Reproductive potential and seed fate of *Sclerocarya birrea* subsp. *caffra* (marula) in the low altitude savannas of South Africa

C.V. Helm *, S.L. Scott, E.T.F. Witkowski

Restoration and Conservation Biology Research Group, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, Johannesburg, South Africa

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 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 minimum 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, which 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 annual 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 seed 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.
© 2011 SAAB. Published by Elsevier B.V. All rights reserved.

Keywords: Dispersal; Elephants; Regeneration; Seedlings; Soil seed banks

1. Introduction

Changes in land use, fragmentation of tree populations and elephant impacts in African savannas have 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 a healthy recruiting tree population). For example, elephants target mid range tree size classes while fire targets the smaller size classes (Midgley et al., 2010). Humans target a wide range of sizes depending on species and uses (e.g. Luoga et al., 2002; Neke et al., 2006). These factors may have already, alone or in combination, resulted in unstable population structures for certain species such as *Acacia tortilis* (Prins and Van der Jeugd, 1993) and *Acacia xanthophloea* (Botha et al., 2002). We know of no study

that has 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 1 m 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 (Gadd, 2002; Jacobs and Biggs, 2002; Shackleton et al., 2003; Walker et al., 1986). 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, the 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. The 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 (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 (Baskin and Baskin, 2001; Von Teichman et al., 1986). 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 (Lewis, 1987; Von Teichman et al., 1986). 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*) (Estes, 1991; Shone, 1979). 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. 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?

2. Methodology

2.1. 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 (Fig. 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 4500 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).

2.2. 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 the 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).
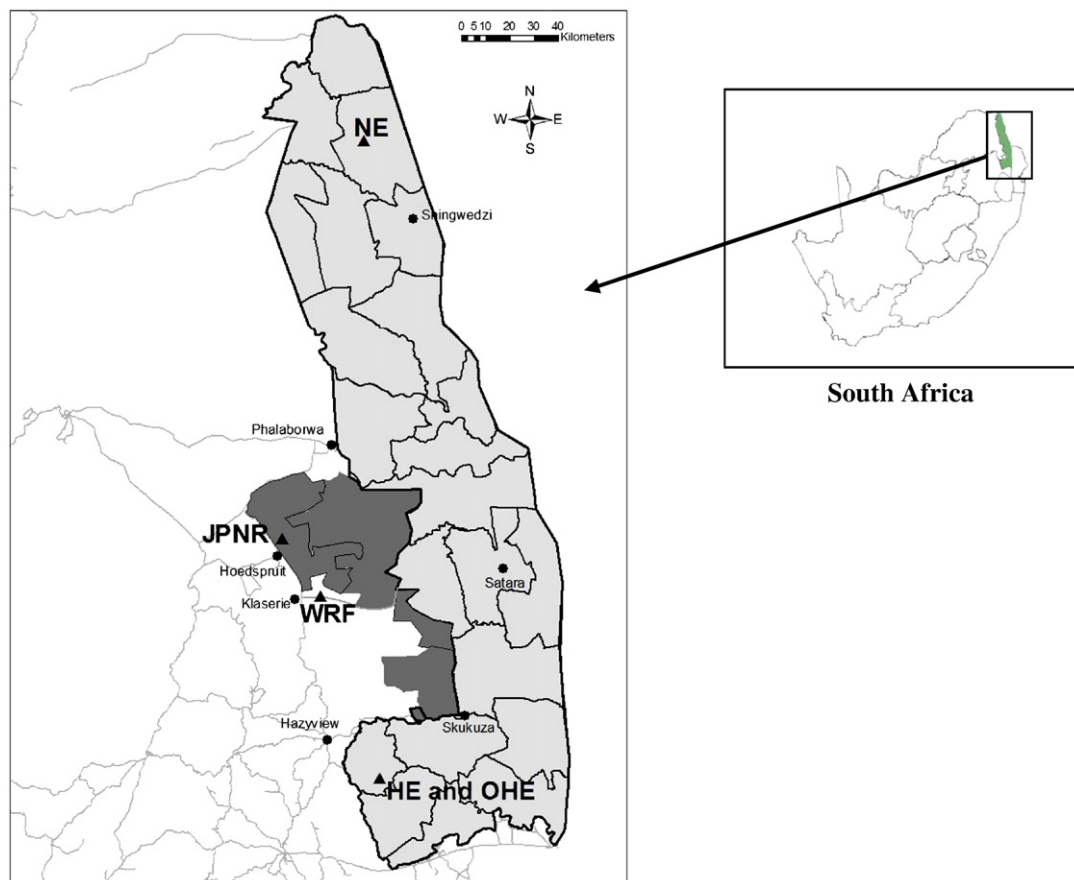

