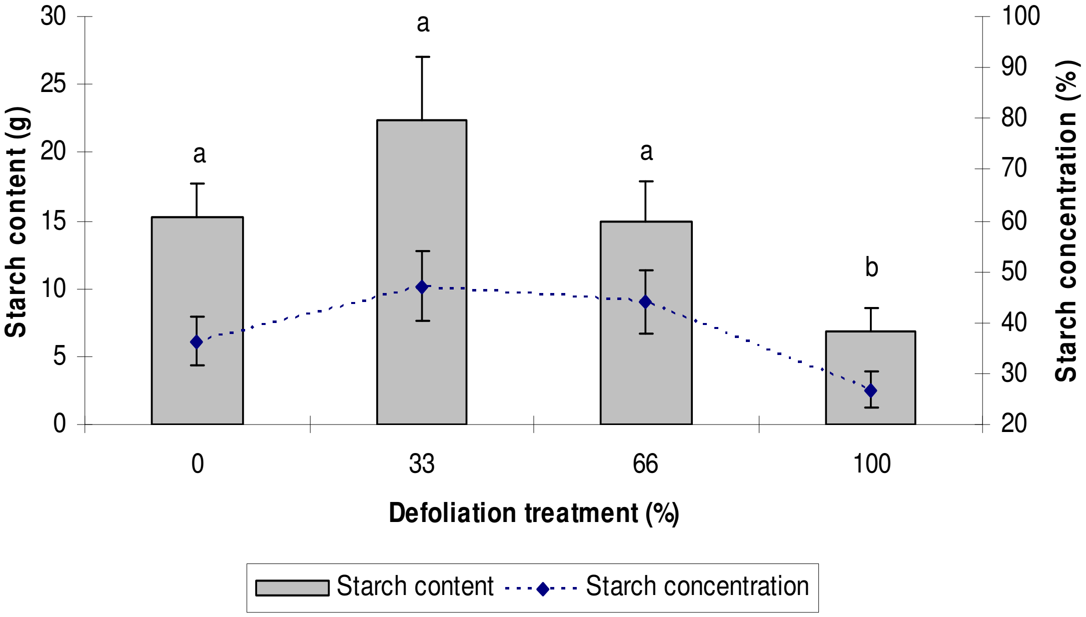

Figure 7: Root starch content (g) and concentration (%) of marula seedlings exposed to four defoliation treatments under glasshouse conditions between October 2007 and December 2008. Different letters indicate significant differences between treatments (LSD; p<0.05).

9.4.1.5. Defence allocation

Contrary to expectation, there was no significant effect of defoliation treatment on leaf phenolic concentration (%) or content (g) of marula seedlings (Table 3; Figure 8). Once again the 33% treatment had slightly elevated levels of phenolics in the leaves compared to the other treatments but this was not significant (p<0.15).

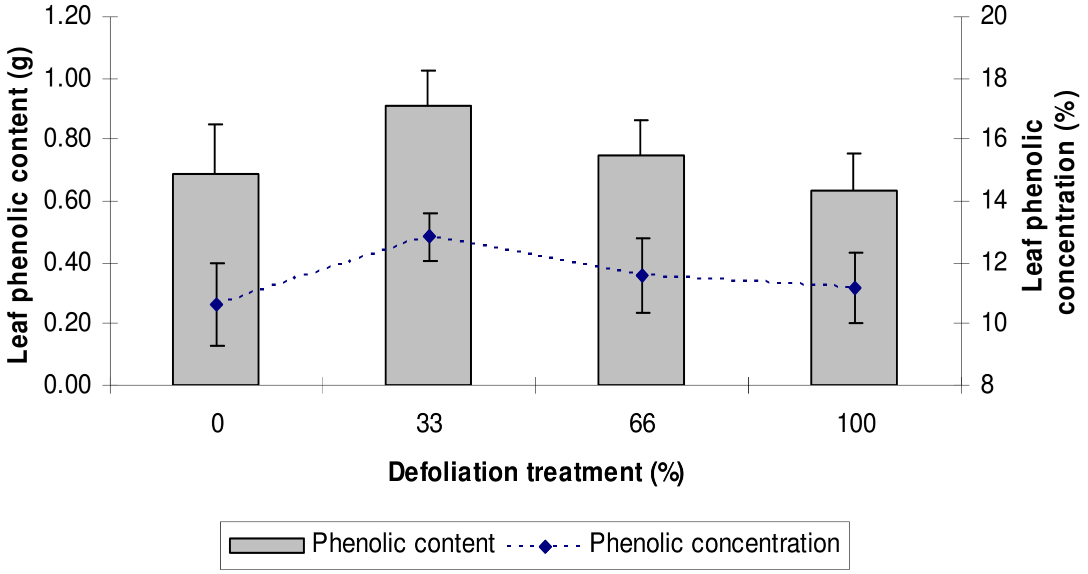

Figure 8: Leaf phenolic content (g) and concentration (%) of marula seedlings exposed to four defoliation treatments under glasshouse conditions between October 2007 and December 2008. No differences across treatments were observed.

Overall, even though not significant (p<0.15), there is a consistent pattern between treatments with 33% > 0%=66% > 100% for both starch and phenolic content as well as RGR.

9.4.2. Comparing the defoliation effects in nutrient-poor sandy granitic versus nutrient-rich clayey basaltic soils (2008/2009)

9.4.2.1. Plant size and biomass allocation

There were no significant blocking effects in pre-treatment growth. Even though the soil treatment had already been applied for the three months acclimatisation prior to the implementation of defoliation treatments, there was no significant initial difference in stem height, stem diameter or leaf number between plants grown in the two soil types (Figure 9, Table 4). Neither were there any differences across defoliation treatments within a soil type (Figure 9, Table 4). At the end of the experiment no blocking effects were found for stem height, diameter or number of leaves. Soil type had an effect on leaf number and the interaction between soil type and treatment tended to affect stem diameter (Figure 9, Table 4).

Table 4: Results of a mixed model two-way ANOVA assessing defoliation treatment (df=3) and soil type (df=1) effects on final stem height, diameter and leaf number. N=54 plants. Type 1 sum of squares. Corresponding data are presented in Figure 9. Significant p values are in bold.

	Stem height		**Stem diameter**		**Leaf number**	
	F	**p**	**F**	**p**	**F**	**p**
Initial measurements						
Soil type	0.83	0.780	0.216	0.651	1.723	0.215
Treatment (trt)	0.279	0.840	0.155	0.926	0.865	0.468
Soil type*trt	0.805	0.499	1.231	0.312	0.533	0.663
Blocking	0.423	0.943	1.273	0.278	0.925	0.534
Final measurements						
Soil type	1.458	0.252	2.419	0.147	5.510	**0.039**
Treatment (trt)	0.192	0.901	0.024	0.995	0.181	0.909
Soil type*trt	0.765	0.521	2.660	**0.062**	0.136	0.938
Blocking	0.713	0.728	1.686	0.114	0.525	0.884

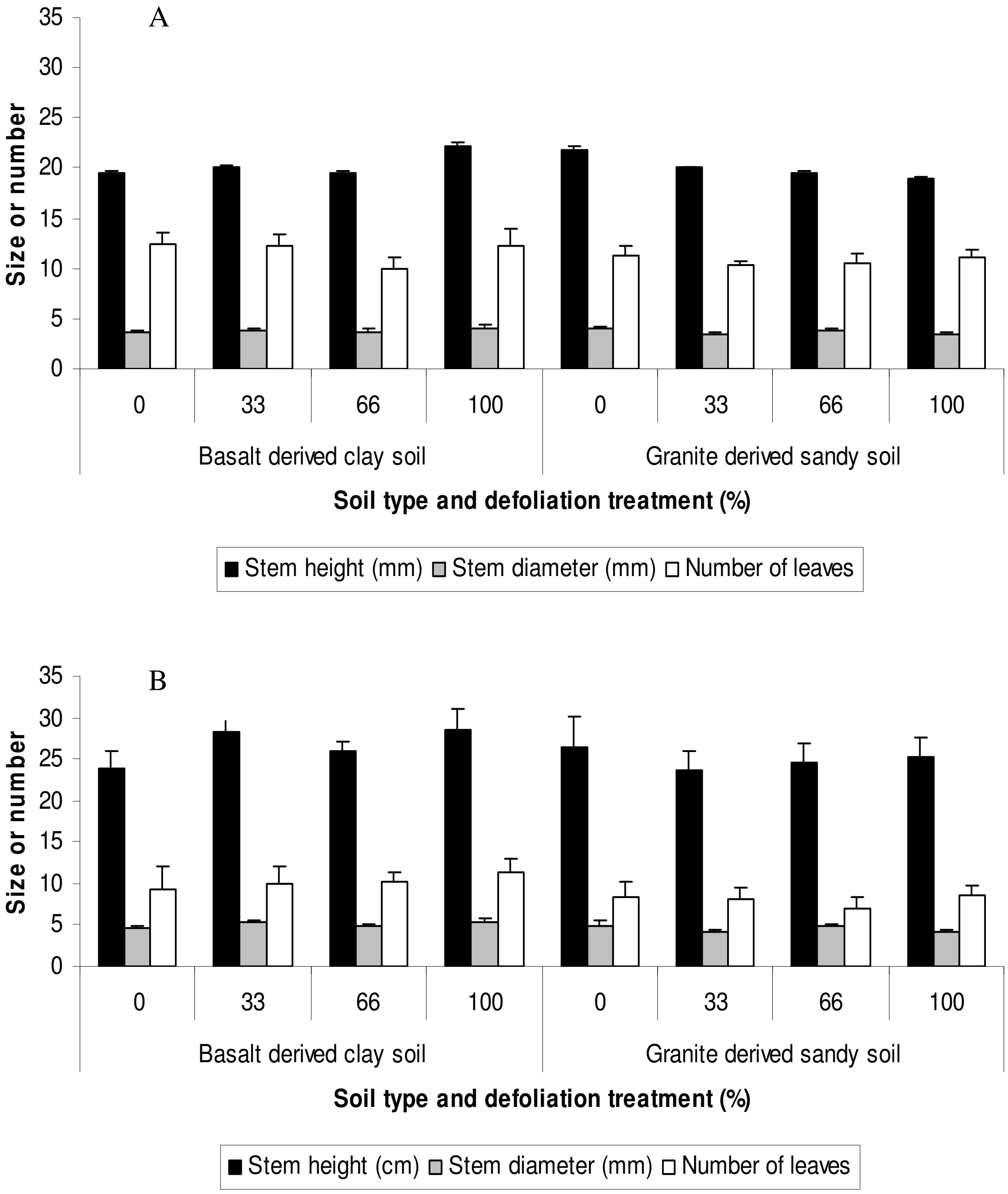

Figure 9: Stem height, diameter and number of leaves (mean ± SE) of marula seedlings grown under glasshouse conditions in nutrient-poor sandy granitics and nutrient-rich clayey basaltic soils between December 2008 and February 2009, a) immediately before and b) twelve months after the application of the first defoliation treatments. No significant differences across treatments were observed.

Plants grown in clay had greater final leaf mass than sandy soils and root mass tended to decrease with increasing levels of defoliation (Figure 10, Table 5). Since there was no difference between the leaf mass of control plants in the two soil types, defoliation appears to modify the relationship between soil type and leaf mass, whereby plants grown in clay compensate more for leaf loss than those grown in sandy soils.

Relative biomass allocation across plant organs was affected by soil type, defoliation treatment and their interactions (Table 5; Figure 11). Plants grown in clay had 7% lower relative allocation to roots and 66% greater allocation to leaves than plants grown in sandy soils. Defoliation increased relative allocation to leaves and the effect was three fold greater in the clay than sandy soils (122% vs 41%). Defoliation decreased relative allocation to roots, but the effect was greater in clay than sandy soils (16% vs 5%). Defoliation increased relative allocation to stems, and the effect was five fold greater in clay than sandy soils (76% vs 15%).

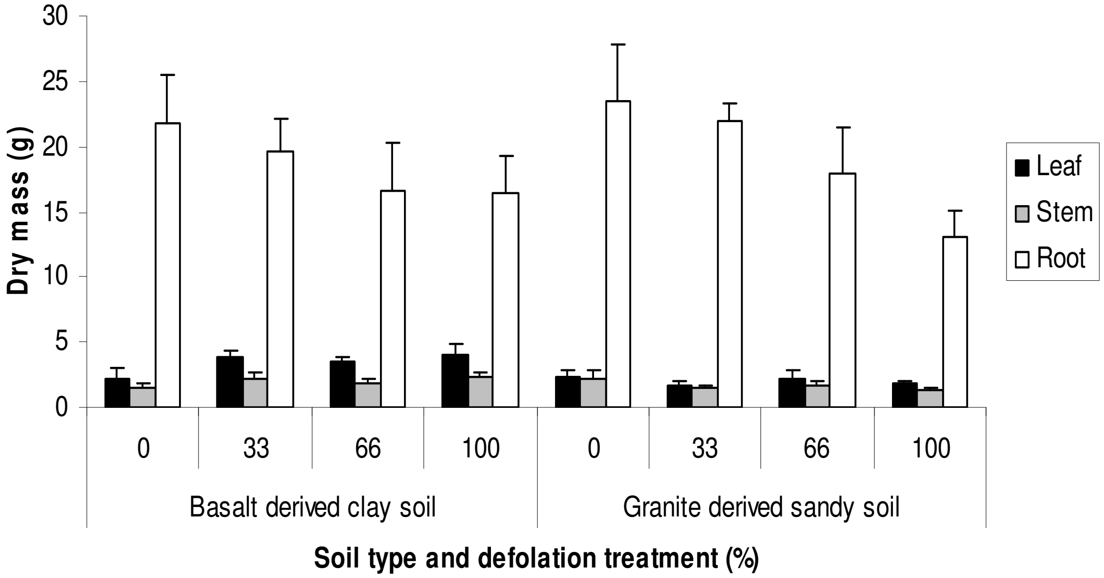

Figure 10: Absolute leaf, stem and root dry mass (mean±SE) of marula seedlings exposed to four defoliation treatments under glasshouse conditions in two soil types between December 2008 and February 2010. No significant differences across treatments within soil types were observed.

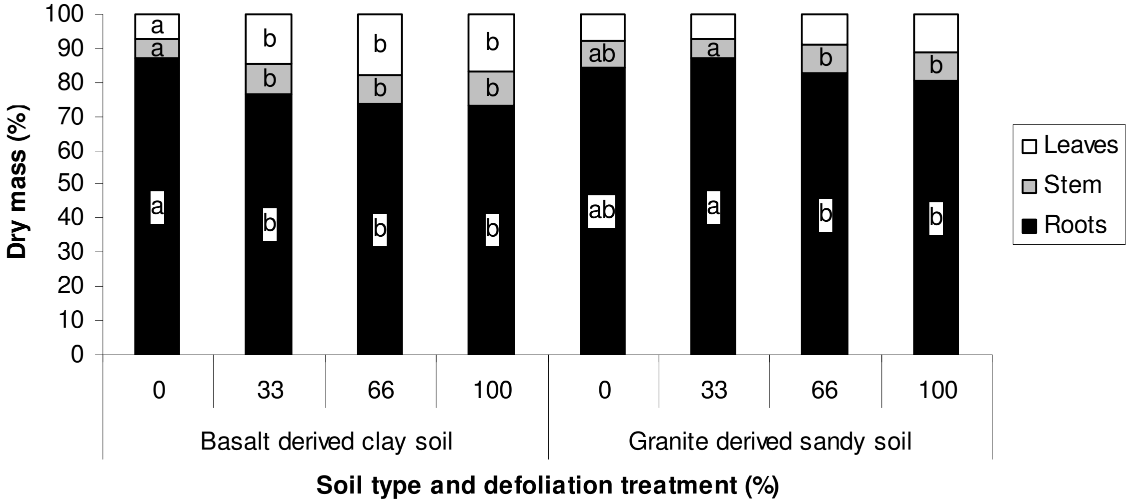

Figure 11: Relative allocation of dry mass to leaves, stem and roots in marula seedlings exposed to four defoliation treatments under glasshouse conditions in two soil types between December 2008 and February 2010. Different letters indicate significant differences between treatments within a soil type (LSD, $p<0.05$).

Soil type had no effect on root: shoot ratio but it did on root: stem ratio (Figure 12, Table 6). Plants grown in sandy soil had higher root: stem ratios than those in clay soil. Root to shoot and root to stem ratios decreased with increasing levels of defoliation. Defoliation appears to modify the relationship between soil type and root: shoot or root: stem ratio through increasing leaf mass in plants grown in clay soils. Above-ground tissue is modified as a whole after defoliation rather than just leaf biomass. With defoliation, plants grown in clay invest more in above-ground tissue than those grown in sandy soil but not at the expense of root biomass. This suggests that starch usage is greater in plants grown in clay soil. It remains to be investigated whether there is an inherent difference in starch allocation in plants grown in basaltic versus those grown in granitic soils.

Table 5: Results of a two factor mixed model ANOVA assessing the soil type (df=1) and defoliation treatment (df=3) and their interaction effects on absolute and relative allocation of total biomass to leaves, stems and roots of marula seedlings. N=54 plants. Corresponding data are shown in Figures 10 and 11. Significant p values are in bold.

	Leaves		**Stems**		**Roots**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
	Absolute dry mass					
Soil type (s)	7.426	**0.019**	0.717	0.414	0.103	0.754
Defoliation treatment (D)	0.424	0.737	0.021	0.996	2.609	0.066
S x D	1.593	0.207	2.065	0.121	0.355	0.785
Blocking	1.636	0.128	1.63	0.129	1.462	0.187
	Relative biomass allocation					
Soil type (s)	17.30	**0.001**	2.376	0.151	14.37	**0.003**
Defoliation treatment (D)	3.50	**0.025**	5.023	**0.005**	4.54	**0.008**
S x D	2.10	0.118	3.569	**0.023**	2.55	0.071
Blocking	0.92	0.536	0.971	0.494	0.91	0.545

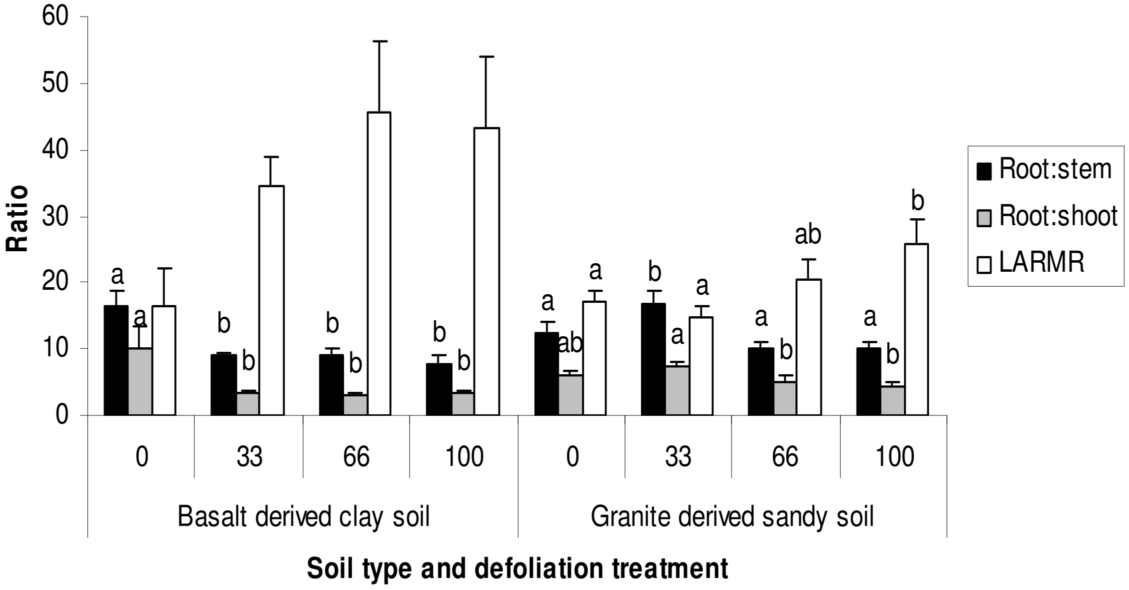

Figure 12: Balance between investment in light intercepting organs versus water and nutrient up-take organs in terms of root/stem and root/shoot ratios and LARMR (cm ^{2}/g) (mean±SE) of marula seedlings exposed to four levels of defoliation under glasshouse conditions in two soil types between December 2008 and February 2010. Different letters indicate significant differences between treatments within a soil type (LSD, p<0.05).

Table 6: Results of a mixed model ANOVA assessing defoliation treatment (df=3) effects on the root/shoot and root/stem ratios as well as LARMR of marula seedlings. Corresponding data are shown in Figure 12. N=54 plants. Significant p values are in bold.

	Root: shoot		**Root:stem**		**LARMR**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
Soil type	1.339	0.271	4.084	0.068	15.13	**0.002**
Defoliation	4.226	**0.012**	5.959	**0.002**	2.97	**0.045**
S*D	3.931	**0.016**	5.309	**0.004**	1.47	0.239
Blocking	0.931	0.528	0.926	0.533	0.85	0.604

9.4.2.3. Allocation to growth post defoliation

There were no differences in relative growth rates between the two soil types in the first three months of growth before the implementation of the defoliation treatments. There was also no significant effect of soil type, treatment or the interaction on relative growth rate (Figure 13; Table 7). There did, however, appear to be a greater above-ground RGR for defoliated plants in basaltic clay compared to the control than in the granite sand. Defoliation in the clay increased RGR by 75% after low levels of defoliation and by 14% in the granitic sandy soil only after high levels of defoliation.

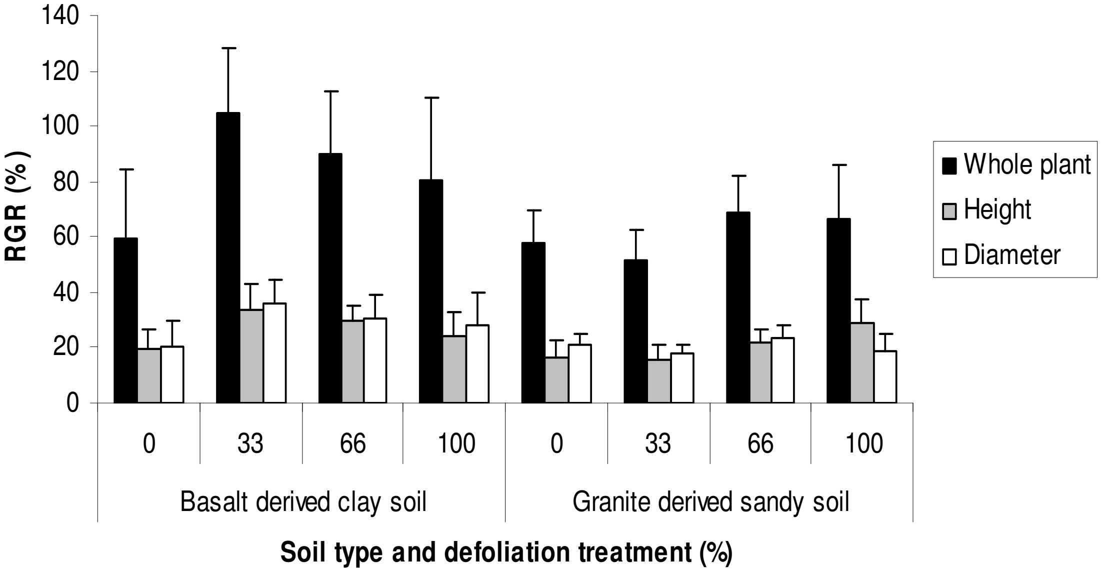

Figure 13: Whole plant, stem height and diameter relative growth rates (RGR, %) (mean±SE) across defoliation treatments of marula seedlings under glasshouse conditions between October 2007 and December 2008. No significant differences across treatments within soil types were observed.

Table 7: Results of a mixed model two way ANOVA assessing defoliation treatment (df=3) and soil type (df=1) effects on the RGR of the whole plant, stem height and diameter in marula seedlings. Corresponding data are shown in Figure 13. N=54 plants. Significant p values are in bold.

	Stem RGR		**Diameter RGR**		**Whole plant RGR**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
Soil type	1.634	0.227	1.978	0.191	1.971	0.187
Defoliation	0.567	0.641	0.315	0.815	0.423	0.738
S*D	0.875	0.463	0.587	0.628	0.665	0.579
Blocking	1.088	0.400	1.515	0.167	1.574	0.146

9.5. Discussion

Marula appears to have a resistant strategy to herbivory in nutrient-poor soils. Under low levels of herbivory, little to no compensation took place. However, herbivory levels above 66% appeared to significantly reduce the fitness of the plant and overall yield declined, i.e. lower stem diameter and number of leaves. Root mass declined significantly with increasing levels of defoliation, indicating a reallocation of biomass from roots to above-ground tissues following severe defoliation. Leaf mass was fully compensated for through this diversion of biomass allocation to leaves. It appears that marula utilises starch reserves to compensate for leaf loss as starch content was reduced to almost 50% of what it would have been if no defoliation had taken place. This starch utilisation reduced root mass. Stem diameter growth rates declined with high levels of herbivory, indicating that only leaf compensation occurred. Levels of defence phenolics remained relatively consistent regardless of defoliation treatment indicating that in marula, chemical defences are constitutive rather than inducible. In nutrient-poor environments the costs of resistance are lower than in nutrient-rich environments as excess carbon can be allocated to defence (Bryant *et al.* 1983; Herms and Mattson 1992). Comparisons with the chemical defence quantities of plants grown in nutrient-rich soils still need to be made.

Low to moderate levels of defoliation have been shown to have little influence on light use efficiency (Watt *et al.* 2007). However, significant gains in light use efficiency have been observed with increasing levels of defoliation in other plant species and this may be related to upward regulation of photosynthesis (Watt *et al.* 2007). Photosynthetic rates often increase with defoliation intensity (Helms 1964; Heichel and Turner 1983). This response is thought to be related to reductions in the source sink ratio which occur as defoliation levels increase (Lavigne *et al.* 2001). Hence marulas in nutrient-poor environments may follow this strategy.

Although roots are generally recognised as important organs for storage, studies on aspen and other species have shown that the stem and branches are a major source of carbohydrates for both initial leaf flush and reflush after defoliation (Landhäusser and Lieffers 2003). Therefore marula may utilise starch reserves from the stem for leaf flush rather than from the roots, whenever possible. Since marulas invest more in stem growth in nutrient-poor than rich soil prior to damage, small amounts of herbivory may result in starch use from the stem rather than depletion of root reserves. Under high levels of defoliation stem starch may no longer be sufficient and hence starch from the roots may then be utilised. The relative proportion of starch in marula roots increased with slight defoliation, but decreased by 25% as the levels of defoliation reached their maximum. The high starch concentration in the roots of marula could also be kept for possible responses to fire or for regrowth in the following growing season post leaf loss in the dormant season. This loss of biomass and relative decrease in starch content indicates that marula will reach a point at which herbivory can no longer be tolerated. It appears, however, that marula seedlings are able to survive the levels of defoliation inflicted on the plants in this experiment, i.e. 100% at the end of the first growing season.

Above-ground growth has been shown to be favoured at the expense of below-ground growth after simulated leaf herbivory (Houle and Simard 1996). This study highlighted the importance of changes in biomass induced by the 66% and 100% defoliation treatments, where root mass decreased by 16% and 36% respectively in sandy soils in 2007/2008. Similarly in 2008/2009 in the same soil type, the root mass decreased by 24% and 44%

respectively. Above-ground biomass decreased negligibly in sandy soils. However, in plants grown in basaltic clay soil which had 66% and 100% defoliation, above-ground biomass increased by 43% and 69% respectively, and root mass decreased by 24 and 25% respectively. Marula plants grown in the nutrient-poor granitic sandy soil, compensated for complete defoliation through reallocation of biomass from the roots to the leaves. In contrast plants grown in nutrient-rich basaltic clay soil overcompensated above-ground tissue. This is assumed to possibly be through increased efficiency in processes such as light use efficiency and photosynthesis (Watt *et al.* 2007) as well as utilisation of stored starch reserves. Defoliation induced increases in allocation to leaves have also been shown in *Eucalyptis nitens* (Pinkard and Beadle 1998) and *Buddleia davidii* (Watt *et al.* 2007). Such overcompensation may not be possible in nutrient-poor soils due to lack of resources. Instead plants grown in such soils will allocate more to stems to compensate for future herbivory at low levels, whereas marulas in nutrient-rich soils will react to even low levels of herbivory through increased growth rates and utilisation of root starch reserves, as well as allocation of biomass to leaves rather than roots. This apparently overactive response to herbivory in nutrient-rich soils may result in the eventual depletion of reserves under continuous herbivory, which would be exacerbated by low moisture availability. In addition, since the response of marula seedlings in nutrient-rich environments is so intense, they may be more vulnerable to subsequent disturbances such as fire. Depletion of root reserves even under low levels of herbivory, would result in a lack of reserves for recovery from fire.

Costs of resistance to herbivory are often modulated by the environment. Marula grows on many soils types (Hall *et al.* 2002) and appears to have a large capacity for phenotypic plasticity since tolerance mechanisms in marula differed between soil types. Mechanisms of tolerance include plant characteristics maintained prior to damage as well as the ability to respond positively to damage (Stevens *et al.* 2008). It is apparent that marula reallocates resources to production and maintenance of photosynthetic tissues to compensate for the impacts of herbivory. In aspen, tolerance was shown to be positively correlated with allocation to stems and negatively correlated with allocation to roots in response to damage under high nutrient conditions, while under low nutrient conditions, tolerance was positively correlated with allocation to stems prior to damage (Stevens *et al.* 2008). Hochwender *et al.* (2000) also found that patterns of biomass distribution prior to damage were predictive of tolerance under low nutrient levels in *Asclepias syriaca*. In contrast, high nutrient levels provide for more rapid compensatory growth, so the genetically determined response to defoliation is more important for tolerance than is biomass distribution prior to damage.

Seedlings of different species and populations of the same species growing on nutrient-poor soil have been shown to develop higher root: shoot ratios than those growing on nutrient-rich soil to compensate for the lower nutrient status (Ladiges 1974). This study did not show any such differences.

Although the 33% defoliation had little effect on the relative biomass allocation patterns in low nutrient sandy soils, this was not the case in high nutrient clay soils. Even small amounts of herbivory impacted on the allocation patterns and the differences between the undefoliated control and the defoliated plants were much more pronounced. The three fold greater increase in relative allocation to leaves in clay versus sandy soils indicates that herbivory results in a much greater compensatory response in clay than sandy soils, i.e. a more competitive response in clay soils versus a relatively stress tolerant response in sandy soils. Relative growth rates after defoliation in clay soils increased slightly and were much greater than those in plants grown in sandy soil. The underlying physiological reasons for this response still require elucidation. Higher nutrient availability results in overcompensation and hence reduces investment in below-ground biomass. This could possibly make marula growing on nutrient-rich soils more vulnerable to fire and subsequent herbivory, due to the reduced investment in below-ground tissue, which would be important for recovery from topkill.

Several plant–herbivore interaction theories, including the continuum of responses model (Maschinski and Whitham 1989) and the growth rate model (Hilbert *et al.* 1981), make conflicting predictions about how plants respond to herbivory under different resource conditions (Hawkes and Sullivan 2001). The continuum of responses model (Maschinski and Whitham 1989) predicts that the probability of compensation for herbivory increases with increasing resource levels. It assumes that at high nutrient levels, tissues can be replaced more easily and plants can grow faster in order to recover more rapidly from damage. In contrast, the growth rate model proposed that plants grow well below their potential RGR_{max} in low resource levels than in enriched resource conditions. Low resource plants will therefore require smaller changes in RGR than high resource plants to compensate for herbivory. Although it is commonly assumed that plants growing in high resource conditions are best able to compensate for or tolerate herbivory (Bryant *et al.* 1983, Coley *et al.* 1985, Bazzaz 1996, Crawley 1997), a meta-analysis of over 200 studies did not support the hypothesis that plants growing in high resource environments can better compensate for or better tolerate herbivory than plants growing in low resource environments (Hawkes and Sullivan 2001). Nevertheless, it appears that marula fits the continuum of responses model since overcompensation only occurred at high resource levels (Figure 14). However, the costs of this overcompensation are likely to be of overall conservation concern, specifically in the KNP where the loss of marula has occurred in the northern basaltic plains and hence require further study.

Mechanical defoliation experiments have been shown to accurately give an indication of the response of plants to various levels of defoliation, but they may not accurately reflect the full range of responses that plants may have to herbivory (Watt *et al.* 2007). Ideally both mechanical and natural herbivory should be studied in conjunction to draw on the strengths of each approach (Watt *et al.* 2007).

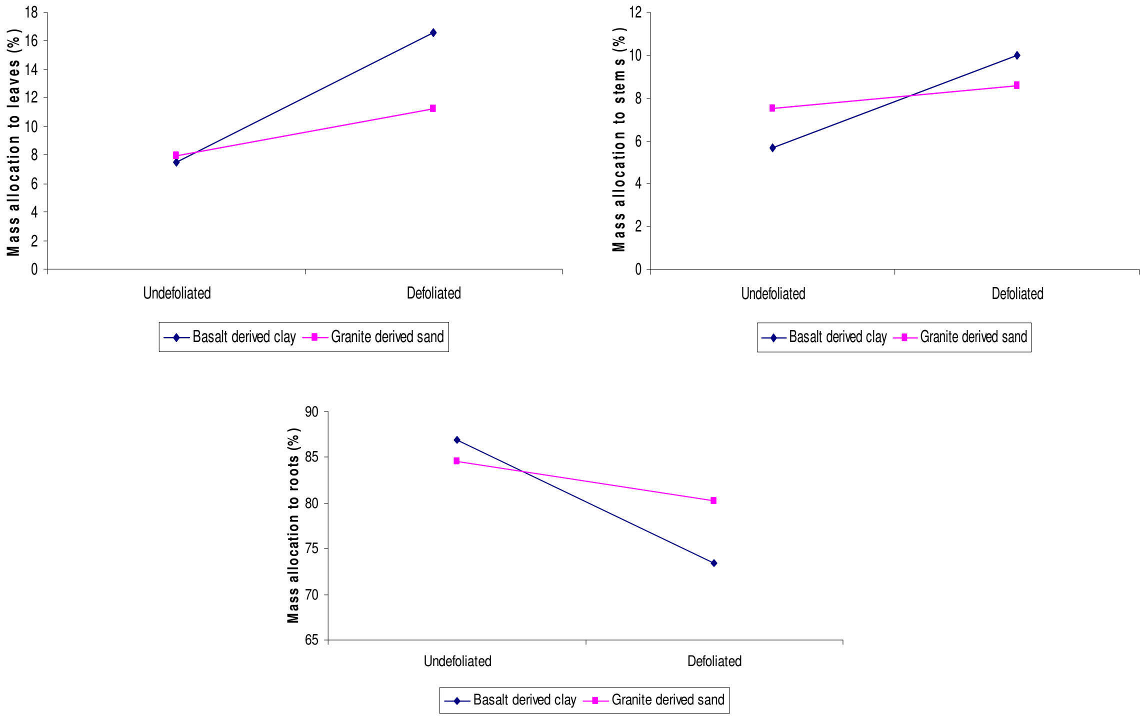

Figure 14: Overall dry mass allocation response trends of marula seedlings to herbivory in different resource conditions.

It is not only the degree of defoliation that may affect the response of plants to herbivory but also the frequency and season (Maze 2001). Seasonal variation in starch reserves have been well documented for a number of species (Kozlowski 1992). In deciduous species, reserves are highest just before leaf drop at the end of the growing season (Chapter 5, Maze 2001). Since seasonal variation exists, then plants should be more susceptible to defoliation when reserves are lowest. Maze (2001) investigated the effect of season of defoliation on recovery of *Acacia karroo, A. nilotica* and *Dichrostachys cinerea*. *A. karroo* was unaffected by season of defoliation and had the same response regardless. Starch content remained relatively high and constant throughout the seasons for this species. However, *A. karoo* is only weekly deciduous. Root reserves in the other two species were depleted after new leaf growth. According to allocation theory (Bond and van Wilgen 1996), deciduous species have their highest root reserves at leaf loss. Species with relatively large root starch concentrations appear to be relatively unaffected by season of defoliation (Maze 2001). Hence it would be expected that marula with its high levels of root reserves may not be affected by season of defoliation as well. However, the large decrease in starch concentration observed at the end of the first growing season in Chapter 5 could be indicative that one year old seedlings may be particularly susceptible to herbivory at this time and this susceptibility may not apply once the seedling is older.

Finally, since the resource environment is only one of many variables known to influence plant responses to herbivory, models which rely solely on plant resources to predict these responses are likely to have limited predictive power (Hawkes and Sullivan 2001). A better understanding of the function of the resource environment may only be possible if an understanding of the fundamental developmental and physiological processes responsible for the observed plant responses is achieved (Hawkes and Sullivan 2001). In addition, an understanding of the effect of herbivory on the overall fitness of the plant is also required in order to determine whether being eaten by herbivores improves fitness or not (Paige and Whitham 1987).

9.6. Conclusion

Marula appears to not only be able to chemically defend its tissues against herbivory, but can apparently also tolerate (i.e. compensate for) tissue loss. The intensity of, and the type of response, is dependent on resource availability (Houle and Simard 1996). The compensatory response of marula is much greater in clay than sandy soils due to higher nutrient availability. This could explain the loss of marula trees in the basalt plains in northern KNP outside of NE relative to sandy granite areas (HE). Overcompensation by marula under high resource conditions could result in negative losses in below-ground biomass which under high and continuous levels of herbivory could result in the death of the plant.

It was initially postulated that tolerance would depend on complex interactions between soil type and herbivory. Final stem diameter, relative stem and root biomass and root: shoot ratio did have such interactions. It appears that these stem traits increase with increasing levels of defoliation in basalt soils but remain relatively constant in granite soils. Root relative biomass allocation decreases more with defoliation in basalt than in granite soils. In the nutrient-poor soils, undefoliated marula plants allocated more to stems than in nutrient-rich soils.

Further experimentation on the effects of soil type coupled with variations in moisture availability and defoliation could elucidate why marulas have all but been extirpated from the arid nutrient-rich environments and not the arid nutrient-poor environments in the KNP. Measurements of starch content and concentration in the roots of plants grown in the two soil types is also required as are the levels of phenolics.

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Chapter 10: Lack of *Sclerocarya birrea* subsp. *caffra* (marula) seedlings in some habitats is not a response to drought or high variability in moisture availability

10.1. Abstract

In certain sites in the lowveld of South Africa that are typical environments for marula trees, there is a lack of seedlings or a regeneration bottleneck. Rainfall variability and summer drought have previously been proposed explanations for episodic marula recruitment. Marula seedlings were grown under glasshouse and growth chamber conditions for one and three months and then watering was stopped entirely. Three month old seedlings could survive for more than 450 days without water while one month old seedlings could survive for 180 days. These results indicate that summer drought is unlikely to affect the survival of marula seedlings directly, but seedlings establishing late in the growing season (such as those germinating in the same season as fruit production after passage through the digestive tract of a mammal) may be less likely to survive an extended dry season than seedlings establishing earlier in the wet season. An investigation into how marula seedlings resist drought was then conducted through glasshouse pot experiments, assessing seedling growth in response to three watering regimes after germination: low, medium and high water availability. Differences between treatments in dry mass, stem height, stem diameter and leaf number were marked after 12 months of growth. Marula seedlings had high allocation to roots regardless of watering regime. Water stress increased allocation to stems, decreased allocation to leaves and resulted in higher root: shoot ratios. Growth rates were also severely reduced. Phenotypic plasticity in biomass allocation appears to be the main mechanism by which marula seedlings are able to survive in arid regions. This study indicates that the lack of marula seedlings in some localities is unlikely to be directly due to summer drought or rainfall variability. However, moisture availability may interact with other constraining variables such as herbivory and fire.

10.2. Introduction

Differences in seedling survival and growth along resource gradients are key factors controlling plant community dynamics (Grubb 1977). Survival and growth at such early stages are major bottlenecks in the recruitment of woody species (Sanchez-Gomez *et al.* 2006). Savannas are characterized by highly variable rainfall regimes (Prins and Loth 1988; Adiku *et al.* 1997). In South African savannas, there is a clear wet season and dry season and the winter months are very dry (Schulze 1997). Mid summer droughts are also common. Seedlings of perennial species inhabiting these environments are regularly exposed to summer drought and are likely to suffer high mortality without specialised drought avoidance or tolerance strategies. Three life-history steps are involved in seedling recruitment: the release and dispersal of seeds, their germination, and their successful establishment (Lamont and Witkowski 1995; Wilson and Witkowski 1998). Drought avoidance can be achieved by spreading the risk of germination over several rainfall events (Clauss and Venable 2000). However, long-lived resprouting, savanna trees can afford to risk germinating nearly all their seeds after one rainfall event, since the adults persist to produce seed in the following year if a germination event is unsuccessful. For example, in Australia, Zammit and Westoby (1987) found risk spreading (more gradual seed release and germination) for the obligate seeder *Banksia ericifolia* but not for the resprouter *B. oblongifolia.* Rainfall variability can be overcome not only through optimal timing of seed germination, i.e. drought avoidance, but also through drought adaptations of the seedlings, i.e. resistance.

Several adaptations may increase a seedlings ability to cope with the consequences of drought and low rainfall including both ecophysiological and morphological adjustments (Kozlowski 1982). Very little is known about these adjustments in tree seedlings (Medina and Silva 1990), but may include deep, dense rooting systems and rapid root growth to exploit the temporally and spatially restricted soil water resources (Witkowski 1991; Leishman and Westoby 1994), high root: shoot ratios (Grime 1979; Otieno *et al.* 2001), stomatal closure and/or leaf shedding to reduce transpiration, or stomatal opening and/or the ability to maintain viability at water potentials below the turgor loss point (Richards and Lamont 1996).

Actual water use depends on the balance between above-ground and below-ground plant dimensions (Schenk and Jackson 2002). Plants are predicted to have larger root: shoot ratios in drier environments (Chapin *et al.* 1993). Higher root: shoot ratios would increase the surface area available for water uptake, as well as reduce transpiration through smaller leaf area (Botha 2006). Partitioning of carbohydrates to processes associated with water uptake instead of carbon acquisition with increased moisture stress has been noted for the seedlings of some savanna woody species such as *Acacia tortilis* and *Colophospermum mopane* (Johnson *et al.* 1996; Otieno *et al.* 2001), but not *A. xanthophloea* (Otieno *et al.* 2001) that is prevalent in wetter savanna areas (Botha *et al.* 2002). Species that are able to shift carbon investment under water stress would have a competitive advantage in arid regions (Otieno *et al.* 2001). The relative ability of seedlings to resist drought stress will determine their relative success under different rainfall variability regimes.

Adult marula trees have been shown to be relatively resistant to drought and water stress (Viljoen 1995; O'Connor 2010). However, seedlings were absent from a number of marula populations in the low altitude savannas of South Africa (Chapter 2), or in other words there is a recruitment bottleneck. Could this be due to lack of moisture in particular seasons? An understanding of the drivers preventing the establishment of seedlings is vital for an understanding of the recruitment bottlenecks in marula populations. Marula seedling establishment has been reported to be episodic by some studies (Walker *et al.* 1986) and continuous by others (Jacobs and Biggs 2002), indicating widespread spatial variability.

Possible explanations for the lack of marula seedlings at some localities include (Wilson and Witkowski 1998; Jacobs and Biggs 2002): 1) lack of rainfall resulting in no germination or high water requirements for germination, 2) high rainfall variability resulting in the death of newly germinated seedlings due to desiccation in summer droughts, 3) high levels of seed predation, 4) high levels of seedling predation and/ or 5) high fire frequency.

It has been argued in Chapter 6 that marula seed predation at some localities such as Jejane Private Nature Reserve (JPNR) is extremely high, indicating that seed predation is a likely candidate. Marula seedlings have also been shown to be highly resilient to the effects of fire from as young as one year of age (Chapter 8), indicating that fire frequency may not be as important a driver in causing the lack of seedlings as proposed by other studies (Jacobs and Biggs 2002). Water requirements for germination are relatively high (Chapter 7), indicating that a decent summer rainfall event is required for germination. Marula seedlings have also been shown to be highly resilient to the effects of herbivory (Chapter 9), further indicating that seedling predation may not be an important driver. Botha (2006) found that marula seedlings can survive for extended periods without water in a glasshouse at the University of Cape Town (lower temperatures than found in the field) indicating that marula seedlings may be highly resistant to drought. This needs to be more thoroughly tested and to include the effects of summer drought on survival and growth.

The aim of this study was therefore to 1) determine the effect of summer drought on the survival of marula seedlings of two different ages and compare these results with those of Botha (2006) and 2) determine the growth and biomass allocation responses of marula seedlings to three different water availability regimes. Investigating the growth and biomass allocation responses of marula seedlings to high (300 ml), medium (150 ml) and low (50 ml) rainfall regimes, and the length of time seedlings can survive under drought conditions, will provide an understanding of whether or not water stress is a significant bottleneck for marula regeneration in the low altitude savannas of South Africa.

10.3. Methodology

Two experiments were conducted in 2008/2009 in a glasshouse at the University of the Witwatersrand, Johannesburg. The first experiment was used to determine the effects of three watering regimes on growth in marula, while the second experiment was used to compare the survival of 1 month and 3 month old seedlings during imposition of drought. The second experiment made use of both a Phytotron growth chamber where temperature and humidity was kept constant, as well as the glasshouse where temperature and humidity were moderated by a heating and cooling system.

Environmental conditions in the glasshouse were the same as those described in Chapter 5. Temperatures in the growth chamber were set at 30 °C for 14 hours of light and 19 °C for 10 hours of dark each day and therefore averaging 25 °C, while humidity was set at 70%.

10.3.1. Survival of marula seedlings exposed to simulated summer drought

109 marula seeds were germinated and the resultant seedlings grown under constant temperature and humidity in a Phytotron growth chamber in 250 ml polystyrene cups with potting soil as the growing medium. During the growth phase the seedlings were watered to saturation three times a week. The drought treatment was implemented on 42 randomly selected seedlings at 1 month of age and on the remaining 67 at 3 months of age. Watering was stopped completely at the start of the drought treatment and no hardening period was implemented. Seedling survival was assessed at 3 day intervals. Seedlings that looked dead were removed and watered to ensure that no resprouting occurred (and it did not). This was repeated until all seedlings were dead.

To determine whether the constant temperature and humidity in the Phytotron, as opposed to natural variations in the field, allowed for survival of droughted plants for longer than expected, the above experiment was repeated in the glasshouse where temperatures and humidity showed typical field variations (Chapter 5). These seedlings were planted 6 months after the start of the first batch of seedlings used in the Phytotron. 103 seedlings were grown in the glasshouse. The drought treatment was implemented on 72 randomly selected seedlings at 1 month of age and on the remaining 31 at 3 months of age. The seedlings were monitored for survival at 30 day intervals as described above.

10.3.2. Growth and biomass allocation responses of seedlings to water availability regimes

Marula fruit collected from JPNR near Hoedspruit in the Limpopo Province of South Africa in January 2007 was used in this trial. Seed treatment, germination and seedling growth followed the same methodology outlined in Chapter 5 unless explicitly stated otherwise. Twenty 10 day old seedlings were transplanted into 2.8 litre pots (20 cm deep) in December 2008. Two watering treatments were allocated randomly to ten plants each. The first treatment represented a high water treatment (similar to that experienced at the

Hlangwine enclosure (HE) near Pretoriuskop, KNP), where 300 ml of water was given every Monday, Wednesday and Friday, and the second represented a more arid treatment (similar to that experienced at the N'washistumbe enclosure (NE) near Shingwedzi, KNP), where 150 ml of water was given on the same days. After assessing the initial response, an additional treatment representing an extremely arid environment was added, where 50 ml was given on the same days at the same frequency. The seedlings for the 50 ml treatment were initially approximately 15 days older than the seedlings for the other two treatments. All plants were assessed for stem height, stem diameter at the cotyledon node and number of leaves before implementation of the treatments. The bottoms of the pots were covered with a pan to ensure that no loss of water via drainage occurred and that each plant received its allocated water. The plants were harvested in December 2009 at 1 year of age.

10.3.3. Data analysis

From the primary data the following variables were derived: percentage biomass allocation to roots (root mass/ total plant mass), stems (stem mass/ total plant mass), and leaves (leaf mass/ total plant mass); absolute dry mass of roots, stems and leaves; leaf area ratio (LAR; leaf area/total plant mass, in cm^2/g); leaf area root mass ratio (LARMR; total leaf area/root mass, in cm^2/g), root to shoot ratio, mean leaf size (MLS; total leaf area/total leaf number, in mm^2) and specific stem length (SSL; stem length/stem mass, in mm/g). These variables refer, respectively, to biomass allocation (% roots, leaves and stems), leaf display (LAR, MLS), the balance between investment in light intercepting organs versus water and nutrient uptake organs (LARMR and root: shoot ratio) and the efficiency of biomass investment for height gain (SSL) (Poorter 1999). One-way ANOVA and Fishers LSD were used to assess differences in water availability regimes. Survival curves for droughted marula seedlings were constructed by dividing the data into 30 day intervals. To statistically compare the survival of the seedlings, the mean number of survival days was calculated from the number of days survived by each plant and then analysed by a one-way ANOVA.