Fig. 1. Location of the five study sites (black triangles) in or near the Kruger National Park (KNP), South Africa. Dark grey indicates the private reserves outside of the lighter grey KNP. NE = N'washitsumbe enclosure, HE = Hlangwine enclosure, OHE = Outside the Hlangwine enclosure, WRF = Wits Rural Facility, and 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; G. Thompson pers. comm. (Manager of JPNR)). Methods for population structure determination and density estimates described in the 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 ($F_{4,23}$=8.49; p=0.0002)	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					

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, opportunistic wandering transects following no fixed route and starting at four random locations, were used to locate a minimum of 50 marula trees, covering the full range of available size classes at a site. 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 recognised 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 h (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 h of light and 19 °C for 10 h 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 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 <0.25 m 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.

2.3. 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 over-lapping microsites were sampled using a total of eight 0.5 × 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 (Fig. 2).

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 m² 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 were 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.
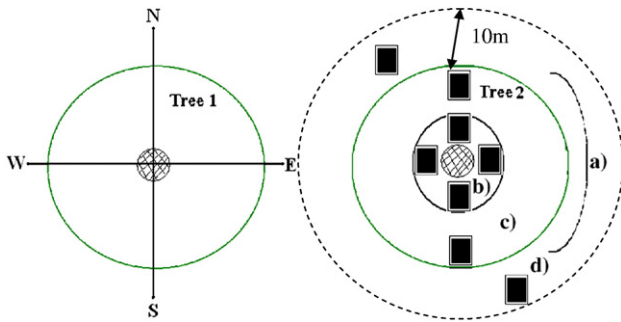
Fig. 2. A birds' eye view of the canopy of two trees with the trunk inserted (shaded centre circle); showing bearing (Tree 1), as well as, the position of the eight quadrats (Tree 2) underneath and around the canopy within which the seed banks were sampled. The four microsites are also shown, a) sub-canopy (area between trunk and canopy edge), b) inner canopy (trunk to 1/2 radius), c) outer canopy (1/2 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 × 0.5 m quadrats sampled.

2.4. 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 the 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 the 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: <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, and >70 cm. 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, the year selected being the year of major fruit production per site. 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).

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 the 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 (0.25 m^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.

3. Results

3.1. 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 density and female 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 were the highest (Tables 1 and 2).

3.2. 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). When including trees with endocarps below the canopy as females, the sex ratio at JPNR was at unity, while the values for 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 more than double that at 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).

3.3. 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 6398 fruit in 2010 but was excluded from the 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. 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).

Marula fruit are large, both in length (30–35 mm) and mass (11–19 g). The majority of marula endocarps (>50%) contained two seeds in both 2009 and 2010 (Table 4); but seeds/endocarp varied between sites. In 2009, a 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.

The initial percentage seed viability (60.3%) was higher than the percentage of seeds that actually germinated (32.4%) (Fig. 3a). Up to 25.5% (WRF) of opercula investigated contained aborted seeds. Most viable seeds germinated within two months of planting (Fig. 3b), leaving <2% of the 15 month

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=13.41 x−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=10.89 x−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=52.62 x−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=2.59 x−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=38.43 x−1014.3	70.45	1,281	<0.0001	0.20