10.4. Results

10.4.1. Survival of marula seedlings exposed to simulated summer drought

All the one month old seedlings had died after 150 days under constant environmental conditions (Phytotron growth chamber) and after 180 days under variable conditions (glasshouse) (Figure 1). Three month old seedlings in the Phytotron and glasshouse survived to the time of writing this chapter, with 80% survival after 180 days and 40% survival after 450 days in the Phytotron and 87% survival after 180 days and 84% after 210 days in the glasshouse.

There was no difference between the number of survival days for the one month old seedlings in the Phytotron versus those in the glasshouse (Figure 2). However, there was a difference between the survival days achieved by the different ages with survival increasing with age (Figure 2).

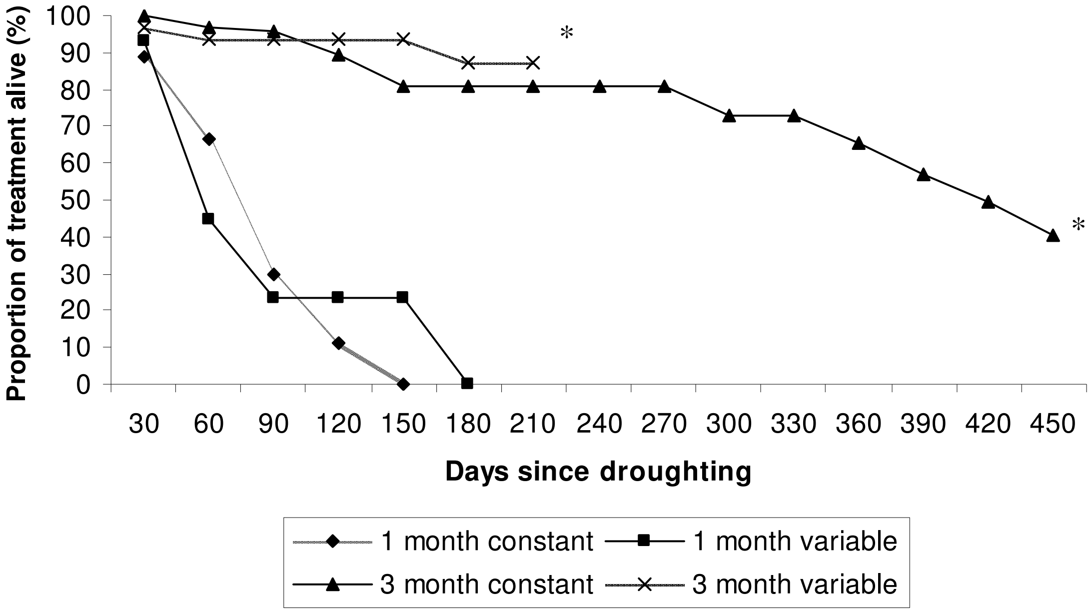

Figure 1: Survival curves of marula seedlings of two ages grown under two environmental conditions (constant versus variable), measured since start of droughting.

* Monitoring continues.

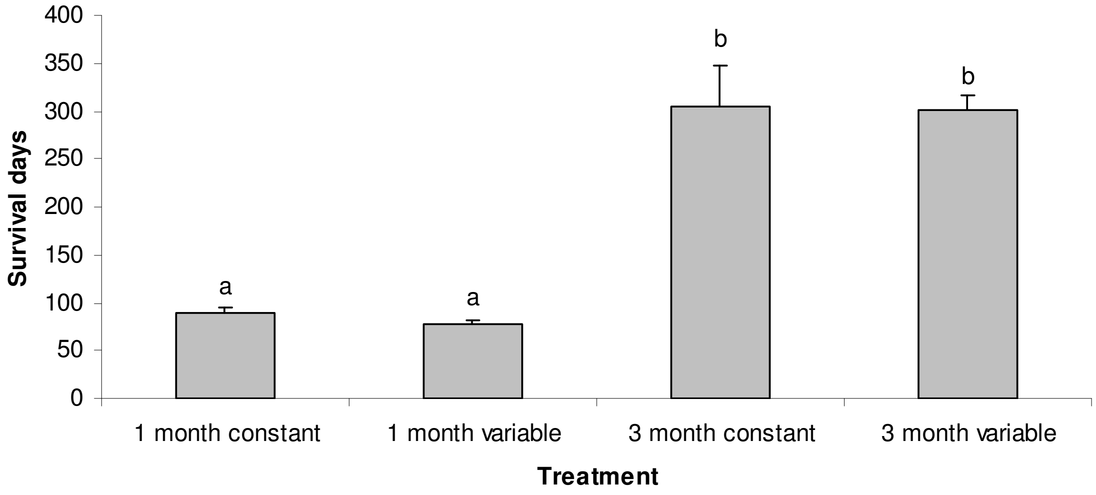

Figure 2: Mean (+SE) number of survival days of marula seedlings in relation to initial age and conditions of growth (Phytotron growth chamber=constant; glasshouse=variable) ($F_{3, 132}$=69.80, p<0.0001). Almost 80% of the 3 month variable seedlings and 40% of the constant seedlings were still alive. Different letters indicate significant differences between treatments (LSD, p<0.05).

10.4.2. Growth and biomass allocation responses of seedlings to water availability regimes

10.4.2.1. Plant size and biomass allocation

Three plants in the 150 ml treatment died during the experiment from unknown causes. As the plants for the low (50 ml) watering regime were 15 days older than the others (see methods), they were initially significantly taller and had more leaves, but were not larger in stem diameter than the other treatments (Figure 3a Table 1). Initial stem height was therefore used as a covariate in all future analyses.

There were significant differences between stem height, stem diameter and number of leaves across treatments, with an increase in each trait with increasing levels of water (Figure 3b, Table 1). Stem height also tended to differ between the 50 ml and 150 ml (LSD, p=0.06).

Table 1: Results of one way ANOVAs assessing the effect of watering regime treatment (df=2) on stem height, diameter and leaf number. Corresponding data are shown in Figure 3. N=27 plants. Initial stem height was used as a covariate for the final measurements. Significant p values are in bold.

	Stem height		**Stem diameter**		**Leaf number**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
	Initial measurements					
Treatment	8.63	**0.0015**	0.17	0.85	16.97	**<0.0001**
	Final measurements					
Treatment	8.71	**0.0015**	23.29	**<0.0001**	20.94	**<0.0001**

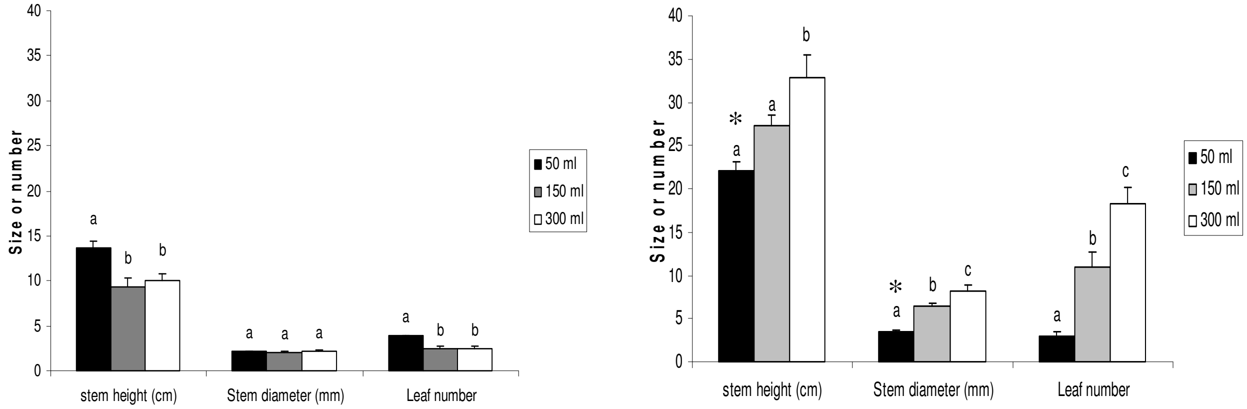

Figure 3: Measurements of seedlings a) before and b) 12 months after the application of the water availability regimes. Letters above the bars represent significant differences between treatments (LSD, p<0.05). * differed at p=0.06. Note 50ml plants were 15 days older than the 150 and 300 ml plants.

After 12 months, absolute leaf, stem and root dry mass differed significantly between all treatments, with all increasing with increasing levels of water (Figure 4, Table 2).

By doubling the water received from 150 to 300 ml, root, stem and leaf mass increased by 79%, 100% and 73% respectively. Overall the plant biomass increased by 80%. By tripling the water received from 50 to 150 ml plant biomass increased by 473% and by increasing the water six fold plant biomass increased by 932%.

Marula seedlings allocated high proportions of their biomass to roots regardless of treatment. Relative allocation of dry mass to stems and leaves differed significantly between the 50 ml and the other two treatments but not between the 150 ml and 300 ml treatments (Figure 5, Table 2). Stem mass relative allocation was much higher when water availability was low.

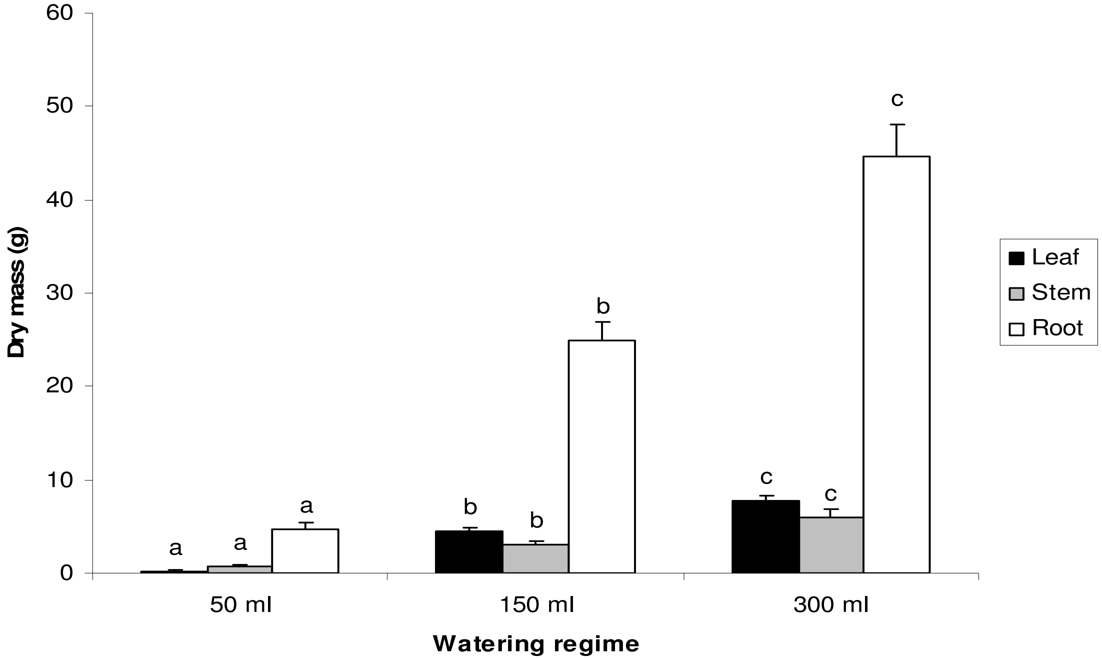

Figure 4: Absolute leaf, stem and root dry mass (mean±SE) of marula seedlings exposed to three watering regimes and grown under glasshouse conditions between December 2008 and December 2009. Different letters indicate significant differences between treatments (LSD, p<0.05).

Table 2: Results of one way ANOVAs assessing the effect of watering regime treatment (df=2) on absolute and relative dry mass allocation to leaves, stems and roots. Corresponding data are shown in Figure 4 and 5. N=27 plants. Significant p values are in bold.

	Leaf mass		**Stem mass**		**Root mass**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
	Absolute allocation					
Treatment	105.55	**<0.0001**	22.18	**<0.0001**	77.07	**<0.0001**
	Relative allocation					
Treatment	19.29	**<0.0001**	4.67	**0.019**	2.13	0.14

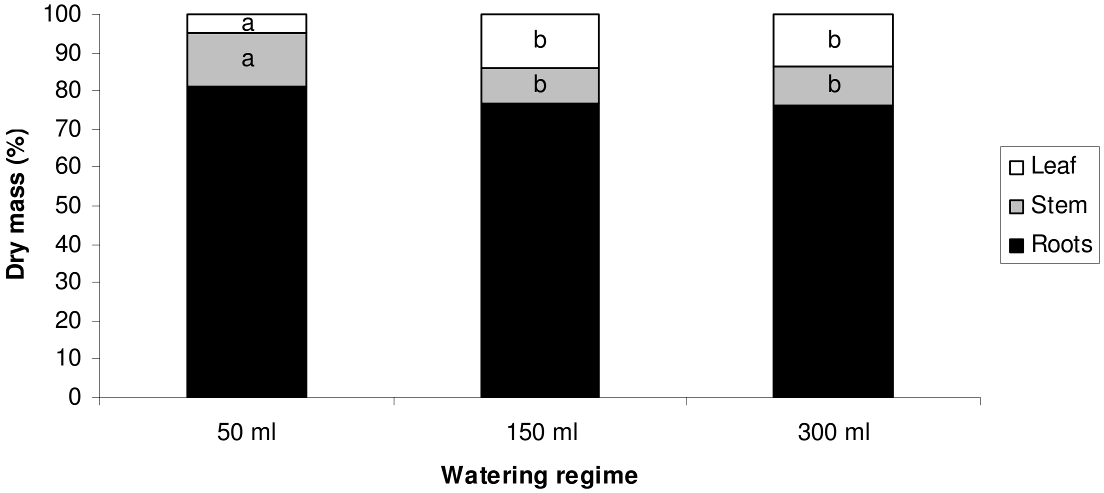

Figure 5: Relative biomass allocation to roots, stems and leaves in marula seedlings exposed to three watering regimes and grown under glasshouse conditions between December 2008 and December 2009. Different letters indicate differences across treatments (LSD, p<0.05), with no differences for root mass allocation.

The significant effect of watering treatment on root: shoot ratio and LARMR (Figure 6, Table 3) indicates that marula responded to water stress by investing more biomass into roots than shoots and hence allocated resources to water uptake at the expense of plant growth.

Table 3: Results of one way ANOVAs assessing the effect of watering regime treatment (df=2) on the root: shoot and root: stem ratios, LARMR, LAR, MLS and SSL. N=27 plants. Significant p values are in bold.

	Root: shoot		**Root:stem**		**LARMR**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
Treatment	3.41	**0.049**	1.85	0.179	4.84	**0.017**
	LAR		**MLS**		**SSL**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
Treatment	6.81	**0.0045**	13.33	**0.00013**	47.22	**<0.0001**

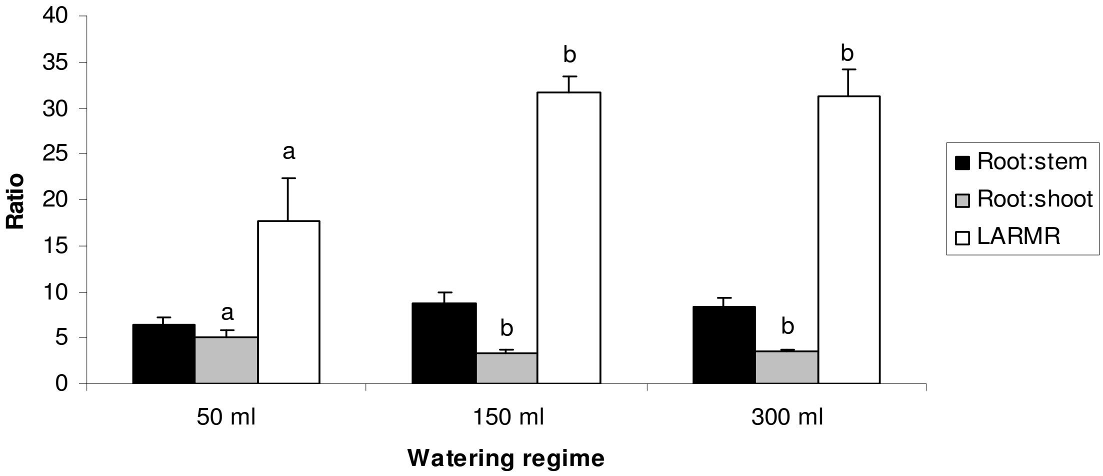

Figure 6: Balance between investment in light intercepting organs versus water and nutrient up-take organs in terms of root/stem and root/shoot ratios and LARMR (cm ^{2}/g) (mean±SE) of marula seedlings exposed to three watering regimes and grown under glasshouse conditions between December 2008 and December 2009. Different letters indicate significant differences (LSD, p<0.05).

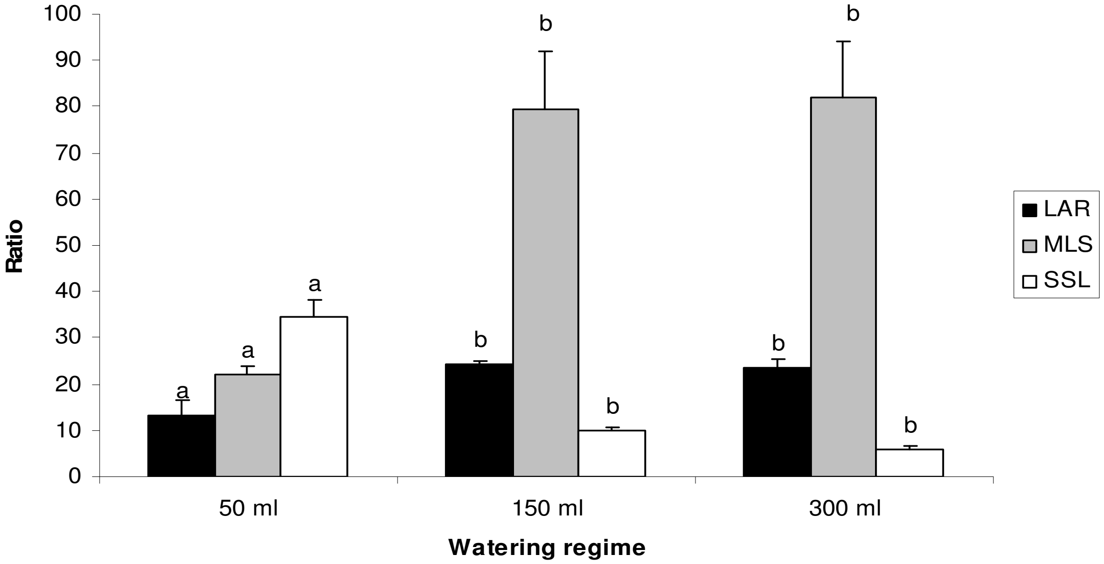

Figure 7: Leaf display and efficiency of biomass investment for height gain (mean±SE) of marula seedlings exposed to three watering regimes and grown under glasshouse conditions between December 2008 and 2009. Different letters indicate significant differences (LSD, $p<0.05$).

10.4.2.2. Allocation to growth

There was a significant effect of watering treatment on relative growth rates with the 50 ml treatment consistently lower than the other two but no difference between the 150 and 300 ml treatments (Table 4; Figure8).

Table 4: Results of one way ANOVAs assessing the effect of watering regime treatment (df=2) on the RGR of marula seedlings. Corresponding data are shown in Figure 8. N=27 plants. Significant p values are in bold.

	Whole plant RGR		**Height RGR**		**Diameter RGR**	
	F-ratio	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
Treatment	52.05	**<0.0001**	20.56	**<0.0001**	56.76	**<0.0001**

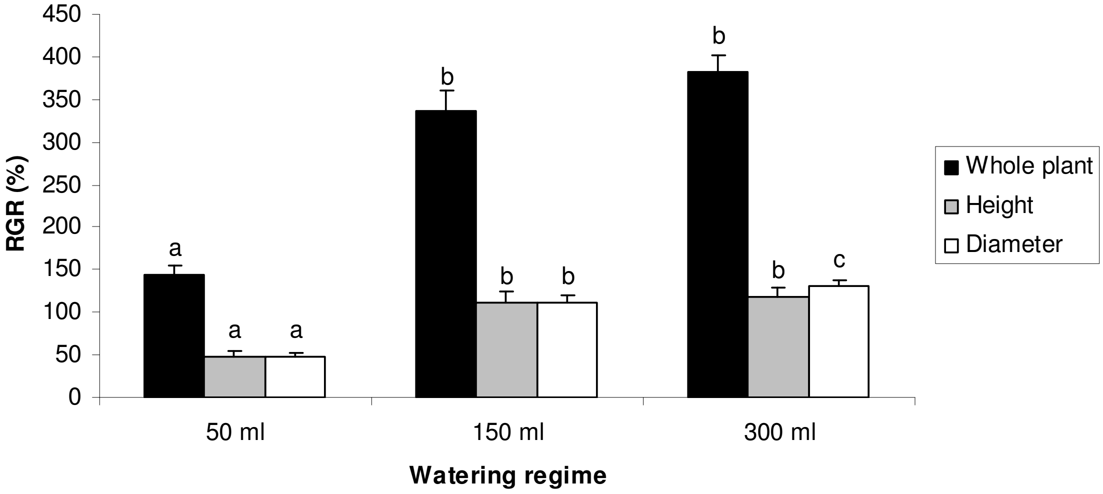

Figure 8: Whole plant, stem height and diameter relative growth rate (RGR, %) across watering regimes of marula seedlings grown under glasshouse conditions between December 2008 and December 2009. Different letters indicate significant differences between treatments (LSD, $p<0.05$).

10.5. Discussion

Up to 40% of the three month old marula seedlings could survive for more than 450 days without water, while 1 month old seedlings could survive up to 180 days. Botha (2006) found that marula seedlings grown in granite soil and which had received no additional water after germination could survive up to 282 days with an average of 171 days and seedlings that had been watered for one month after germination could survive for longer than 370 days without further water. These results indicate that the environmental conditions experienced by the seedlings used in this experiment were more severe than those used by Botha (2006). In particular, summer drought occurs when temperatures are high and hence evaporative demand will also be higher. Regardless of these differences, such an extremely long period of survival without water indicates that marula seedlings can easily survive the winter dry months in South African savannas and indeed any summer droughts on record. It appears, however, that seedlings establishing late in the growing season would be less likely to survive an extended dry season beyond 180 days than those that establish earlier in the growing season. However, only when a seedling has been well watered for three months of growth will it survive without water for over a year, indicating a high level of inherent drought resistance.

Marula survivability of drought is much higher than for savanna tree legumes. Firstly, Wilson (2002) found that 80% of 9 week old *Burkea africana* seedlings could survive a droughting period of 11 weeks. Secondly, Wilson and Witkowski (1998) illustrated the vulnerability of three week old seedlings, finding that *Acacia nilotica* and *Mundulea sericea* seedlings had 20% and 0% survivability after 5 weeks of watering at 12.5% field capacity, and no seedlings survived a periodicity of more than 12 days between watering treatments to field capacity. These results indicate that marula seedlings are much more resistant to drought than other common savannas tree species. Botha (2006) also found that marula could survive for significantly longer periods than another common savanna tree, *Terminalia sericea.*

Younger seedlings were much less resistant to drought than older seedlings. It has been postulated that germinants and seedlings have different abilities to tolerate drought and the size/age at which a germinant becomes an established seedling may be the age/size at which the plant becomes independent of its cotyledon reserves (Stock *et al.* 1990), and have a greater ability to resist or tolerate drought. Of the one month old marula seedlings used in this study, 46% of them had already lost their cotyledons and all plants were without cotyledons by two months of age. Hence marula seedlings could become established relatively quickly.

Despite the findings of Botha (2006), one would logically expect that seedlings would take advantage of the whole rainy season for growth and germinate as soon as sufficient rainfall has fallen at the beginning of the growing season. This would allow for the build up of sufficient reserves and biomass to resist late season drought. Seedlings that may have germinated late in the wet season, such as those from fruit ingested by mammalian herbivores like elephants, may be less likely to survive the winter dry season. This ingestion has been shown to increase the rate of germination and hence allow for germination of the seed in the same growing season as the fruit was produced rather than the delayed germination often observed (von Teichman *et al.* 1986), only occurring at the start of the next growing season (Chapter 6). However seeds that are ingested and hence are able to germinate in the same season only have a short period to establish before the onset of the dry season. In years with below average rainfall, such seedlings may not survive. Hence, if all potential seeds are dispersed by mammalian ingestion and hence the germination rate increased in a dry year, there would be no seeds left at the beginning of the next wet season to germinate and hence the fruit crop from that year could be lost.

The remarkable ability of marula seedlings to survive drought has been shown by this study to be through inherently high investment in below-ground organs and leaf loss to reduce the rate of transpiration. A sacrifice of new leaves and continued physiological activity in mature leaves, facilitated by osmotic adjustment, may also allow prolonged investment in root growth in droughted plants (Williams 2007). The rate of cell elongation in roots is also much less affected by water deficit than in leaves (Saab *et al.* 1990), hence allowing for root growth even under extreme water stress.