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 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 ($F_{4,225}$=9.05, p<0.0001)			2010 ($F_{3,145}$=11.09, p<0.0001)		
		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 ± 17.8^{b} (178)	150.0 ± 40.2	9 (20)	11.1 ± 5.9^{b} (160)	24.0 ± 12.8
<30 cm		13	$39.2\pm15.9^{a}_{a}$	88.5 ± 36.0	3 (23)	$18.7\pm17.7^{b}_{a}$	40.3 ± 38.1
30–40 cm		10	$36.9\pm47.2^{a}_{a}$	83.2 ± 33.7	0 (0)	–	–
>40 cm		21	$97.5\pm34.6^{b}_{a}$	220.0 ± 77.9	6 (29)	$7.3\pm3.7^{b}_{a}$	15.8 ± 7.9
Hlangwine enclosure (HE)	530; 957; 668	39	204.2 ± 46.9^{a} (137)	497.5 ± 114.4	37 (95)	36.3 ± 13.2^{b} (221)	88.4 ± 32.1
<30 cm		18	$59.0\pm12.2^{a}_{b}$	143.6 ± 29.8	17 (94)	$28.9\pm11.8^{b}_{a}$	70.4 ± 28.9
30–40 cm		10	$153.0\pm41.4^{a}_{b}$	372.8 ± 100.9	9 (90)	$14.2\pm5.3^{b}_{a}$	34.7 ± 12.9
>40 cm		11	$508.0\pm117.7^{a}_{a}$	1237.7 ± 286.7	11 (100)	$65.7\pm40.1^{b}_{a}$	160.1 ± 97.6
Outside Hlangwine enclosure (OHE)	530; 957; 668	37	188.5 ± 35.8^{a} (115)	457.8 ± 86.9	33 (89)	96.3 ± 19.9^{b} (118)	233.9 ± 48.2
<30 cm		12	$100.5\pm35.6^{a}_{b}$	244.1 ± 88.8	10 (83)	$29.7\pm9.1^{b}_{a}$	72.1 ± 22.2
30–40 cm		15	$118.4\pm29.7^{a}_{b}$	287.5 ± 72.1	13 (87)	$63.7\pm11.4^{b}_{a}$	154.7 ± 27.7
>40 cm		10	$399.3\pm90.2^{a}_{a}$	969.7 ± 219.0	10 (100)	$205.3\pm48.4^{b}_{a}$	498.6 ± 117.6
Wits Rural Facility (WRF)	570; 987; 750	16	84.9 ± 47.8^{b} (225)	183.1 ± 103.0	70 (100)	1502.1 ± 264.1^{a} (147)	3238.8 ± 569.5
<30 cm		3	$14.3\pm6.3^{a}_{a}$	30.9 ± 13.7	22 (100)	$311.3\pm82.6^{a}_{c}$	671.3 ± 178.2
30–40 cm		1	4.0_{a}	8.6	8 (100)	$663.6\pm223.4^{a}_{b}$	1430.9 ± 481.6
>40 cm		12	$109.3\pm62.7^{b}_{a}$	235.8 ± 135.1	40 (100)	$2324.7\pm413.8^{a}_{a}$	5102.6 ± 892.2
Jejane Private Nature Reserve (JPNR)*	437; 116; 520	94	53.7 ± 5.3^{b} (97)	114.2 ± 11.4	–	–	–
<30 cm		23	$36.8\pm6.9^{a}_{a}$	178.7 ± 42.9	–	–	–
30–40 cm		55	$51.9\pm6.0^{b}_{a}$	110.4 ± 12.8	–	–	–
>40 cm		16	$84.0\pm20.2^{b}_{a}$	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

old seeds viable but dormant (range 0 (WRF and NE) to 4.5% (OHE)) (Fig. 3c). Only 16.2% of the NE seeds germinated, compared to 44.4% at JPNR. The low number of NE germinants could be due to the 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 (Fig. 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) (Fig. 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.

3.4. Seed bank dynamics

Endocarp density (within soil and litter) under the canopy differed across sites (Fig. 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 (Fig. 5).

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±SE seeds/endocarp
2009						
N'washitsumbe enclosure (NE)	15; 175	1	72	27	0	2.3±0.04
Hlangwine enclosure (HE)	15; 161	3	51	46	0	2.4±0.05
Outside Hlangwine enclosure (OHE)	16; 196	3	51	45	1	2.5±0.04
Wits Rural Facility (WRF)	5; 65	4	76	20	0	2.1±0.06
Jejane Private Nature Reserve (JPNR)	30; 332	7	74	19	0	2.1±0.03
2010						
N'watshitshumbe enclosure (NE)	1; 20	0	75	25	0	2.3±0.10
Hlangwine enclosure (HE)	2; 4	0	50	50	0	2.5±0.30
Outside Hlangwine enclosure (OHE)	14; 87	4	68	28	0	2.2±0.10
Wits Rural Facility (WRF)	26; 144	3	70	26	1	2.3±0.04
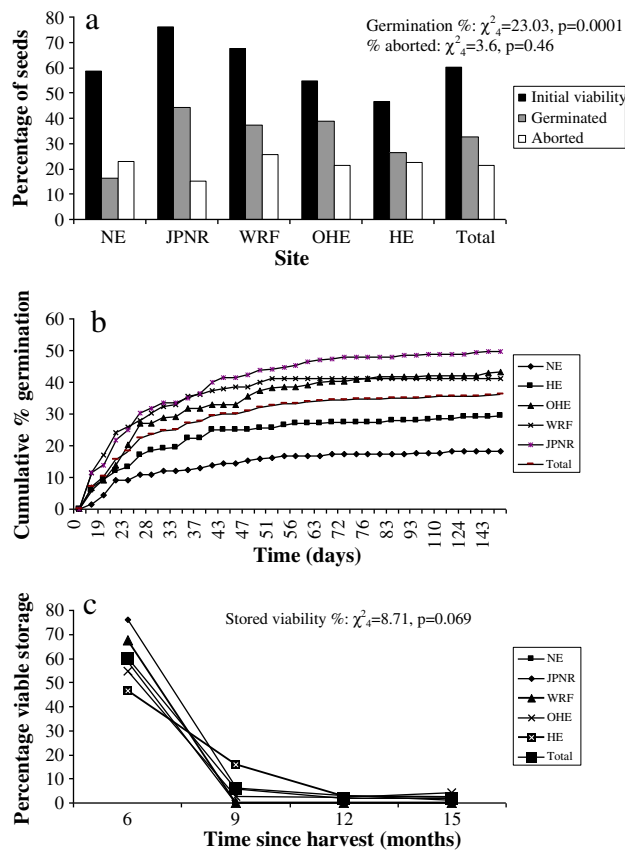
Fig. 3. a) Seed abortion, initial percentage viability and germinability from five sites and in total (N=1058) after 15 months in the soil, b) cumulative percentage germination over time and c) percentage of viable seeds stored over time. Seeds were collected in January 2009 in the low altitude savannas of South Africa and germinated in July 2009 after 6 months storage in the laboratory.