Stem height, diameter and number of leaves of marula were all affected by the watering regime, as was dry mass of leaves, stems and roots. More available resources were directly translated into greater biomass acquisition. Marula seedlings receiving lower levels of rainfall also had greater root: shoot ratios, indicating that a relationship between belowground and above-ground ratios is highly plastic and responsive to watering regime. However, Botha (2006) found that there was no tendency of any species studied (*Acacia nigrescens, Philenoptera violacea, Sclerocarya birrea* and *Terminalia sericea*) to invest proportionally more carbon to roots than shoots when kept at field capacity for a longer period before water was withheld. Marula seedlings have an inherently high allocation to root biomass regardless of water treatment, ranging from 78-82% across treatment. This allocation did not change under water stress but rather an increase in allocation to stem biomass coupled with a decrease in leaf biomass allocation was evident.

Although it has been predicted that root: shoot ratio should increase with increasing levels of aridity (Wilson 1988), Schenk and Jackson (2002) only found an increase for the herbaceous species but not for the woody species studied. However, their results were confounded by differences in temperatures. Root: shoot ratios tend to be higher under suboptimal growing temperatures (Davidson 1969). The current study indicated a small, increase in root: shoot ratio with increasing water stress, however the root: stem ratio did not change. This indicates that the increase was due to leaf loss rather than increased allocation to roots. Marula seedlings are therefore inherently very well adapted to dry conditions.

Differences in relative growth rate between the 50 ml and the other two treatments were obvious. However, the increase in water from 150ml to 300ml resulted in a significant increase in stem diameter relative growth rate but not height or whole plant growth rate. This indicates that marulas grown in mesic areas may become fire resistant faster than those growing in less mesic areas due to an increased allocation to stem thickness and by extension bark thickness, offering a further explanation for the loss of marulas outside the NE.

Ladiges (1974) found several differences in the ability of *Eucalyptus viminalis* from different provenances to survive drought and moisture stress. Seedlings grown from seed from populations from higher rainfall areas were less drought resistant than those from drier areas. Also seedlings from low rainfall areas with basalt soils were less drought resistant than those from low rainfall areas with granite soils. Due to lower nutrients in granite soils, plants are expected to grow slower than in basalt soils and hence may be able to have greater drought resistance. Other studies have also found different drought resistance in populations of the same species including species of pine (Chambel *et al.* 2007) and *Eucalyptus microtheca* (Li 2000). Hence the provenance of the seedlings used in this experiment may also have affected the results. Since root systems respond to soil texture by changes in root density rather than changes in rooting depth and lateral spread (Schenk and Jackson 2002), drought resistance may not necessarily be influenced by the soil type used. Future studies should include both soils types and various provenances to determine whether the plasticity observed in this study is not restricted to the granite soils and the provenance used.

Ontogenetic changes in allocation may also affect the response of a plant to stress (Chambel *et al.* 2007). More frequent harvests of the plants at younger and older ages may have revealed different dynamics to those observed in this study. The pot size may also influence the sensitivity of the plant to water stress (Ray and Sinclair 1998). However, the overriding factor determining the response of plants grown in pots to drought stress was soil water content (Ray and Sinclair 1998), not pot size.

10.6. Conclusions

Given the above findings, the lack of marula seedlings in various populations in the low altitude savannas of South Africa (Chapter 2) are unlikely to be due to seedling death through summer drought *per se*. Marula seedlings also appear inherently adapted to dry conditions due primarily to high allocation to root biomass. Such extreme levels of resistance make the lack of seedlings observed in some areas all the more perplexing.

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PART D: MODELLING AND SYNTHESIS

Chapter 11: A simple stochastic demographic model to investigate the effects of rainfall, fire and elephants on *Sclerocarya birrea* subsp. *caffra* (marula) population structure

11.1. Abstract

To understand the complex, dynamics of woody plant populations in African savannas, models are required. Marula populations in the low altitude savannas of South Africa have wide spatial variability in size structure. Unstable population structures are common. A simple, stochastic, demographic model (MARULA1) was developed to investigate the effects of rainfall, fire and elephant on marula population structure. The model simulates marula population structure in the low altitude savannas of South Africa at a medium rainfall site (MAR: 650 mm), taking into account the recruitment dynamics, growth dynamics and mortality rates described in previous studies. The model used an annual time step in order to incorporate annual recruitment dynamics. Rainfall was applied as a stochastic environmental variable and mortality dynamics were related to rainfall. Using default parameter levels for recruitment rates, mortality rates and growth derived in previous chapters of this thesis, the model was able to simulate the stable population structures observed at sites such as Wits Rural Facility, where there are no elephants or fire. Stochastic runs of the model indicated that marula populations were not sensitive to fluctuations in the long-term rainfall unless it is within 75% of the long-term mean of 650 mm (490-813 mm). The model output further indicated that marula populations cannot survive annual fire. Triennial fire supported stable populations, but with increased density of individuals held within the fire trap. Elephant impact alone reduced population density, increased the density of individuals between 5 and 20 cm, but decreased those >40 cm in stem diameter. Marula populations could only stabilise in the presence of elephants if the fire interval was >7 years. These results indicate that the current combination of frequent fire and elephant impact in the Kruger National Park (KNP) may eventually result in the relegation of this dominant species in many landscapes to refugia, where elephants are unable to access the tree or where fire is very infrequent. The model was also able to simulate the main population structure groups identified in Chapter 2, namely: adult dominated, juvenile dominated, "missing size class" and stable structures. A number of gaps in our knowledge on marula biology were highlighted through running the model, most importantly a) the response of damaged adult size classes to fire, b) how low rainfall affects the mortality of damaged trees and c) how fire interacts with this relationship, as well as d) the extent of elephant utilisation of juvenile trees. Although the model is

parameterised from data mainly from the lowveld of South Africa, it is flexible enough to be utilised for other woody species across Africa. The major shortcoming of the model in its present form, is the lack of explicit consideration of spatial variability in fire and elephant impacts. The model formulation exercise proved useful for synthesising the information gathered by this study and our present understanding of savanna woody plant dynamics in general, as well as highlighting where the emphasis for future studies on marula and other savanna woody species needs to be placed.

11.2. Introduction

There is no clear understanding of the ecological drivers resulting in the contrasting marula population structures observed at different localities in the lowveld of South Africa (Chapter 2). Some sites have stable reverse J-shaped size class distributions (SCD), others are dominated by adults, others by juveniles and other sites show a “missing size class” between 2-8 m in height (Chapter 2). These variable structures lend support for both episodic and continuous recruitment in marula (Walker *et al.* 1986; Jacobs and Biggs 2002). Such site variability also indicates substantial spatial variability in marula population dynamics, further indicating that marula may have many stages of potential vulnerability that require elucidation.

Models are needed to synthesise the information we have on savanna woody plant dynamics and to ultimately understand the system. Throughout this thesis vital rates have been determined, including growth rates, mortality rates and fecundity, as well as how these respond to disturbances such as herbivory and fire and their variability across soil types and rainfall regimes. In savannas the main drivers are considered to be nutrients, fire, herbivory, and rainfall (Scholes and Walker 1993, Solbrig *et al.* 1996, Rutherford 1997, Scholes 1997). Hence this study forms an almost complete basis from which parameterisation of a population model for a typical African savanna tree species may be made.

A number of models have attempted to predict the future dynamics of vegetation in response to heavy elephant utilisation, fire and disturbances in African savannas (Caughley 1976; Pellew 1983; van Wijngaarden 1985; Dublin *et al.* 1990; Ben-Shahar 1996; Duffy *et* *al.* 2000; Baxter and Getz 2005; Holdo 2007). Problems with these models, such as ignoring spatial heterogeneity have been highlighted. Spatial vegetation models have included both individual-based models and grid-based approximations to individual based models and have explored processes by modelling at fine spatial resolutions (Baxter and Getz 2005). Such models are not readily expandable to larger spatial scales where elephants are expected to impact. Some models have ignored the vertical structure of woody vegetation, while others have excluded the effects of climate, rainfall variability, grass competition, fire and density dependence. Many of these models have tended to rely on fragmented data sources to produce parameters, often from multiple studies and habitats (Higgins *et al.* 2000; Baxter and Getz 2005; Holdo 2007). As a result they do not control for unmeasured variables that may differ among studies. Comprehensive datasets are also rarely available for a single site, and thus it is not possible to perform a comparative analysis of the role of different disturbance types, or the interactive effects between them (Holdo 2007). In addition models that have examined the elephant-fire interaction have tended to focus on the issue of tree-grass coexistence, and have often ignored variation in response to disturbance among tree species. This variation may be just as important for ecosystem function as the tree-grass interaction (Holdo 2007). Kruger National Park (KNP) and other large conservation areas in Africa with elephant-fire dynamics, require a comprehensive model which can be used to predict the future persistence of woody vegetation in response to elephant and fire impacts. The “Baxter Model” (Baxter and Getz 2005) has been given support as a useful model in this regard, but it needs re-parameterisation, specifically in terms of woody plant growth rates, and it also needs to be applied at larger spatial scales to be useful in the KNP context (R. N. Owen-Smith pers. comm.).

Given the vital rates and responses of marula to various disturbances investigated in this study, an ideal opportunity now exists in which to utilise these findings to parameterise previous models such as those of Baxter and Getz (2005). Improved understanding of the most important bottlenecks in the life history of marula and what population drivers are most important in contributing to the observed population structures can also be explored in more detail, thus investigating the central question of this thesis further: how do marula populations persist in areas with intense and frequent disturbance? A simple, demographic model was therefore developed and the findings on marula biology reported in this study were used for model parameterisation unless otherwise specified.

The main aim of this modelling exercise was to integrate the information presented in this thesis and attempt to understand what this information means for marula population dynamics in the face of fire and elephant impacts. In running the model, a secondary aim was to explore what processes and parameter values might generate the four main marula size structure patterns observed across the low altitude savannas of South Africa (Chapter 2), taking into account fire, rainfall and elephant influences. In addition, identification of the major gaps in our understanding of marula biology may also be revealed in the process of developing and running the model.

11.3. Methodology

11.3.1. Model formulation

Design information

The simple model presented here is a stochastic, size-structured, demographic, EXCEL spreadsheet-based model, using an annual time step and incorporating a gap dynamics sub-model which allows for the interaction between disturbances and recruitment events to generate locations that have no established trees. Since marula is dioecious, only the female segment was considered and sex ratio at unity was assumed. Environmental variability was introduced through randomly varying rainfall (within its known range), fixing the frequency of fire according to historical records and linking elephant impact and mortality to rainfall. In order to incorporate patch dynamics at some level, different scenarios were introduced using the conditions prevalent at a representative site from each of the four main population structures described in Chapter 2.

Stage/size classes

The stage classes were based on stem diameter as this measure is the best indicator of the actual size independent of damage, fire vulnerability and reproductive capacity of marula. Some classes have two subclasses. The first subclass represents the undamaged state of the individual. If the individual is damaged in some way then it moves to the second subclass within a class and will transition up in parallel to the undamaged class. This enables different mortality dynamics to be applied to the different subclasses as damage by elephant and fire usually results in subsequent increased vulnerability to fire and further herbivory. The classes were specified using biologically meaningful traits such as fire resistance and reproductive maturity (Figure 1 and 2). The following nine classes were used and described:

1. **Seeds:** One fruit represents a single seed.
2. **Unestablished seedlings**: newly germinated seedlings, highly susceptible to fire and herbivory and summer drought (<0.5cm in stem diameter, <0.25 m in height).
3. **Established seedlings within the fire trap** (2.75 m high; Jacobs and Biggs 2001): resprout from the base when topkilled by fire or damaged by elephant reverting to the coppicing size class (0.5-5 cm in stem diameter, <3 m in height).
4. **Non-reproductive saplings out of the fire trap** (2 subclasses: a and b): Subclass a: Undamaged marula stems have been found to be resistant to stem death by fire when >5 cm in diameter (Chapter 8) (5-14 cm in stem diameter, ~3-5 m in height). If the stem is toppled but the canopy survives on the ground or is pollarded by elephants, then the individual reverts to subclass b, indicating its increased vulnerability to fire. If the stem is toppled and the canopy dies and the individual resprouts from the base or if it is topkilled by fire, then the individual transitions to the coppicing size class.
5. **Repro 1** (2 subclasses: a and b): Reproduction was shown to start at 14 cm in stem diameter (Shackleton 2003; Emanuel et al. 2005; Chapter 6). This class has a low fruit yield (14-20 cm in diameter, ~5-6 m in height). If toppled (canopy survives on ground) or pollarded by elephants reverts to subclass b which is more vulnerable to fire. If toppled (canopy dies and resprouts from base) or if topkilled by fire, it reverts to coppicing size class.
6. **Repro 2** (2 subclasses: a and b): 20-30 cm in stem diameter, ~6-8 m in height. If toppled (canopy survives on ground) or pollarded by elephants, reverts to subclass b which is more vulnerable to fire. If toppled (canopy dies and resprouts from base) or if topkilled by fire, it reverts to coppicing size class.

7. **Repro 3** (2 subclasses: a and b): 30-40 cm in diameter, ~8-10 m in height. If toppled (canopy survives on ground) or pollarded by elephants, reverts to subclass b which is more vulnerable to fire. If toppled (canopy dies and resprouts from base) or if topkilled by fire (unlikely), it reverts to coppicing size class.
8. **Repro 4** (2 subclasses: a and b): >40 cm in diameter, >10 m in height. Plants up to 1 m in stem diameter have been observed. If toppled (canopy survives on ground) or pollarded by elephants, reverts to subclass b which is more vulnerable to fire. This class cannot revert to the coppicing class.
9. **Coppicing class**: All plants in classes 3, 4, 5, 6 and 7 in both subclasses that are topkilled by fire or elephant and resprout at the base, revert to this class. It consists of individuals that look like established seedlings but are usually multi-stemmed and retain the underground root structure of the original plant.

The overall model has a built in recruitment dynamics submodel, which describes the process of conversion of seeds produced to established seedlings (Figure 1). This process depends on a variety of factors including seed predation, seed dispersal, rainfall, summer drought, fire, mammalian seed ingestion and seedling predation or trampling. Each class from the established seedling class upward, is subjected to either a class specific background mortality or a fire or elephant induced mortality based on parameters estimated in previous chapters of this thesis and contingent on rainfall. Details of these are described below. All classes can revert to the coppicing class if topkilled, toppled or damaged in such a way that the canopy dies, except for the largest class, Repro 4. These reversion rates are class specific. Individuals in the coppicing class grow much faster than the established seedlings, even though their sizes are equivalent, due to stored root reserves. Table 1 indicates the full range of possible transitions between classes.

A null model was initially developed to represent a site with medium to high rainfall (650 mm), no recent fire and no elephants, similar to the situation in the Hlangwine enclosure (HE) near Pretoriuskop in the KNP and at Wits Rural Facility (WRF) (Chapter 2). This model should produce a stable size structure as observed at these sites (Chapter 2).

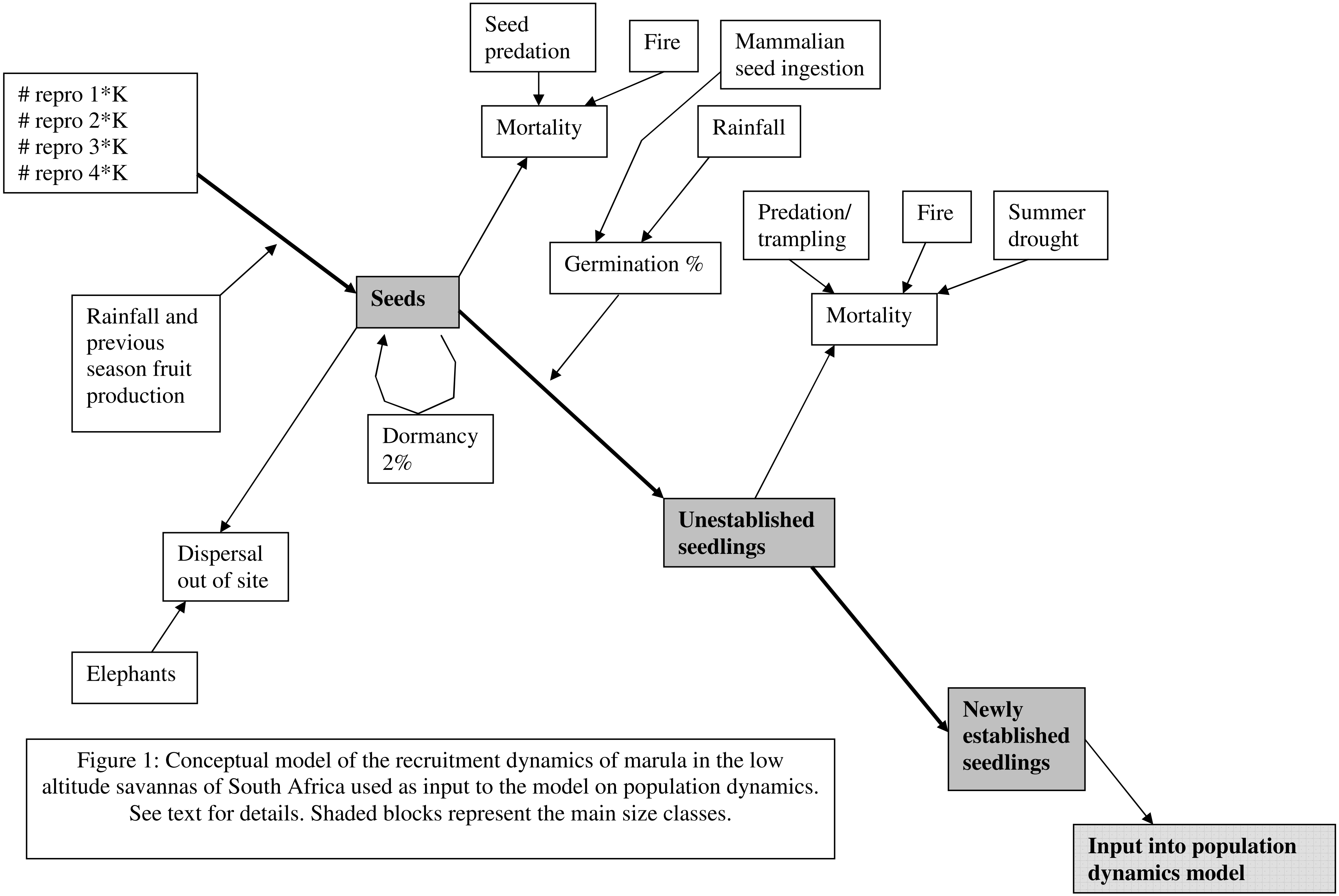


Figure 1: Conceptual model of the recruitment dynamics of marula in the low altitude savannas of South Africa used as input to the model on population dynamics. See text for details. Shaded blocks represent the main size classes.

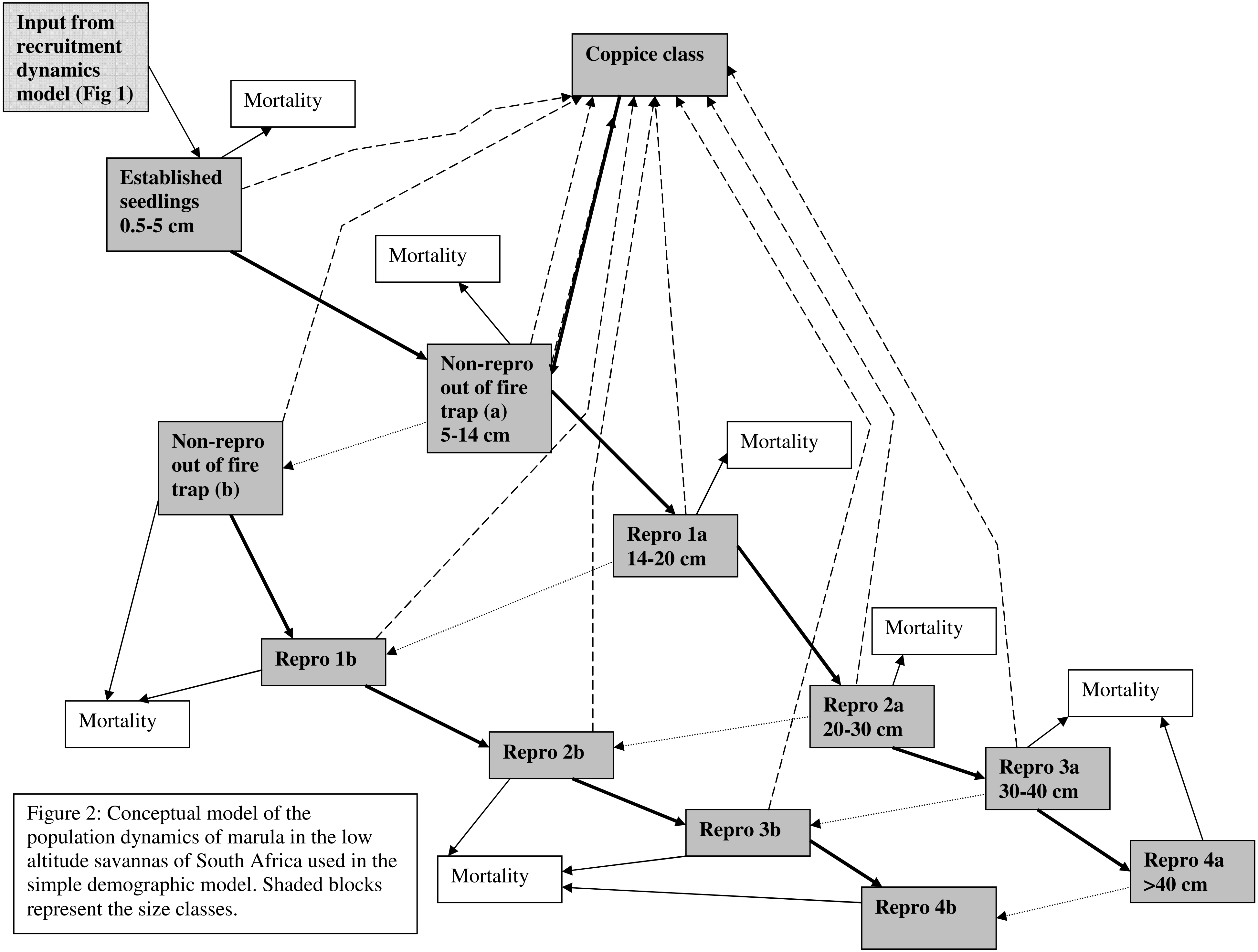


Figure 2: Conceptual model of the population dynamics of marula in the low altitude savannas of South Africa used in the simple demographic model. Shaded blocks represent the size classes.

Table 1: Projection matrix indicating the full range of possible transitions between classes. See text for more details.

	Seed (s)	**Seedling (se)**	**Fire trap (fi)**	**Coppice (co)**	**Non repro a (noa)**	**Non repro b (nob)**	**Repro 1a (R1a)**	**Repro 1b (R1b)**	**Repro 2a (R2a)**	**Repro 2b (R2b)**	**Repro 3a (R3a)**	**Repro 3b (R3b)**	**Repro 4a (R4a)**	**Repro 4b (R4b)**
Seed (s)	s,s						F1	F1/2	F2	F2/2	F3	F3/2	F4	F4/2
Seedling (se)	s,se													
Fire trap (fi)		se,fi	fi,fi											
Coppice (co)			fi,co	co,co	noa,co	nob,co	R1,co	R1b,co	R2a,co	R2b,co	R3a,co	R3b,co	R4a,co	RC4b,co
Nonrepro a (noa)			fi,noa	co,noa	noa,noa									
Nonrepro b (nob)					noa,nob	nob,nob								
Repro 1a (R1a)					no,R1a		R1a,R1a							
Repro 1b (R1b)						nob,R1b	R1a,R1b	R1b,R1b						
Repro 2a (R2a)							R1a,R2a		R2a,R2a					
Repro 2b (R2b)								R1b,R2b	R2a,R2b	R2b,R2b				
Repro 3a (R3a)									R2a,R3a		R3a,R3a			
Repro 3b (R3b)										R2b,R3b	R3a,R3b	R3b,R3b		
Repro 4a (R4a)											R3a,R4a		R4a,R4a	
Repro 4b (R4b)												R3b,RC4b	R4a,RC4b	RC4b,RC4b

11.3.2. Variables and parameter estimation

11.3.2.1. Rainfall

Mean annual rainfall was modelled following Higgins *et al.* (2000). Therefore a sine wave function was used to generate the annual rainfall each year, incorporating a random variable to generate stochastic dynamics:

$$R=x(R_x,R_{sd})+\sin(2\pi*y/l)s; \qquad R\geq 0$$

Where R is the annual rainfall (mm); x is a normally distributed random number defined by the mean (R_x; mm) and standard deviation (R_{sd}; mm) of annual rainfall; s is the effect of the long-term periodicity of rainfall (mm); l is the frequency of periodicity and y is the simulation year (Table 2).

11.3.2.2. Fire

Fire occurrence in a year was modelled using a binary variable f(t) and for a fire to occur f(t)=1. No attempt was made to model fire intensity in relation to grass biomass or rainfall and hence it was applied as a blanket mortality agent when it occurred. Fire affected seedling mortality, topkill of vulnerable classes and the mortality of subclass b in each class.

11.3.2.3. Wet season (summer) drought

Summer drought was defined as 6 weeks without rainfall. The probability of a summer drought was estimated to be a function of the annual rainfall following Higgins *et al.* (2000):

$$P_d=1/(1+\exp((R-R_{0.5})/v_d))$$

Where $R_{0.5}$ is the annual rainfall at which there is a 0.5 chance of a wet season drought, and v_d is a constant that describes the rate at which the probability of wet season drought changes with rainfall (R). Summer drought affected the survival of newly germinated seedlings.

Table 2: Default overall annual parameter estimates used in the basic demographic marula model.

Parameter	Symbol	Default value	Source
Mean annual rainfall (mm)	R_x	650	Higgins *et al.* 2000
Standard deviation of mean annual rainfall (mm)	R_{sd}	280	Higgins *et al.* 2000
Strength of periodicity in rainfall (mm)	s	188	Higgins *et al.* 2000
Period length of periodicity in rainfall (years)	l	20	Higgins *et al.* 2000
Annual rainfall for 50% chance of wet season drought (mm)	$R_{0.5}$	300	Higgins *et al.* 2000
Rate of change of wet season drought probability with annual rainfall (v_d)	v_d	50	Higgins *et al.* 2000
Total number of gaps/ 100 ha	G	4500	Chapter 6
Number of gaps for 50% chance of a gap being filled / 100 ha	$G_{0.5}$	2000	Estimate
Rate of change of filling a gap probability with number of gaps available	v_g	1000	Estimate
Presence of mammalian fruit dispersers	d	1	Estimate
Seed dormancy rate (%)		2	Chapter 6
Seed predation rate (%)		85	Chapter 6
Seedling predation rate (%)		95	Chapter 6
Germination (%)		40	Chapter 6 and 7

11.3.2.4. Elephant utilisation

Elephants in protected areas have been implicated in reducing structural, compositional and possibly functional diversity in such ecosystems (Baxter and Getz 2005). Although elephants are known to be facilitators in seed dispersal, nutrient cycling and provision of gaps for new germinants (Owen-Smith 1988), the loss of canopy trees through severe elephant damage may result in the loss of woody species populations if there are no recruits, or may result in state transitions to shrubveld through prevention of tree recruitment (Baxter and Getz 2005).

Elephant herbivory was modelled using a random binary variable e(t). IF e(t)=1 then the annual probabilities of toppling, pollarding or bark stripping were applied based on rates identified in the KNP between 2001 and 2010 (described in Chapter 4 and listed in Table 3). If there was no topkill due to toppling and if the tree was pollarded above 1 m, then these individuals reverted to subclass b within their size class. Bark stripping also increases the vulnerability of stems to subsequent borer attack and ultimately to fire (Chapter 8). Bark stripping prevalence on marula in the KNP was high (Chapter 8), but high intensity bark stripping (>50% of the stem circumference) only occurred in 6% of the population surveyed. Annual rates are probably low but an estimate was incorporated into the reversion rate of trees damaged by elephant to subclass b. Subclass b would then transition parallel to the main subclass trajectory and be more vulnerable to fire (Chapter 8), and would also have reduced fruit production- less than half of its potential. Those trees pollarded below 1 m and toppled but resprouting from the base reverted to the coppice class. Basal diameter growth rates in subclass b were slower than those in subclass a due to the damage incurred during the disturbance event by a factor of 0.25.

11.3.2.5. Fruit/seed production

Fruit production is highly variable between years and assumed to depend on a combination of the previous year's rainfall and whether the tree produced fruits the previous year. Fruit production is also size dependent (Chapter 6). Only the female portion of the population was modelled, so therefore the fruit number used as a default parameter was taken as half of the actual fruit production observed in Chapter 6, since half of the germinants are assumed to be male. Even though marula endocarps (dispersal unit) contain from 1-4 seeds (von Teichman *et al.* 1986), one endocarp was assumed to represent only one potential seed. Even if more than one seed germinates from a single endocarp, only one seedling will probably survive due to resource constraints. Therefore fruit number was used instead of seed number for potential seeds. Seed production was calculated as follows:

Table 3: Class specific parameter symbols, names and default annual values used for the simulation runs in the demographic marula population model. Parameter values sourced from results obtained in the previous chapters of this thesis, while numbers in grey have been estimated. See methods for more details. Subclass b is for individuals damaged by elephants.

	Seeds	Seedlings	Seedling established fire trap	Coppice	Non reproductive out of fire trap	Subclass b	Repro1	Subclass b	Repro2	Subclass b	Repro3	Subclass b	Repro4	Subclass b
Stem diameter (cm)		<0.5	0.5-5	0.5-5	5-14	5-14	14-20	14-20	20-30	20-30	30-40	30-40	>40	>40
Starting values (Stable size distribution) trees/100 ha	2 000	90 000	1 141	0	861	0	363	0	364	0	326	0	729	0
Fecundity K (#fruit=#seeds)							25	12	250	125	750	375	1500	750
Annual stem diameter growth rate (mm)			0.2		3.1		2.8		0.3		0.2		0.2	
Time in this stage without disturbance given annual growth rate (years)	1	1	16	12	30	38	21	26	32	40	36	43	163	200
Background mortality good years			0.06	0.06	0.04	0.08	0.02	0.04	0.01	0.02	0.006	0.012	0.01	0.02
Background mortality bad years			0.12	0.12	0.08	0.15	0.04	0.09	0.02	0.05	0.012	0.024	0.02	0.04
Elephant mortality good years			0.05	0.05	0.01	0.1	0.036	0.1	0.036	0.1	0.039	0.1	0.029	0.1
Elephant mortality bad years			0.075	0.075	0.015	0.15	0.054	0.15	0.054	0.15	0.059	0.15	0.043	0.15
Fire mortality	0.5	0.95	0.05	0.05	0.01	0.1	0.001	0.1	0.001	0.1	0.001	0.1	0.001	0.1
Elephant pollarding and toppling-coppice class reversion rate			0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Elephant pollarding and toppling-vulnerable subclass reversion rate					0.001		0.058		0.058		0.056		0.04	
Fire topkill rate			1	1	0.05	0.1	0	0.001	0	0.001	0	0.001	0	0.001

If (t-1) rainfall is >0.6 of the long-term mean rainfall and (t-1) fruit production is <K/2, then fruit (t) =K. Otherwise if rainfall is >600 mm and (t-1) fruit production is >K/2, then fruit production (t) = K/2. However, if the rainfall is <0.6 of the long-term mean rainfall, then fruit production (t)=0.1*K. K is the maximum fruit production. All plants in subclass b of a size class were assumed to have reduced fecundity by a factor of 0.5.

In dioecious species, there is also a need for a minimum number of plants for pollination to be successful, hence it was assumed that if the number of reproductive plants was <8, then fruit production =0.

11.3.2.6. Mortality rates

Shackleton (1997) estimated a background mortality rate for savanna woody plants based on the following formula: Mortality rate; $y = e^{-0.0232x+2:018}$ where x=average circumference in the size class (cm). This was used as annual background mortality rate across all size classes and was assumed to include herbivory influences (including the effects of borers and natural senescence), but excluded mortality caused by elephants or fire. The Repro 4 class had a higher mortality rate due to natural senescence through doubling the mortality rate obtained from Shackleton's (1997) estimate. Low rainfall would increase this mortality rate as herbivores would be more likely to utilise the plant and resprouting ability would be hampered. Hence a function linking this rate to rainfall was applied where, if the rainfall in the previous year was less than 80% of the long-term mean (bad year) then the mortality rate increased by a factor of 0.5. If elephants were present the mortality rates associated with utilisation were linked to rainfall in the same way as the background mortality rates.

The total mortality for classes 3–9 (from established seedlings upwards, including the coppicing class and subclass b of each class) = background mortality (includes rainfall and browser effects) or elephant induced mortality if elephants are present + fire mortality. The mortality rate applied when elephants were present included background mortality.

11.3.2.7. Seed fate

Germination percentages were found to be relatively low, with a maximum of 50% of the fruit produced producing a germinant (Chapter 7). Germination rates may be improved by mammalian ingestion of the seed (Lewis 1987), but overall germination percentages remain the same over time for a specific provenance (Chapter 6). Since the water requirements for germination and successful seedling establishment in marula are very low, it was assumed that seeds could germinate every year and an average percentage of 40% was assumed (Chapter 7 and 10). Marula seed predation rates range between 70-95% (Gallaher 2010; Chapter 6) but are highly variable. Predation rates are highest under the canopy of mature trees and where dispersers are absent, predation rates may be expected to be much higher than when present. Fire also kills the exposed seeds on the surface and the annual fire kill rate was assumed to be 0.5 if a fire occurred in that year (Chapter 8). Up to 2% of marula seeds remain dormant in any particular year (Chapter 6). At the Hangwine enclosure (HE) near Pretoriuskop, KNP, where there are no elephants and little fire, approximately 5.3% of the seeds produced become seedlings annually (Chapter 6).

11.3.2.8. Seedling fate

Mortality of seedlings is mostly due to predation/trampling of seedlings by impala (Haig 1999) and other herbivores. To provide an estimate of the annual seedling mortality rate due to predation/trampling, counts of one year old seedlings around parent trees inside (3 seedlings/tree) and outside (2 seedlings/tree) HE in the KNP (Chapter 6) were made. Inside the enclosure there are no mammalian seedling predators but outside these are present. If three seedlings per tree germinated per year, then after 16 years (estimated time in fire trap) there should be 48 seedlings in the fire trap. However, if we assume that two fires occurred in those 16 years killing all the seedlings in those years (leaving a possible 42 seedlings), then the rate of throughput outside the enclosure would be 2/42=0.047 (since there were only 2 seedlings / tree) and predation/trampling rate=95.3%.

Summer drought affects newly germinated (unestablished) seedling survival. Marulas, three months of age, were found to be extremely drought resistant, with up to 40% surviving for >450 days without water (Chapter 10). If summer drought was found to occur during the first year of growth, 30% of the seedlings were assumed to die before predation rates were applied (Chapter 10).

Since fire would probably only occur in the dry season, it would only affect seedlings that had already survived predation and summer drought. Even though it has been found that marula seedlings can resprout after only 1 month of growth (pers. obs.), it is not known how this interacts with rainfall. It was therefore assumed that these seedlings would not survive fire, assuming all fires were during the dry season. Since fire is patchy in nature, a small percentage of the seedlings in safe sites may escape the fire. Hence if fire occurred in a year, the mortality rate of surviving seedlings = 95%.

11.3.2.9. Resprouting

Plants in class 3, 4a and b, 5a and b, 6a and b, and 7a and b can revert to the coppicing class through fire and elephant induced topkill, resulting in basal resprouting after the disturbance event. This class has a greater growth rate than that of the established seedling class (Class 3) due to the presence of an extensive root stock.

11.3.2.10. Growth rates

Marula growth rates for various size classes and at three sites have been determined (Chapter 4 and 5; Table 3). These were used to determine the length of time that a plant was expected to remain in a size class. Transitions between size classes were calculated by dividing the possible number transitioning by the number of years that they were meant to remain in a size class. Subclass b within a class had slower growth rates due to recovery from damage and were reduced by a factor of 0.75

11.3.2.11. Dispersal and gap dynamics

A maximum number of gaps were assigned to a site depending on the maximum density assumed to be possible at NE, where there is no herbivory or elephants and high rainfall (9000 trees/100ha). Since this model is only incorporating the female segment, the maximum possible gaps in the default model was 4500 trees/ha. Site gap numbers could be modified based on rainfall and density observed. The default area modelled was 100 ha and all parameter estimates were based on trees/100 ha. The seedlings that emerge can survive their first year regardless of how many gaps are available. However, the number of seedlings that become established depends on the number of gaps available and the probability of a seed being dispersed to the open gap. This probability will decrease exponentially as the number of gaps decrease. The probability of a seedling establishing in a gap was estimated to be a function of the number of total gaps possible and the number that had been filled:

$$P_g=1/(1+\exp(-((G-G_{0.5})*d)/v_g))$$

Where $G_{0.5}$ is the number of filled gaps at which there is a 0.5 chance of a gap being filled by a newly germinated seedling, and v_d is a constant that describes the rate at which the probability of a gap being filled changes with the number of gaps already filled (G), and d is a constant indicating whether possible dispersers are present (1) or absent (1/2) at a site. The presence of dispersers increases the likelihood of a seed finding a suitable gap.

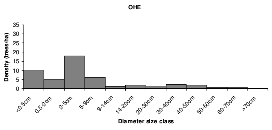
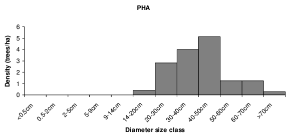
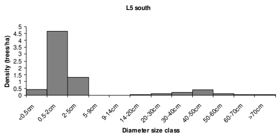
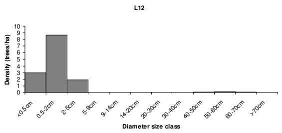
11.3.3. Sensitivity analysis

The sensitivity of the basic model to changes in rainfall, fire frequency and elephant presence or not and all the other parameters was investigated by changing the parameter values incrementally both directions from the mean value.

11.3.4. Scenarios

Four sites on granite derived nutrient-poor soils in the low altitude savannas of South Africa, each representing one of the four population groups identified in Chapter 2 were selected (Table 4) and the parameter estimates were modified according to the values for each site. The different parameters for each site were run as four different simulations starting with the default parameters, and then parameters were adjusted in order to try and obtain the population structures observed at the sites. Once this had been achieved, the population was projected into the future to determine what would most likely be the future direction that the population will take given the same parameters that had resulted in the current population structure. Rainfall for each site was obtained from rain stations in the KNP and the fire return interval was estimated from van Wilgen *et al.* (2000).

Table 4: Characteristics and parameter values used for the simulation runs to describe the four main marula population structures on granite soils in the KNP (see Chapter 2). Other parameters are set to the values in Table 2 and 3.

	OHE	**PHA**	**L5 south**	**L12**
Population group	Stable size structure	Adult dominated	“missing size class”	Juvenile dominated
Mean annual rainfall (mm; R_x)	753	670	562	515
Standard deviation of mean annual rainfall (mm; R_{sd})	360	212	200	220
Elephants	yes	yes	yes	yes
Fire return interval (years)	3	4	6	6
Marula female tree density (trees/100 ha)	2 400	750	315	700
Size class distribution				

11.4. Results

The model developed here shall henceforth be named MARULA1. The null model was used to simulate the trajectory of a marula population over 500 years. The annual rainfall generated by the model has a stochastic component and is very variable (Figure 3). Figure 4a shows the output of the model with the default parameters and no fire or elephants. There is a weak stochastic signal in the model for the smaller size classes, so the output is averaged over 100 simulations (Figure 4b). Over 500 years marula develops a size structure represented by an inverse J shaped curve for individuals <40cm in basal diameter (Repro 4). Individuals >40cm stabilise at numbers greater than Repro 1, 2 and 3, probably due to the long lifespan of Repro 4 and because a large proportion of the remaining sizes are lumped into one class (40-100 cm in stem diameter) (Figure 5).

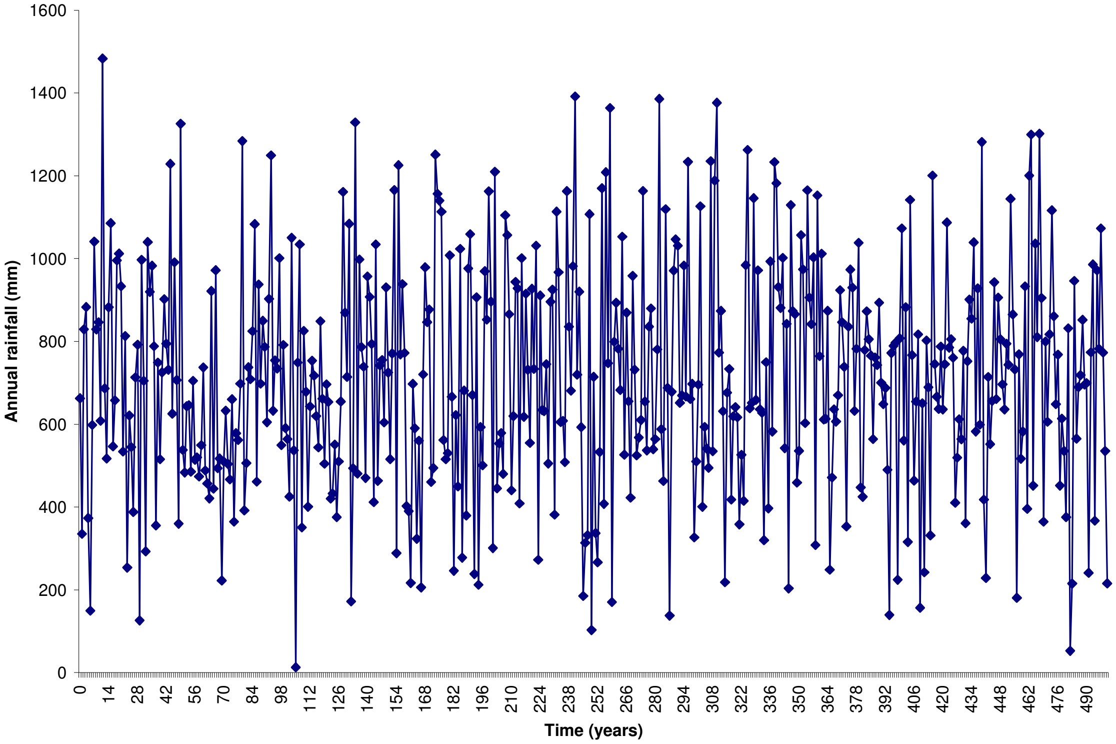


Figure 3: Model annual rainfall output from one stochastic run over 500 years.

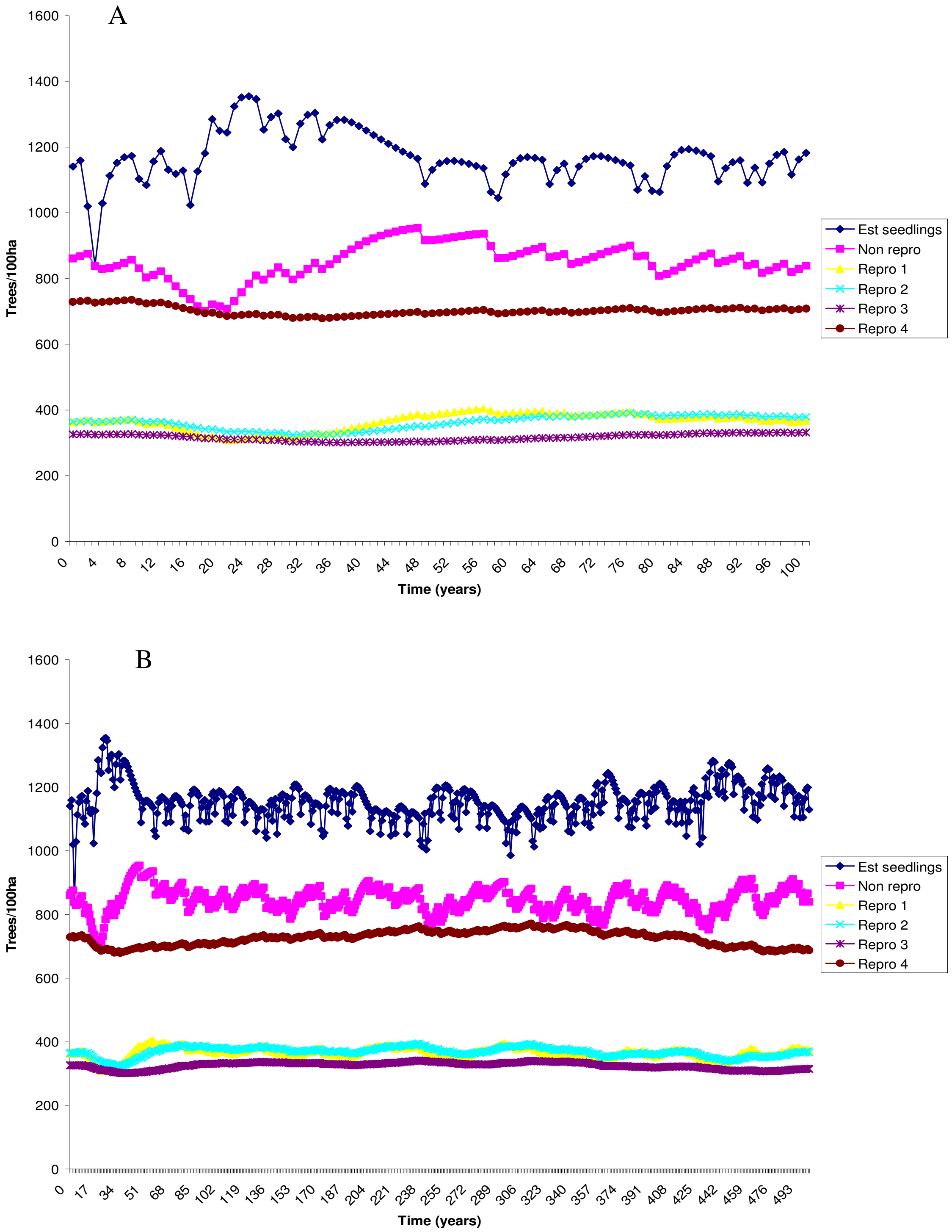
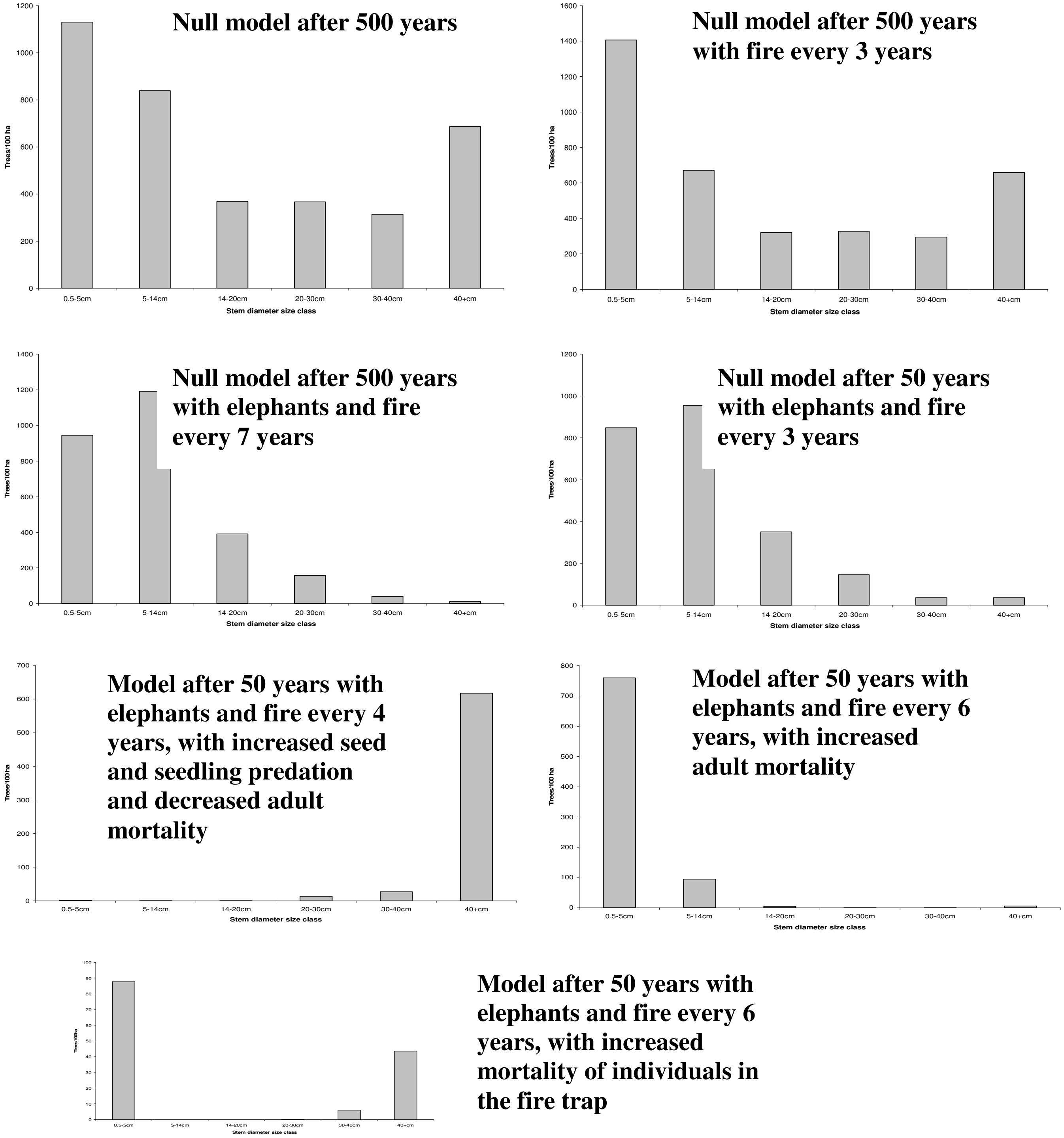


Figure 4: Model results using the default parameter set showing the density of individuals in each class with no elephants or fire. a) Output is from a single stochastic run over 100 years. b) Mean output from 100 stochastic runs over 500 years.


Null model after 500 years

Null model after 500 years with fire every 3 years

Null model after 500 years with elephants and fire every 7 years

Null model after 50 years with elephants and fire every 3 years

Model after 50 years with elephants and fire every 4 years, with increased seed and seedling predation and decreased adult mortality

Model after 50 years with elephants and fire every 6 years, with increased adult mortality

Model after 50 years with elephants and fire every 6 years, with increased mortality of individuals in the fire trap

Figure 5: Size class distribution output of the model run with various combinations of elephants and fire frequency and with altering the default parameters. Note change in scale of y axis for each graph. All x axes are the same.

11.4.1. Model sensitivity

Rainfall regime was altered by simply multiplying $r(t)$ by 1.2 or 0.8, to give a “wet” and a “dry” scenario (nominally these perturbations are referred to as a 20% increase or decrease in rainfall, although actual averages will be more of an increase and less of a decrease because $r(t)$ is constrained to be non-negative). The periodicity (w) of long-term cycles were not altered, nor was the standard deviation of annual rainfall (R_{sd}). The mean trajectories obtained are shown in Figure 6.

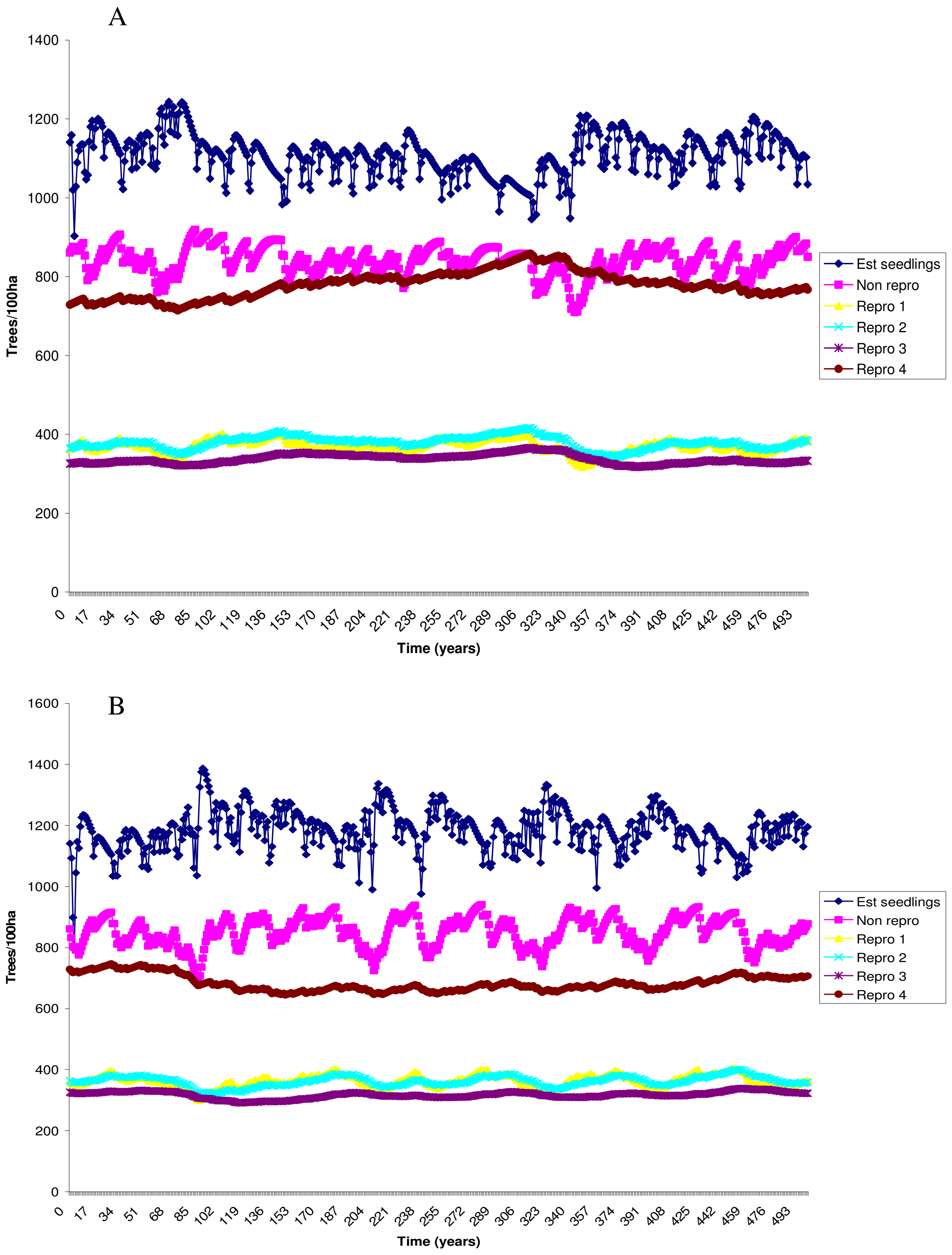


Figure 6: Mean trajectories for a) dry (80% of mean annual rainfall) and b) wet (120% of mean annual rainfall) scenarios.

The density of reproductive trees is not very sensitive to rainfall (Figure 7). Marula is relatively drought tolerant. Growth rates were not linked to rainfall in this model.

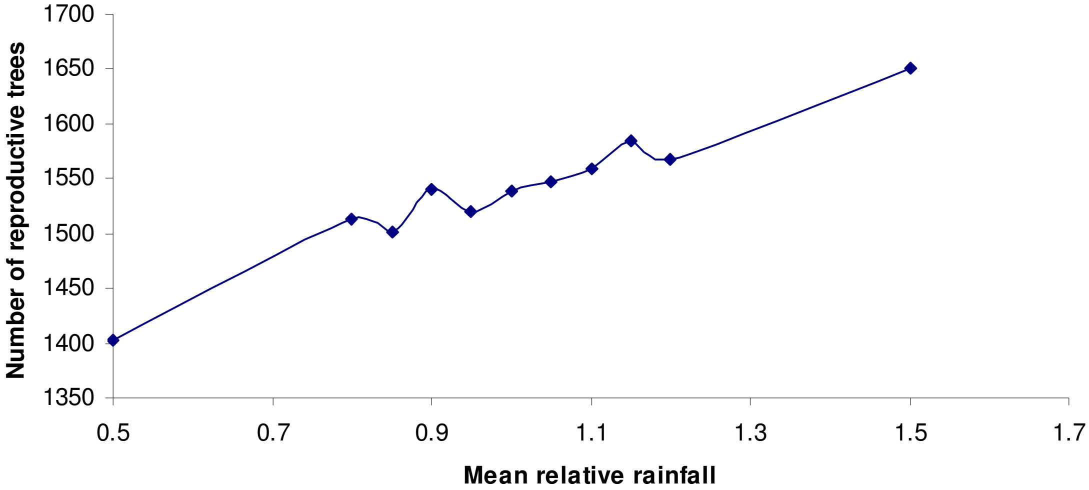


Figure 7: Sensitivity of number of reproductive trees to mean relative rainfall (default =1; 650 mm)

11.4.2. Fire influences

The fire return interval was varied in order to determine the effect on the marula population (Figure 8). Fire was introduced after 100 years. The probability of quasi removal (defined following Baxter and Getz (2005) as less than 0.01 reproductive trees per hectare (1/100ha)) was determined given a specific fire return interval. Annual fire resulted in quasi removal of trees. Marula populations were able to stabilise with fire every 3 years after 500 years with densities similar to those before the fires started. The SCD after 500 years was very similar to the stable SCD (Figure 5). Fire every three years inhibited escape from the fire trap, increasing the number of individuals <5 cm in stem diameter and decreasing the density of the non reproductive individuals out of the fire trap. Based on these results, marula populations appear to be relatively resistant to frequent fire.

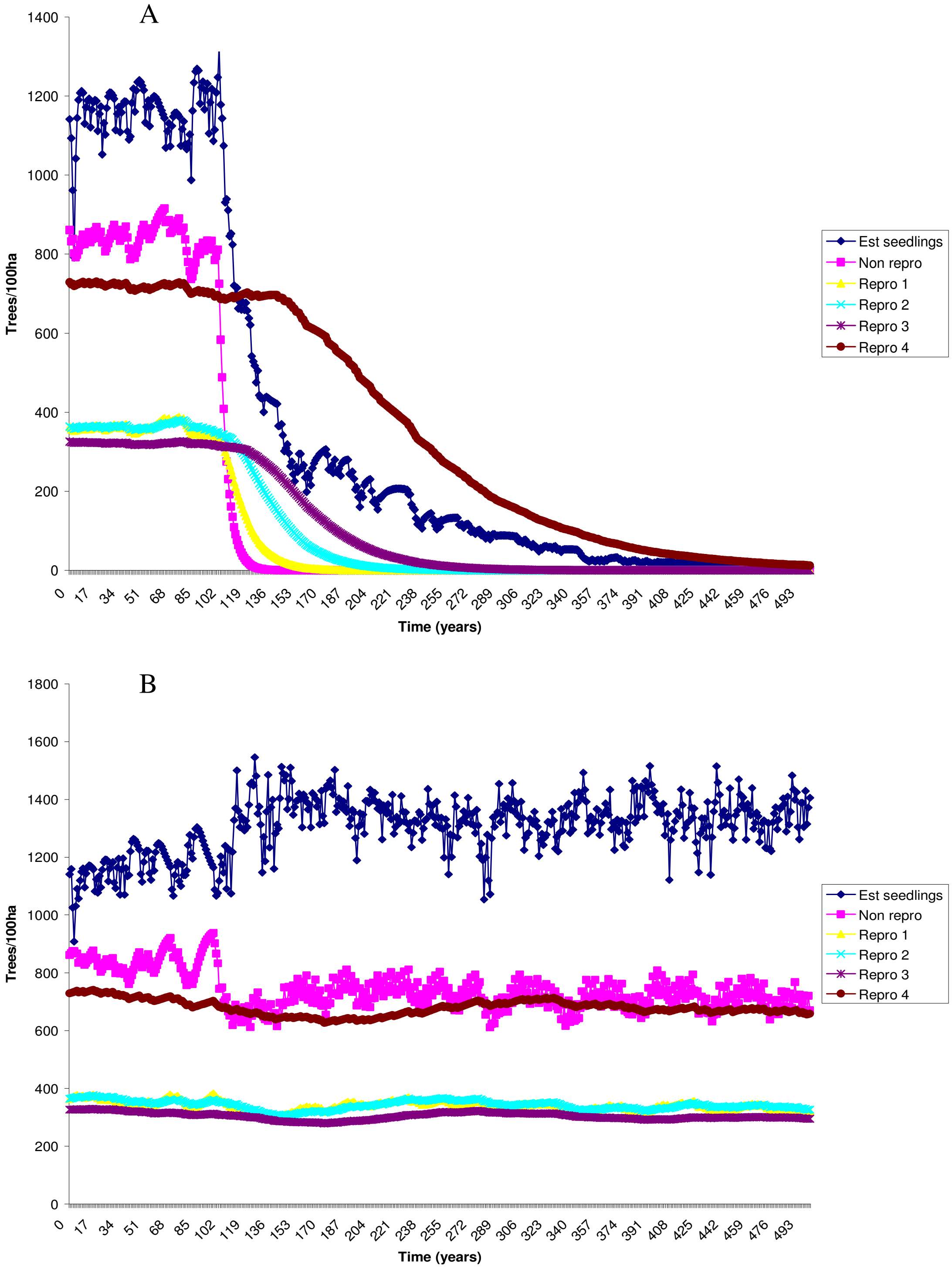


Figure 8: Response of the marula population to the introduction of fire after 100 years with an a) annual and b) every 3 year return period. The initial differences between a and b in the first 100 years is due to the stochastic nature of the model.

11.4.3 Elephant introductions

Upon introduction of an elephant population, it was assumed to remain at constant density and hence all rates remained constant over the simulation period. When an elephant population was introduced after 100 years, with no fire and the default values in Table 3; the marula population declined in density drastically, but then stabilised (Figure 9a). The presence of elephants severely reduced the density of large trees (Repro 2, 3 and 4; >20 cm in stem diameter), but the density of the individuals between 5 and 20 cm increased (Non repro and Repro 1). Trees >20 cm stabilised at less than half their initial density. Trees >40 cm declined by 97% with the introduction of elephants. By increasing the pollarding and toppling rates, by a factor of 0.1, the marula population crashed immediately (Figure 9b).

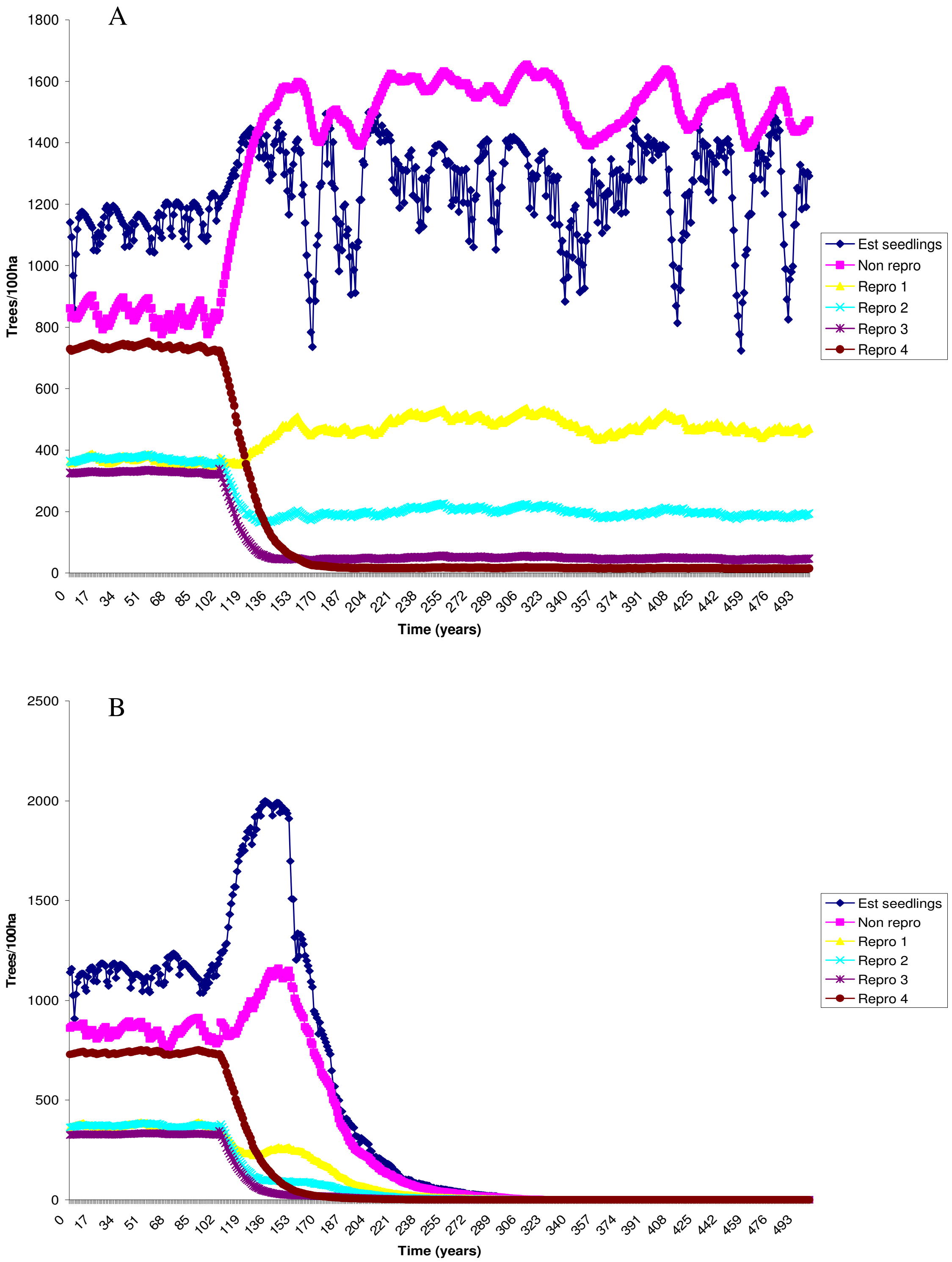


Figure 9: Effect of elephant introduction at constant stocking density based on a) the default elephant mortality and damage rates in the KNP between 2001 and 2010 and b) doubling the elephant toppling and pollarding default rate in only one class (Non repro).

11.4.4. Elephant and fire influences

When elephants and fire every 3 years were introduced after 100 years, the marula population crashed within the next 300 years (Figure 10a). Only with a fire interval of 7 years do all size classes stabilise (Figure 10b). This indicates that marula populations are susceptible to the effects of fire and elephant in combination and with the default values used, the fire regime within the KNP between 1902 and 1998 (2-6 years; van Wilgen *et al*. 2000) was too frequent for the continued existence of marula populations in the KNP. The SCD for the model output with elephant and fire every seven years indicates much lower stable densities, with a dominance of individuals between 5 and 14 cm in stem diameter (Figure 5).

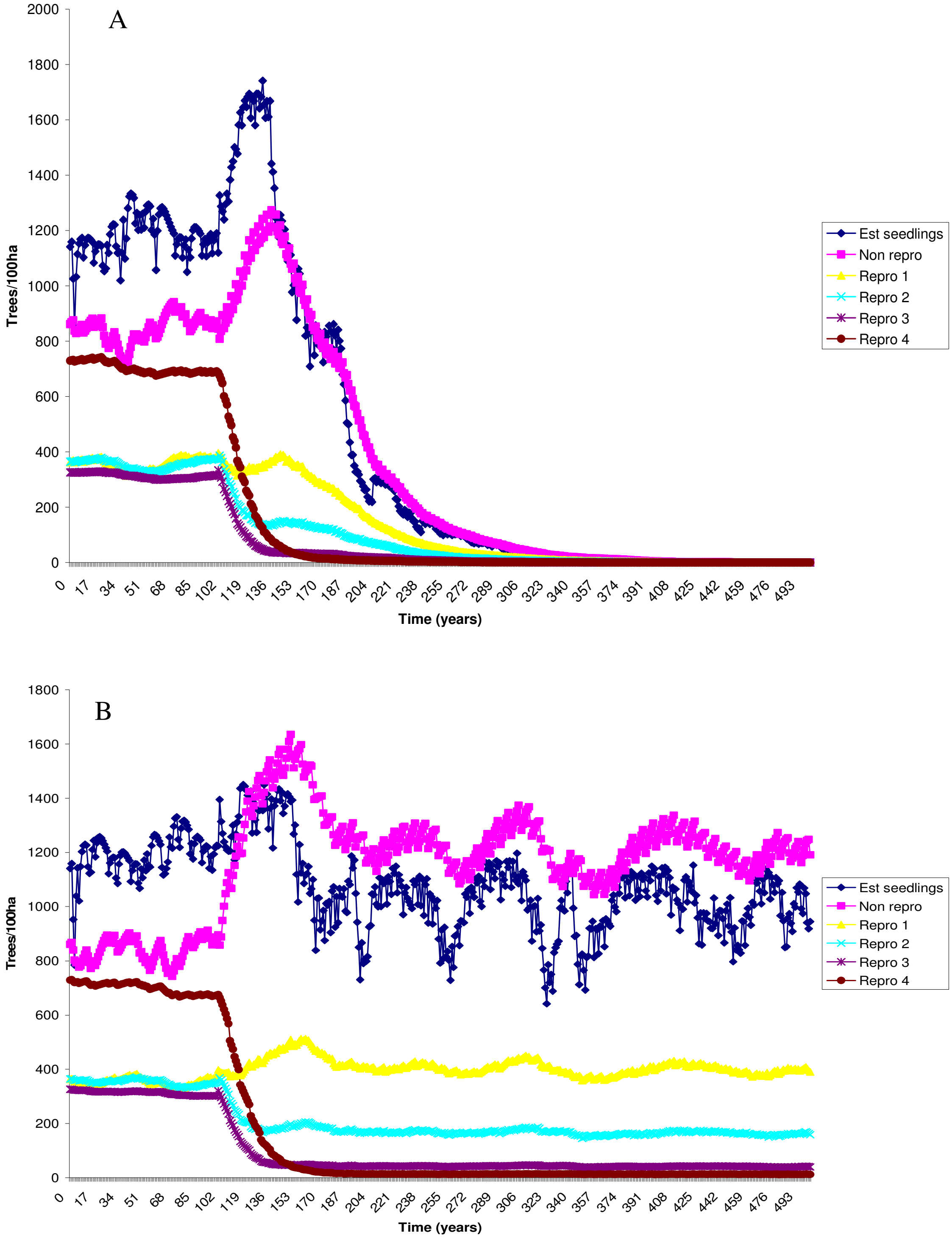


Figure 10: Response of the marula population to the introduction of elephant after 100 years in conjunction with fire a) every 3 years and b) every 7 years.

11.4.5. Scenarios

The stable structure observed outside the Hlangwine enclosure near Pretoriuskop in the KNP (OHE; Table 4) appears to only be transient. With fire every three years and elephants present the population structure observed at this site can be replicated by the model using the default values after 50 years (Figure 11). However, as described above, by 100 years with the same conditions, the population crashes (Figure 11). The crash takes slightly longer than it would at a lower rainfall (Figure 10a).

To replicate the adult dominated population structure at Phabeni in the KNP (PHA; Table 4), adult mortality rates were decreased and seed and seedling predation rates increased. An adult dominated population could be generated after 50 years (Figure 14), but over time, if no recruitment occurs the population dwindled to below 4 trees/100 ha over the following years. This therefore indicates that rodent predation and herbivore utilisation of the seedlings is very high at PHA and adult mortality has been relatively low.

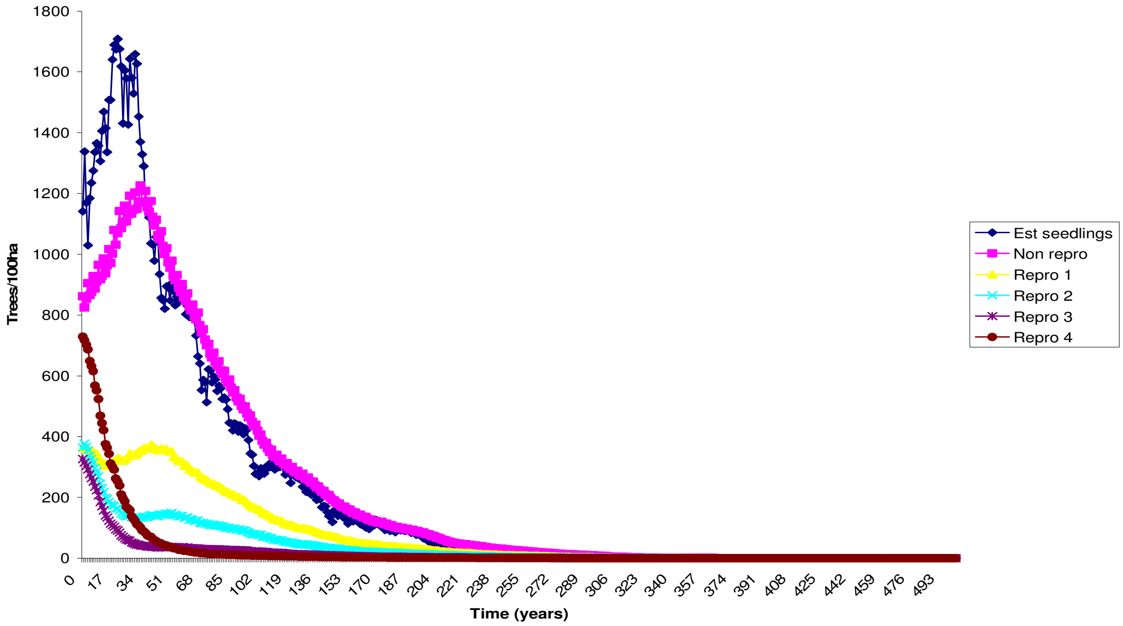


Figure 11: Projection of the marula population with fire every 3 years and elephant impacts using the parameters at OHE.

To replicate the juvenile dominated population structure found in Landscape 12 in the KNP (L12; Table 4), adult mortality rates need to be extremely high (Figure 5). No reproductive input results in the eventual extinction of the population unless the high mortality rate drivers are removed.

To replicate the "missing size class" population structure as observed in Landscape 5 in the KNP (L5; Table 4), the mortality rate of individuals in the fire trap (coppicing class and established seedlings) as well as non-reproductive individuals had to be increased substantially (Figure 5). This structure was unstable and will crash after 100 years.

11.5. Discussion

According to MARULA1, marula and elephants can co-exist without fire, if the mortality and utilisation rates observed between 2001 and 2008 continue. However, when fire is introduced, coexistence can only take place with a fire return interval of seven years. This fire interval is much higher than the mean fire interval of 4.5 years experienced in the KNP between 1941 and 1996 (van Wilgen *et al.* 2000). Fire alone, unless applied annually, could not suppress the marula population. These results contradict those of Dublin *et al.* (1990), whose tree population dynamics model found that elephants could not reduce recruitment rates below mortality rates and hence cause a decline in woodland vegetation in the Serengeti-Mara ecosystem in the 1960s and 1970s. Their findings indicated that fire was more important and it was hypothesised to be the driver responsible for woodland declines. However in the 1980s, their model indicated that elephants alone were preventing woodland regeneration as the loss of large trees resulted in a change in elephant feeding behaviour from utilisation of mainly large trees to utilisation of seedlings (Dublin 1986). Their overall hypothesis indicated that fire initially changed the woodland to grassland, but once the woodland was reduced to low enough densities, elephants were able to keep the vegetation in a grassland state.

MARULA1 indicates that the stable marula population structure observed at high rainfall sites in the KNP, such as at OHE, is a transitory phase on a progression towards severe reduction in the population size and instability. As already understood, the four main population SCDs observed, i.e. adult dominated, juvenile dominated and those with a “missing size class” (Chapter 2), are also shown by the model to be unstable, and unless the pressures of high adult mortality and seed and seedling predation are reduced, the model predicts that these populations will eventually crash. The model is sufficiently detailed to be able to model all these different scenarios, albeit relatively crudely.

Baxter and Getz (2005) found that the overriding disturbance factor in maintaining tree grass coexistence was not fire but drought in their model. MARULA1 shows that marula is relatively insensitive to drought. However, drought was not as explicitly modelled here as it was in the Baxter and Getz (2005) model, where if rainfall was below the long-term mean for three consecutive years, additional morality was applied to the trees. Viljoen (1988) indicated that the loss of large trees between 1944 and 1981 was due to drought in the 1960s interacting with fire effects. However, in light of the relative invulnerability of marula seedlings to drought (Chapter 10), the fact that so few marula adult trees died during a severe drought in the KNP in the 1990s (Viljoen 1995), and after a drought in Botswana (O’Connor 2010), it is assumed that marula is sufficiently drought tolerant not to be impacted in this way and that drought is not as important for marula as it may be for other species modelled by Baxter and Getz (2005).

MARULA1 has taken into account the faster growth rates of coppicing trees due to stored starch reserves which Baxter and Getz (2005) did not take into account. This inclusion increases the recovery time of woody plants after fire and probably has a significant effect on the overall woody biomass (Menaut *et al.* 1990).

The model of Pellew (1983) proposes that other browsers and fire play a role in preventing recovery of damaged trees, eventually causing the woody component to reduce in density. However, according to Baxter and Getz (2005), a decrease in the woody plant density will result in a concomitant reduction in the browser guild and heavy herbivore utilisation of the grass component may reduce the frequency of fire. The effects of herbivory besides elephant herbivory is explicitly incorporated in MARULA1 through the mortality rates and predation rates of seeds and seedlings. More detailed incorporation of the effects of small browsers such as impala may further modify the results since their effects may be more important than what the model was able to incorporate at this stage (Moe *et al.* 2009). The effects of soil type also still need to be explicitly incorporated, especially the interesting response of marula seedlings to herbivory on basalt soils observed in Chapter 9, making them more vulnerable on these soil types.

Baxter and Getz (2005) predicted that an elephant density >1 km^{-1}, would result in a 70% probability of quasi removal of trees within a century. However they stated that their model did not incorporate the possible invasion of a faster growing less palatable shrub species into the space opening up as reported in Botswana (Teren and Owen-Smith 2010), where an understory shrub, *Combretum mossambicense* has replaced an *Acacia* spp. canopy due to elephant impact. Since marula is not an unpalatable invasive shrubby species, and is actually highly favoured by elephant and heavily utilised (Shannon *et al.* 2008), it would be expected to follow the predictions of the Baxter and Getz (2005) model. The results from MARULA1 support this contention based on a uniform elephant density prevalent between 2001 and 2010 in the southern KNP (0.8 to >2.0 km^{-1}).

A positive aspect of the model was the inclusion of actual growth rates of marula individuals of different sizes based on empirical data. Many previous modelling attempts have estimated annual growth rates and the lack of growth rate data has been highlighted as a major missing parameter for previous models (Baxter and Getz 2005). However, growth rates vary with rainfall and levels of damage and this variability still needs to be incorporated into the current model.

The positive role that mammalian dispersers may play in dispersal of seeds and increase in germination rate in marula (Lewis 1987) was not considered in this model. It was assumed that mammalian ingestion did not result in increased germination percentage following the results obtained in Chapter 6, which indicated that given sufficient moisture and scarification (achieved through the dry season), all potential seeds will germinate. Chapter 7 indicated that acid digestion did increase the germination rate but not necessarily the germination percentage. The possibility that marula fruit ingested by elephant may be able to germinate in the same season as fruit production (Chapter 6) was not incorporated. A source sink situation may also apply with elephant dispersal. Removal of fruit from marula trees growing in favourable environments, to habitats normally favoured by elephants, but which may not be suitable for marula seed germination and seedling survival, also requires careful consideration when deciding to incorporate the possible benefits that mammalian ingestion of seeds may have on marula population dynamics.

A number of gaps in our knowledge of marula biology remain. These include: 1) the response of damaged adult size classes to fire, 2) growth rates of damaged trees, 3) effect of low rainfall on the mortality of damaged trees and how fire interacts with this relationship, 4) fate of dispersed seeds, 5) tree longevity and natural senescence; 6) how borers into the heartwood affect tree longevity and senescence, and 7) prevalence of elephant utilisation of juvenile marula trees.

Major shortcomings of MARULA1 in its present form, include the lack of spatial variability in elephant utilisation and fire effects, which are important factors in terms of tree mortality, but currently MARULA1 uses mean values from a large area (Chapter 3). Neither does it incorporate rainfall effects on growth rates but uses mean values across sites varying in rainfall (Chapter 4). These shortcomings limit the utility and predictive capacity of the model. Nevertheless the model in its present form does allow for the exploration of the possible factors and their relevant combinations that may result in a stable population structure, adult dominated or juvenile dominated as well as a structure with a “missing size class” (Chapter 2), by incorporating marula specific vital rates investigated in this study. Many more scenarios could be explored, but they were limited to the four presented here.

Further development of the model, as a tool for investigating woody plant strategies in response to disturbance is required in order to verify these results. Validation of the model output with real world data also requires further exploration. This chapter therefore represents only a preliminary foray into the modelling possibilities around the substantial amount of data collected in this study. Future presentations of this model will also utilise the standard format for documenting models and their analyses: transparent and comprehensive ecological modelling (TRACE) documentation proposed by Schmolke *et al.* (2010).

11.6. Conclusions

Even though this is a simple model which does not incorporate large scale spatial variability and is not grid based, it is parameterised using detailed data collected on the species in question and provides valuable insights into the knowledge gaps still remaining. It also aids in an understanding of how the various population structures came about and the thresholds in disturbances such as elephant damage and fire frequency which may result in an unstable marula population structure, i.e. elephant presence and fire every seven years. The integration of the information derived from this entire study is valuable in its own right. Of course the model requires improvement and as with any model, is a work in progress. Future anticipated adaptations for MARULA2 include making the model grid based, introducing greater stochasticity in rainfall, fire regime and elephant impacts based on elephant densities, vegetation biomass and patch dynamics, tree-grass interactions, as well as small scale habitat variations brought on by herbivore utilisation and water provision.

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Chapter 12: General discussion and conclusions

12.1. Aims

This chapter sets out to:

- Integrate the findings of the study's components and their synthesis in the model developed in Chapter 11.
- Discuss the contributions made by this study in the understanding of the responses of marula to disturbances and why so many different types of population structures, usually unstable, exist in the low altitude savannas of South Africa.
- Identify potential management recommendations based on the findings of the study to ensure the continued persistence of marula in the low altitude savannas of South Africa in light of its importance for rural livelihoods and in natural ecosystems.
- Identify the remaining gaps in our knowledge on marula biology and make recommendations for future studies on this species.

12.2. Introduction

This study has provided detailed insight into the population structures, life history and responses to disturbance in marula. It appears that different factors affect marula populations in different places, but most populations exposed to disturbance of one form or another, show classically unstable population structures. Outside of conservation areas, human utilisation of the fruits for food and beer making (Emanuel *et al.* 2005), wood for carving (Lawes *et al.* 2004) and bark for medicinal purposes (W. Twine pers. comm.), require monitoring to ensure sustainability (Emanuel *et al.* 2005). Although in the past, adult marulas were not damaged in communal areas due to cultural rules, increasing human densities are resulting in the utilisation of large trees more and more frequently (Neke 2005; pers. obs.). In protected areas with fire, high browser densities and elephant such as the Kruger National Park (KNP), marula is prone to unstable population structures and has even become locally extirpated in some areas, specifically those with low rainfall (eg. outside the N'washitsumbe enclosure near Shingwedzi (NE) (Jacobs and Biggs 2002a)). However, in protected areas without elephants, where fire frequency is low, rainfall is sufficient for growth and browser intensity is low, such as at Wits Rural Facility (WRF), marula populations thrive in suitable habitats. This indicates that a temporary removal of the factors mentioned above could result in a dominance shift to marula. This may have happened in the past (Hall *et al.* 2002), in places such as Jejane Private Nature Reserve (JPNR) and in the southern parts of the KNP where high density, adult dominated populations exist.

In Chapter 2, highly variable marula population structures were described and placed into four main groups: 1) those dominated by adults, 2) those dominated by juveniles, 3) those with a "missing size class" and 4) stable population structures. Populations consisting only of adults such as at JPNR, Albasini in the southern KNP (ALB) and Phabeni in the southern KNP (PHA) may have become established due to human habitation in these areas. This is evident from the recruitment failure for at least the last 60 years (no individuals <20 cm in stem diameter). Such sites may not ordinarily be suitable for marula recruitment due to unsuitable soil type or low rainfall combined with heavy herbivore utilisation of any germinating seedlings or heavy seed predation by rodents. The loss of such populations is probably of little concern since they are essentially an artefact of past conditions. Such a loss is most likely inevitable in the KNP, where annual mortality rates of up to 4.6% in adult trees, due to a combination of elephant damage and fire are prevalent. Unless a massive recruitment event takes place, the remaining adults may become relegated to refugia such as rocky outcrops. Continued elephant utilisation and frequent fire will continue this trend.

At sites where only juvenile marulas trapped within the fire trap remain, the long-term effects of elephant impact and fire are evident. The loss of the adult trees in these areas prevents any new input of seed. Whether this is mitigated by mammalian dispersal of the seeds from sites with adults is still unknown. The longevity of the seedlings in this seedling bank is also unknown. However, gradual loss of the remaining individuals over time is expected to occur unless fire suppression is practiced in conjunction with a reduction in browser numbers.

Marula has been extirpated from the semi-arid northern regions of the KNP with mean annual rainfall (MAR) of approximately 450 mm, (Jacobs and Biggs 2002a), as well as from the arid Tuli Game Reserve in Botswana with MAR of 370 mm (O'Connor *et al.* 2007). It appears that elephant impacts increase mortality of marula in dry areas relative to wetter areas. Loss of the species from the dry northern basalt plains (Jacobs and Biggs 2002), indicates that the pattern of marula population extirpation may occur first on basalt in the remaining dry areas in the KNP and then later on granite in the drier areas. It is interesting to note that there are sites in the KNP, which have the same stem diameter structure on both basalt and granite soils, indicating that the same drivers are structuring the marula populations on both substrates in the KNP. However, adult tree heights were lower on the basalt than on the granite, indicating that more elephant damage in the form of pollarding was present on the basalts, reducing the heights of the established trees.

It appears that in the Pretoriuskop region, which has high rainfall, marula is still able to maintain a stable population structure, however densities are significantly curtailed compared to those within an enclosure (HE), with no browsing or elephant (Chapter 6). Continued utilisation of individuals within the "missing size class" and prevention of recruitment of saplings by fire and herbivory may, however, destabilise these populations as well.

It must be noted that many of the population structures assessed in Chapter 2 in the KNP were based on surveys done in 2001 or 2002. In Chapter 3 it became evident that these population structures were highly modified over the last decade due to high annual mortality rates as well as high annual toppling and pollarding rates. Recruitment out of the fire trap did not occur and very little regeneration was evident, and mainly confined to the basalt ecozones studied. No new recruits were found in the drier granitic or gabbroic soils east and west of Skukuza. There was however evidence of continuous recruitment at higher rainfall sites such as Pretoriuskop (pers. obs.). Between 2001 and 2008, at sites with a "missing size class", the number of individuals in this class decreased and the class width increased to include larger trees, indicating that these populations are progressing to a state similar to the juvenile dominated populations mentioned above.

It must also be taken into consideration that many past studies on marula conducted during the dry season, mistake false marula (*Lannea schweinfurthii*) for marula, due to their very similar dormant form, especially in juveniles and hence, those in the "missing size class". For many previous data sets, where I have personally gone and tried to find previously marked marulas, I found a false marula in its place. This indicates that the "missing size class" phenomenon and hence unstable population structures may be more prevalent than the literature or previous studies may imply.

12.3. Integration of ecological findings and additional insights

12.3.1. Marula life history strategy and responses to disturbance

Marula allocates vast amounts of biomass to its roots and into starch storage, making it extremely drought resistant and provides a strong basis from which to resprout after fire or after some other disturbance event. Such investment can also make it vulnerable to root removal by baboons and elephants, especially during the dry season, due to this high quality resource. Growth rates are relatively fast but differ across size classes and across years following rainfall. Marula have slow height growth rates but relatively quick diameter growth rates. Allocation to defence in terms of phenolics is relatively high in seedlings, however no trade-off between growth, defence and storage could be discerned.

Strong seasonal shifts in starch allocation during the juvenile phase are evident, indicating that marula seedlings are particularly vulnerable to fire and herbivory after leaf flush when starch reserves are low. Soil type does not appear to affect growth dynamics but does affect the response of seedlings to herbivory. Marula has a resistant strategy in response to herbivory, coupled with overcompensation even at low herbivory on nutrient-rich soils. Provenance of the seeds is important as different provenances have different growth characteristics and perhaps different responses to disturbance. Competition does not appear to affect the growth dynamics of marula trees in the field.

Marula seedlings invest in stem diameter growth at the expense of height gain and use a well developed thick bark to become resistant to fire at relatively small sizes, even if the apical buds are within the fire trap. Marula seedlings are also vigorous basal resprouters.

However, once fire resistance has been achieved, epicormic resprouting occurs on burnt stems >2 cm in diameter, indicating that stem diameter is more important than stem height in fire resistance. Low wood density results in faster diameter growth rates, which results in early fire resistance. Marula is sufficiently resistant to fire as a single disturbance. However, when combined with subsequent herbivory, resources are significantly depleted. Being one of the first species to resprout after a fire, even before the rains, makes it particularly vulnerable to herbivory, as the succulent shoots are sought after and are at ground level, and hence very accessible to a number of herbivores. On nutrient-rich basalt soils, this effect is amplified.

Fruit is not produced annually and there is only a small persistant seed bank. Recruitment relies almost entirely on the current season's fruit crop. However during low rainfall years no seeds will germinate, carrying them over to the next year when rainfall may be higher. Marula endocarps stored in ideal conditions remain viable for at least 10 years. However, those in the field exposed to temperature and moisture extremes would breakdown relatively quickly.

Given the soft wood of marula, they would be expected to be relatively short lived trees, compared to more dense wooded species such as *Combretum imberbe*. Preliminary results indicate that once a mature marula has been damaged by an elephant (either by bark removal or branch damage), the likelihood of insect borers attacking the heartwood increases regardless of the level of recovery of the bark or branch. Recovery from the initial elephant damage is fast. However, the damage to the heartwood by the insect borers is further exacerbated by woodpeckers and other birds boring into the trunk to eat these insects. After a number of years, the inside of the marula trunk may be sufficiently weakened, that in the event of a fire, that tree has a very high likelihood of being felled by the fire. Recovery after such an event depends on the height at which the damage occurred, the size of the stem and the time allowed for the borers to do the damage. The soft wood of marula makes it particularly vulnerable to the effects of damage and most damaged trees, although they resprout vigorously and regrow lost tissue, have been invaded by borers making them particularly susceptible to future fires (Chapter 8).

Although many life history traits were elucidated in this study, it must be remembered that many life history processes are thought to be highly stochastic, and driven by spatiotemporal rainfall variability (Higgins *et al.* 2000). Hence long-term monitoring is imperative before the predictions made above can be verified.

12.3.2. Marula population model and the gaps in our understanding

All modelling exercises not only synthesis the information gained from a study, but are also valuable in identifying the major gaps in our understanding. Despite undertaking a comprehensive study of marula biology, many gaps in our understanding and considerable uncertainty of its vulnerability to disturbances still exist.

The most important gaps in our understanding which should be emphasised in future research include:

1) The effects of repeated utilisation on resprouting and survival
2) Length of time for the plant to regain biomass after a disturbance and reach reproductive maturity
3) Longevity of marula seedlings in the seedling bank
4) Longevity and onset of natural senescence in adult trees in the presence or absence of borers
5) Time interval between successive episodic recruitment events
6) The level of damage that will result in mortality
7) The effect of stochasticity in rainfall on elephant utilisation of marula and subsequent mortality

12.3.3. Comparisons with other species

Compared to other co-occurring savanna tree species, marula has very low wood density. This variable indicates fast growth rates (Enquist *et al.* 1999). Marula appears to have one of the fastest growth rates of any African savanna tree, even when compared with woodland and forest tree species. However, they do appear to have slower growth rates than *Acacias* which are leguminous species. This could be due to *Acacias* preferring more nutrient-rich soils and having the ability to fix nitrogen. The relatively fast growth rates for marula therefore follow from that predicted by its low wood density.

Plant ecology strategy schemes arrange species into categories according to their ecological attributes (Westoby 1998). Westoby (1998) proposed the leaf-height-seed (LHS) scheme to categorise species. This particular scheme consists of three axes: specific leaf area (SLA) of mature leaves, height of the canopy at maturity and seed mass. The plant strategy is characterised in the scheme by a position in a 3-D volume. For marula these values are relatively low (18.1 mm^2/mg), high (up to 18 m) and low (0.3 g) respectively compared to other species (Moog *et al.* 2005). Species with low SLA have generally less palatable leaves which increases their resistance to herbivory (Moog *et al.* 2005). Low seed mass is more competitive in a high disturbance environment, while high canopy height indicates the potential for dominance.

Marula saplings become fire resistant at smaller diameters than other co-occurring savanna species (Appendix 1) and this was attributed to a high allocation to bark very early on in its development. The extremely high, non-plastic allocation to roots from germination is an important trait for survival from drought and fire and yet comparisons with other savanna species are difficult due to the lack of equivalent information in the literature.

Marula falls within the fourth largest tree family in southern Africa, comprising of 80 tree species and many shrubs. Family characteristics include leaves with watery latex, the absence of stipules, the strong resinous odour of the crushed leaves and the small unisexual flowers (Schmidt *et al.* 2002). Marulas lack spines, a trait that runs through the entire family and instead are chemically well defended. In savannas often dominated by spinescent *Acacias*, which reduce intake of browse by smaller browsers, marula leaves appear to be palatable enough to be heavily utilised by wild animals to such an extent that recently respouting individuals have all their leaves eaten (pers. obs.). Marula has compound leaves, while the remaining members of the Anacardiaceae are evenly split between compound and simple leaves. The evolutionary advantage of compound leaves over simple leaves is unknown and does not confer decreased vulnerability to herbivores or decreased light interception (Warman *et al.* 2010).

12.4. Management implications and recommendations

Given the high utilisation of marula trees evident in the KNP (Shannon *et al.* 2008), marulas may show negative responses, despite their reported resilience (Coetzee *et al.* 1979; Gadd 2002; Jacobs and Biggs 2002b) to elephant impacts, long before other less utilised species, and hence could be used as an indicator species (for example, when setting thresholds of potential concern (TPC) for elephant impacts on woody vegetation in the KNP). The KNP has various TPC's for the woody component. The ones applicable to marula include: 1) dominant species composition; 2) diagnostic or differentiating species; 3) woody structure or abundance. For the dominant species composition TPC to be reached within a landscape, the dominance ranking of the first or second ranked species descends to the third or lower position. A drop of less than 3% proportional abundance will not be considered a drop. For the diagnostic species TPC to be reached in a landscape, the proportional abundance of marula will have to drop by more than 50% from that recorded by Gertenbach (1978; 1987). For the woody structure TPC to be reached per landscape, the proportional abundance of the <1 m in height trees should not drop by more than 40% and the >5 m class should not have dropped by more than 10% of their proportions since the benchmark was set (Gertenbach 1978; 1987; Van Rooyen 1978; Coetzee 1983; Venter 1990), or individuals in the >5 m class have dropped by 1% for 3 consecutive surveys.

In the KNP, marula has surpassed the last TPC, however it is one species and this TPC refers to the entire woody component of the vegetation. For the dominance TPC it is difficult to tell whether marula has dropped a dominance ranking in any landscape as other dominant species such as *Acacia nigrescens* have also declined (Moncrieff *et al.* 2008). Whether marula's proportional abundance has dropped by more than 50% from that recorded by Gertenbach (1978; 1987), is also relative to other species. Hence the current study focussed on marula alone is unable to determine whether this TPC has been surpassed as other species were not assessed. A relative measure for a TPC may be confounded by the concurrent decrease or increase of other species in the habitat. Since marula is a keystone species in the KNP and heavily utilised, combined with the evidence of local extirpation of the species in some areas of the park, it may be more appropriate to set an absolute TPC for marula based on the loss of certain size classes as is done for the vegetation structure TPC.

An overriding management recommendation for KNP is to erect similar sized exclosures to the HE and NE in all landscapes in the KNP. These should be used to assess changes in the vegetation brought on by fire and herbivory. Each exclosure should have one section with the same fire regime as that outside the exclosure and another section should be protected from fire. Although this is a costly experiment, it is a worthwhile exercise and will in the long-term be able to go a long way toward answering many of the questions related to the effects of fire and herbivory on vegetation. The current enclosures should be seen for what they are, jewels of information even though their initial purpose was not for observations of vegetation change. The abandonment of these enclosures will be a serious loss for the future understanding of vegetation dynamics. A management plan for these enclosures should be drawn up. Blocks within the enclosure should be burnt under a specific plan with at least one block being burnt at the same time as the area surrounding the enclosure is burnt as well as a block where fire is suppressed.

The marula population structures observed are snap shots in time and long-term studies on permanently marked marula populations are required to verify the model developed to predict the population dynamics of marula into the future. The marula trees marked in this study with GPS co-ordinates should be relocated in five years time in order to assess changes in the population structures.

Further investigations into the effects of rainfall, herbivory, fire and soil type are still needed for marula. The interesting findings from this study indicate that marula reacts more negatively to herbivory on basalt soils than on granite soils. How this interacts with rainfall is unknown, but based on observations of marula extirpation from dry basalt areas in the northern KNP, this interaction is very important. A factorial glasshouse experiment needs to be conducted to tease out these interactive effects and what they mean for marula juveniles. The responses of adults to herbivory, fire and drought also still need further elucidation. Pruning experiments on large trees or following damaged trees over time is essential to determine what combination of factors will result in the death of adult trees. The responses of different provenances to disturbance also require further study. The responses of seedlings to herbivory at different times in the season also require further investigation.

The response of marula to insect herbivory is an entirely unexplored avenue of research. As already mentioned marula is heavily utilised by insects, from moths to fruit flies to borers. The Coleoptera that utilise the soft wood tissue after damage are very important in marula dynamics but receive little attention in the literature. Moth species whose caterpillar stage utilise the leaves can wreak havoc in years of abundance. In the 2009 rainy season many trees of adult size had become engulfed in a web created by the Ermine moth, resulting in complete leaf loss and fruiting failure. The costs of leaf reflush and how this impacts on growth and future dynamics is a fresh avenue for investigation, as well as the prevalence of these outbreaks. In addition to this, other caterpillars utilise the leaves in the wetter parts of the marula range around Pretoriuskop, resulting in brown, dried, rolled up leaves full of caterpillar faeces. In January 2009, in up to 50% of the smaller marulas (<5 m in height) the entire canopy of leaves was in this state, resulting in complete reflush of new leaves by the time the trees were revisited in May that year. The impacts of this reflush starch storage require further investigation,

Further investigation into recruitment processes are required, including fruit production and its drivers, as well as the longevity of seeds in the seed bank if sufficient rainfall does not result in germination. Pollination biology also requires further investigation in terms of possible pollinator limitation in homogeneous vegetation, such as at NE as proposed in Chapter 6, as well as in terms of the effects of pollen limitation.

Isoptope studies of the water and nutrient use of marula trees of all ages still require investigation. Such studies will provide insight into the partitioning behaviour of the plants, as well as its habitat requirements. They may even be able to tease out the reasons why marula is more vulnerable on nutrient-rich basalt soils versus nutrient-poor granite soils.

The effect of climate change on marula growth rates and responses to disturbance also require further investigation. Indications that trees will be favoured under increasing levels of CO_2 may mitigate the negative effects that elephants and browsers may have on the rate of tree loss. Whether this will apply to marula requires further study. Its drought resistance strategy does, however, bode well for its continued existence under future climate change scenarios.

12.5. Conclusion

The overall aim of this study was to explore the possible explanatory mechanisms for 1) how marula populations may be able to sustain themselves in environments prone to disturbance and 2) why marula has such variable and unstable population structures, by assessing the adaptive responses and vulnerability of marula to disturbance in its natural habitat as well as under artificial conditions.

Even though marula is highly resilient to damage from either herbivory or fire alone, the combination of frequent fire and heavy utilisation is proving fatal for marula populations in the KNP and elsewhere. The unstable population structures prevalent in the KNP are the first indication that marula populations are no longer self-sustaining in these environments. It appears that the combination of elephant and fires more frequent than seven year intervals will result in crashes in marula populations, which is further exacerbated by low rainfall.

In terms of other savanna tree species, marula is an outlier in its life history strategy, being extremely well adapted to the effects of fire with very thick bark, extensive resprouting ability and fast growth rates, combined with very high levels of storage and chemical defence. This indicates that the stresses on marula populations in the wild must be overwhelming for unstable population structures to develop. However its soft wood, which is susceptible to wood borers, appears to be the main weakness of adult trees in the light of both elephant and fire impacts. The lack of recruitment in some sites is perplexing given the extraordinary ability of marula seedlings to resprout from an early age, withstand extensive drought, have fast root penetration rates, extremely high root reserve storage and resistance to fire at small stem diameters, combined with high levels of fruit production and low water requirements for germination. It can only be construed that the lack of a dense persistent seed bank, high inter-annual variability in fruit production, and low germination percentages combined with high seed and /or seedling predation rates and possibly dispersal of seeds away from suitable habitats, are the main drivers behind the lack of recruitment observed at many sites.

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APPENDICES

Appendix 1: *Sclerocarya birrea* subsp. *caffra* (marula) allometry in the low altitude savannas of South Africa

1.1. Methodology

1.1.1. Tree sampling

Trees between 1 and 8 m in height were selected in three protected areas and the height and basal diameter as well as diameter at breast height (1.3 m) were measured with a height metre and tree calliper respectively (Chapter 4). Additional allometry data was obtained from trees surveyed in transects in the KNP by M. Hofmeyr in 2001 (Chapter 2), as well as additional transects surveyed in this study in 2008 and 2009 (Chapter 2). Data from these various sources was combined to determine the various relationships described below. Bark thickness measurements were also obtained from various data sources, measured using a Bartack bark metre on two opposite sides of the stem at 30 cm above the ground at the same height as basal diameter measurements and averaged. Leaf area estimations were made using 50 plant leaf samples harvested in the glasshouse and leaf area calculated using PCI software package to assess scaled digital photos of the leaves as described in Chapter 5.

1.1.2. Data analysis

All analyses were at the plant level. Only intact woody plants were used in the analyses, i.e. plants with evidence of cutting or substantial breakages were excluded, since allometry (height to diameter relationship) and resource allocation to growth and reproduction are likely to have been affected.

1.2. Results

1.2.1. Basal diameter and diameter at breast height

In many studies various methods of stem diameter assessment have been used. The most common are basal diameter and diameter at breast height (usually 1.3 m). In order to make comparisons between studies, the relationship between these two measurements is useful to formulate. For marula in the low altitude savannas of South Africa, the relationship between basal diameter and diameter at breast height is:

Basal diameter (cm) = 1.1*diameter at breast height (cm)+1.7002

Less than 1.5% of the variation could not be explained by this regression and hence it is an accurate relationship (Figure 1).

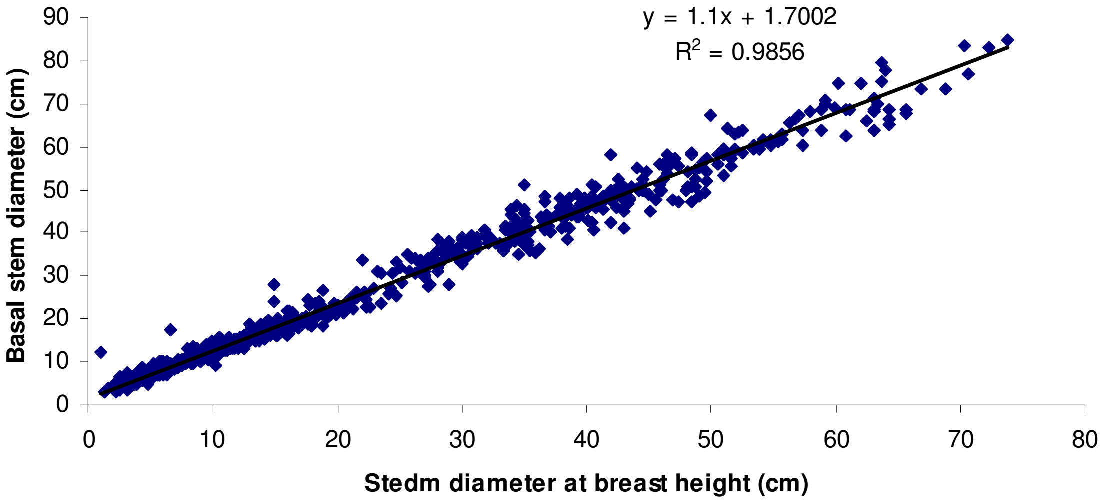

Figure 1: Relationship between diameter at breast height (1.3 m) and basal diameter in marula in the low altitude savannas of South Africa. N=726.

1.2.2. Basal diameter and height

There was no evidence to suggest that the growth strategy of marula changes with increasing size as height gain was constant with increasing basal diameter. A piece wise regression did not increase the R^2 obtained and hence was not a better fit to the data than the linear relationship, which was:

Log (Height (m)) = 0.6751*log (basal diameter (cm))-0.1005

Only 16% of the variation could not be explained by this regression (Figure 2).

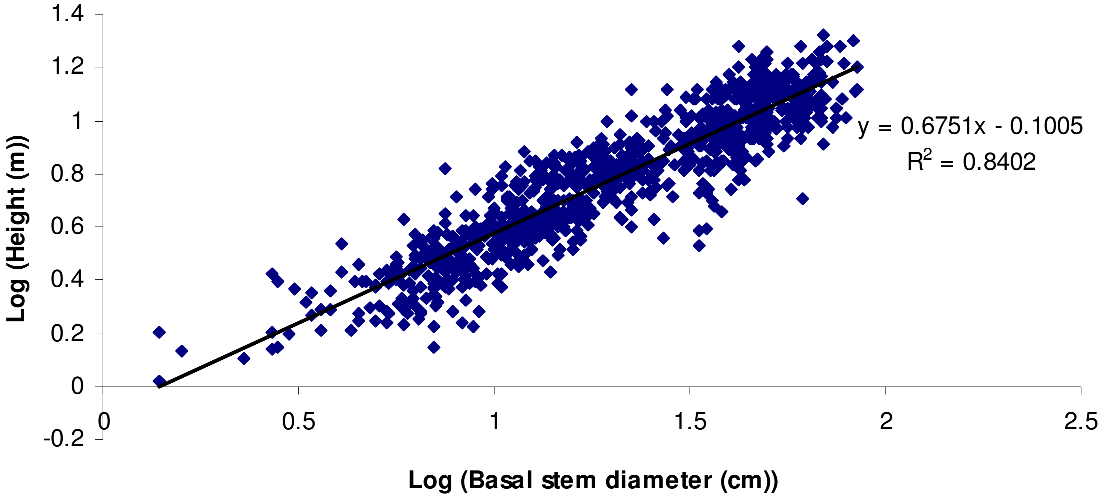

Figure 2: Relationship between basal diameter and height in marula in the low altitude savannas of South Africa. N=969

1.2.3. Bark thickness and basal diameter

A significant positive relationship between bark thickness and basal diameter in marula was found (Figure 3). Marula has a higher allocation to bark growth in smaller stems relative to the other species for which similar data was available from the Organisation of Tropical Studies (sweet thorn, scented thorn and mopane). An average thickness of 4.6 mm was found for marula individuals between 1.5 and 3 cm in diameter, whereas the other species had an average bark thickness of 1.2 mm at similar diameters (calculated from actual values). Analysis of covariance indicated that the slopes were significantly different ($F_{3,239}$=466.781; $p<0.001$) from each other. Bark thickness has a similarly significant linear relationship with height for individuals <3 m in height (Height (cm) = 0.007*bark thickness (mm) + 6.47; r^2=0.16; p=0.00001)).

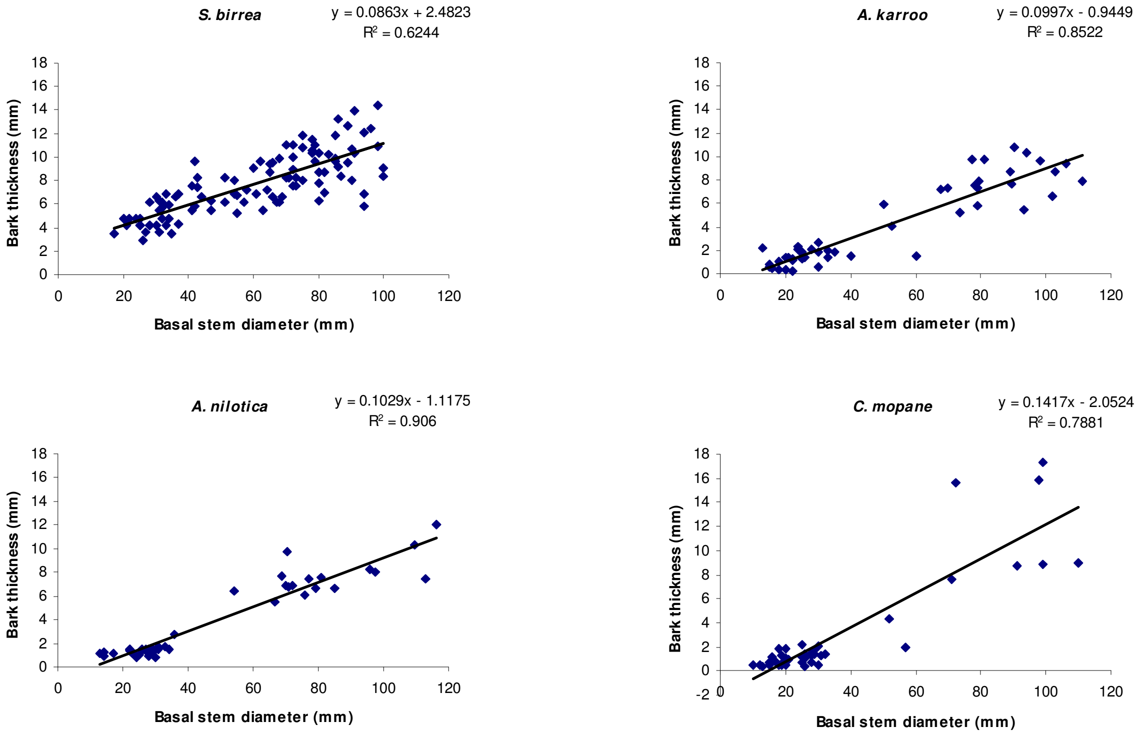

Figure 3: The relationship between bark thickness (mm) and stem diameter (mm) for individuals from 15 to 120 mm in basal diameter for four savanna tree species: *Sclerocarya birrea* subsp. *caffra* (n=108; p<0.0001); *Acacia karroo* (n=50, p<0.0001); *Acacia nilotica* (n=47; p<0.0001); and *Colophospermum mopane* (n=44, p<0.0001). Stem diameter and bark thickness were both measured at the base.

1.2.4. Relationship between leaf area and leaf mass

A strong positive linear relationship between leaf area and leaf mass was evident from the 50 leaf samples harvested from marulas grown under glasshouse conditions in Chapter 5 (Figure 4).

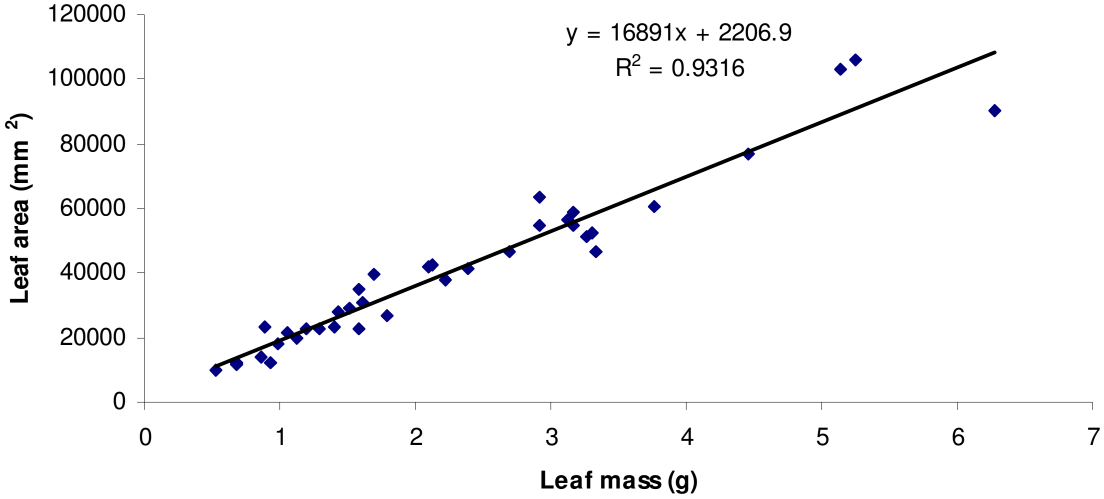

Figure 4: The relationship between leaf area and leaf mass in marula seedlings grown under glasshouse conditions (Chapter 5).

Appendix 2: Detailed description of the laboratory techniques utilised in the soil, starch and phenolic analyses

2.1. Soil texture and fertility analysis

This section described the methodology used by the Soil Fertility and Analytical Services, Kwazulu-Natal Department of Agriculture and Environmental Affairs for soil sample analyses (Manson and Roberts 2000). Soil samples were spread onto drying trays and dried at room temperature. Once the soil was dry, samples were crushed using rubber belts and the soil was then passed through a 1 mm sieve. Samples were placed into trays that contained 11, 70 ml PVC cups. Nine of the cups were each filled with one of the unknown soil samples, one with a standard soil sample for quality control and one blank. Dispensers and diluter/dispensers were used to dispense aliquots of extractant or reagent to three samples at a time. Organic carbon and clay contents were determined utilizing near-infrared spectroscopy. Total carbon and nitrogen were analyzed by the Automated Dumas dry combustion method using a LECO CNS 2000. Weighed soil samples are deposited into ceramic crucibles containing 0.5 g vanadium pentoxide (a combustion catalyst). The crucibles were placed in a horizontal furnace, and burned in a stream of oxygen at 1 350°C. The gases produced were passed through infrared cells where the carbon (as CO_2) was determined. Nitrogen was determined as N_2 in a thermal conductivity cell. Effective cation exchange capacity was calculated as the sum of extractable Ca, Mg, K and acidity. For pH in KCl, ten ml of soil was placed in each PVC cup and 25 ml of one molar KCl added. The suspension was stirred at 400 r.p.m. for 5 minutes using a multiple stirrer. The suspension was allowed to stand for 30 minutes before the pH was measured using a gel-filled combination glass electrode while stirring. For calcium, magnesium and acidity, 2.5 ml of soil were placed in each PVC cup and 25 ml of 1 molar KCl solution was added. The suspension was stirred at 400 r.p.m. for 10 minutes using a multiple stirrer. The extracts were filtered using Whatman No. 1 filter paper. Five milliliters of the filtrate was diluted with 20 ml of 0.0356 molar $SrCl_2$. Calcium and Mg were determined by atomic absorption. For extractable phosphorus and potassium, the extracting solution consisted of 0.25 molar NH_4CO_3, 0.01 molar Na_2EDTA, 0.01 molar NH_4F and 0.05 g/l Superfloc adjusted to pH 8 with a concentrated ammonia solution. Twenty-five milliliters of this solution were added to 2.5 ml of soil. The suspension was stirred at 400 r.p.m. for 10 minutes using a multiple stirrer. The extracts were filtered using Whatman No.1 filter paper. Phosphorus was determined on a 2 ml aliquot of filtrate using a modification of the molybdenum blue procedure. Potassium was determined by atomic absorption on a 5 ml aliquot of the filtrate after dilution with 20 ml de-ionised water.

Bulk density is not measured in routine soil analysis for soil fertility. The density of the soil samples once they have been dried and milled was measured. This yielded the 'sample density' (mg/l). From there, a conversion to a mass basis (mg/kg) was undertaken. For soil texture, suspended clay and fine silt were determined after dispersion and sedimentation, whereas sand fractions were determined by sieving. Textural class was determined by means of a textural triangle, which defines particle size limits of the various textural classes (Soil Classification Working Group 1991).

2.2. Starch assay procedure using the Megazyme kit

Milled samples were passed through a 0.5 mm screen and 100 mg subsamples (accurately weighed), were then added to centrifuge test tubes. To remove D-glucose and/or maltodextrins possibly present in the sample, 5 ml of aqueous 80% ethanol was added to the tube and the contents incubated at 80-85 °C for 5 min using an aluminium heating block. The contents were then mixed with a vortex mixer and a further 5 ml of 80% aqueous ethanol added. The tubes were centrifuged for 10 min at 3000 rpm using a bench centrifuge (Universal 320 Hettich Zentrifuge). The supernatant was discarded. The pellet was then resuspended in 10 ml of 80% aqueous ethanol and stirred on a vortex mixer. The sample was then again centrifuged as described above and the supernatant carefully poured off.

Thereafter, 0.2 ml of 80% aqueous ethanol was added and the tube stirred on a vortex mixer. Immediately thereafter, 3 ml of thermostable α-amylase was added. The tube was then incubated for 12 min at 100 °C with vigorous stirring on a vortex mixer after 4, 8 and 12 min. The tubes were then placed in a 50 °C water bath (manufacturer) and then 0.1 ml of amyloglucosidase was added. The tube was then stirred on a vortex mixer and incubated for 30 min. The volume was then adjusted to 10 ml with distilled water and then centrifuged at 3000 rpm for 10 min. Since the samples were expected to contain >10% starch, 1 ml of the solution was diluted to 10 ml with distilled water. From this solution, 0.1 ml was transferred to a glass test tube. 3 ml of GOPOD reagent was then added and the solution incubated at 50 °C in a water bath for 20 min. Two controls were also prepared, the D-glucose control consisted of 0.1 ml D-glucose standard solution (1 mg/ml) and 3 ml of GOPOD reagent. Reagent blank solutions consisted of 0.1 ml distilled water and 3 ml of GOPOD reagent. The absorbance of each sample and the D-glucose control was measured at 510 nm against the reagent blank using a Helios Gamma UV-Vis spectrophotometer (Thermo Scientific, Inc, Waltham, MA, USA).

2.3. Phenolic analysis

Leaf samples were dried in a convection oven at 50 °C for five days, then ground in a mill to pass through a 0.5 mm sieve. Different plants with different phenolics of different polarities will all require extraction procedures that differ (Waterman and Mole 1994). Since no standard universal technique is applicable for all species, initial experimentation with extraction methods, initial sample weight, types of extractants (and dilution), levels of dilution and the assay procedure was undertaken and a standard methodology adopted based on the precision of the results obtained. The extractant that extracted the most phenolics from the same marula leaf sample, all else being equal, was 70% acetone. Three replicate 0.05 g subsamples per leaf sample were weighed and placed in 20 ml test tubes and 10 ml of 70% acetone was then added. The extraction procedure found to be best involved repeated extraction with fresh solvent to maintain a steep diffusion gradient and was fully optimized to ensure maximum extraction by comparing multiple alternate procedures.

After the addition of the 10 ml of acetone, the samples were then mixed using a vortex mixer for 1 min, and then centrifuged at 2000 rpm for 10 min at 4 °C on a bench centrifuge (Universal 320 Hettich Zentrifuge). The supernatant was carefully removed and the pellet resuspended in 10 ml of 70% acetone, mixed for 1 minute with a vortex mixer and then centrifuged again for 10 min. The supernatant was carefully removed and combined with that of the first extract. This was mixed for 1 min and 10 ml of this sample was recentrifuged for 5 min. This supernatant was then carefully poured into clean test tubes and used for phenolic analysis.

1 ml of the sample extract was then placed in 20 ml of distilled water and mixed. 1 ml of 1N Folin Ciocalteau reagent was then added and the solution mixed again. After 1 min and before 8 min, 5 ml of sodium carbonate (40g of anhydrous sodium carbonate dissolved in 200 ml of distilled water) was added and the solution was mixed again. After 40 minutes the absorbance was read at 760 nm using a Helios Gamma UV-Vis spectrophotometer (Thermo Scientific, Inc, Waltham, MA, USA). A sample blank was also prepared at the same time following the exact same procedures but using 70% acetone instead of the sample extract. This blank was used to zero the spectrophotometer.

Since ascorbic acid is known to interfere with the Folin Ciocalteau method by increasing the absorbance value (Waterman and Mole 1994), the levels of ascorbic acid in the leaf material of one marula plant was measured by ARC-Irene Analytical Services (SANAS accredited laboratory) using HPLC (ASM 057) and found to be 3.45 mg/100 g. To test whether this significantly affected the phenolic content measured in leaf samples, two additional tannic acid standard curves were prepared, the first used only tannic acid at various concentrations obtained through serial dilution of a solution with tannic acid concentration of 0.1 mg/ml. The second used the same tannic acid concentration but with 10 mg of ascorbic acid added to the initial tannic acid solution. There tended to be a slightly higher absorbance for samples with ascorbic acid added (Figure 1). All leaf samples were assumed to have equivalent concentrations of ascorbic acid.

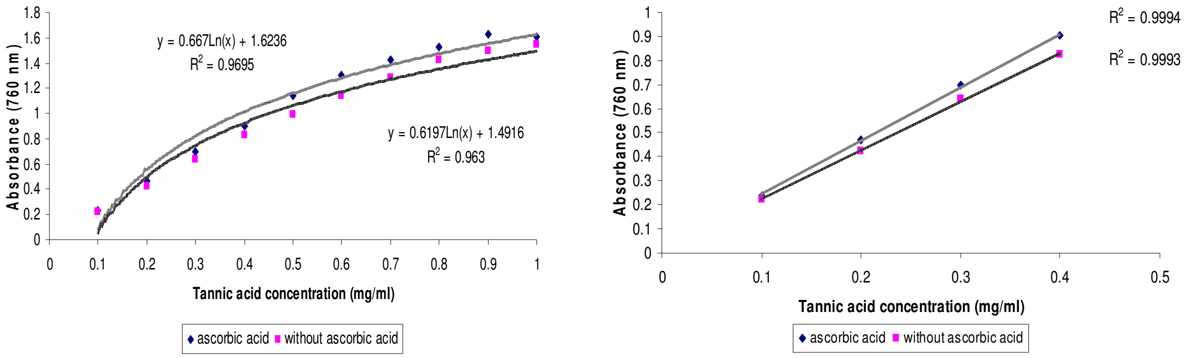

Figure 1: Tannic acid standard curves with and without ascorbic acid added (mg/ml) for a) all concentrations of tannic acid assessed and b) only those tannic acid concentrations reflecting absorbance values <1 and from where most absorbance values obtained were restricted through dilution.

2.4. References

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