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) (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
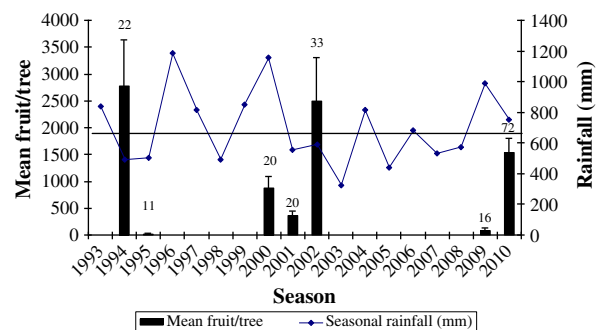

Fig. 4. Annual variation in fruit/tree in relation to rainfall at Wits Rural Facility (WRF) in the low altitude savannas of South Africa. The horizontal line indicates the long term mean seasonal (July to June) rainfall. Numbers above the bars represent the number of trees assessed.
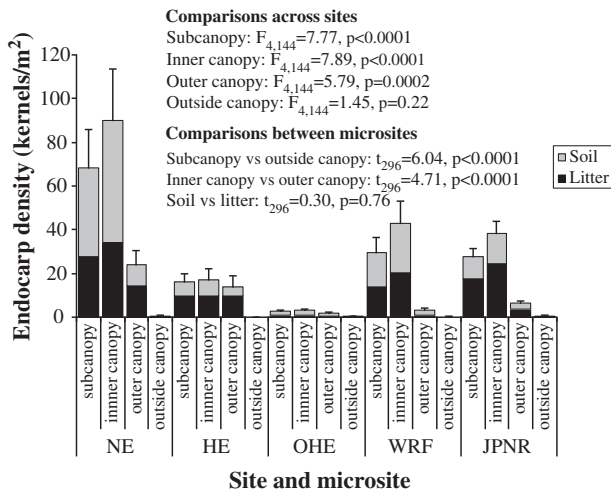
Fig. 5. A comparison between endocarp density (endocarps/m^2; mean±SE) within the seed bank (litter and soil) between four microsites for five sites in the low altitude savannas of South Africa.

(p<0.05). Both enclosures (NE and HE) had the highest viable seed bank density whilst OHE had the lowest (Table 5). 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 small persistent seed bank (Table 5). Only trees in JPNR had viable "old" seeds in the outside canopy microsite, indicating some short distance primary dispersal. Only 9% of the assessed trees had a persistent seed bank, with trees in JPNR and NE having the highest (17%).

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).

3.5. Throughput of seeds into seedlings

The number of seedlings associated with each fruit-producing tree varied between sites, with NE consistently having the least number of seedlings (Table 7).

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/tree. 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).

3.6. 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.

4. Discussion

4.1. Sex ratio

Our study found a male-biased sex ratio for marula at all sites, except WRF and at JPNR if trees with endocarps below the canopy were classified as females. Todd (2002) observed a 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

Table 5

Number 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 superscript 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	280 (0–2541)	53 (0–1980) N=34	11.2	6 (0–208) N=14
Comparisons between sites		$F_{4,145}=2.61; p=0.038$		$F_{4,145}=1.68; p=0.16$

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)
Comparison between sites	$H_{4,150}=29.96$, p<0.0001		$H_{4,65}=18.02$, p=0.0012

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 (De Jong and Van der Meijden, 2004; Opler and Bawa, 1978). 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, as 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.

4.2. 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). Marula reaches reproductive maturity at relatively larger stem diameters than other common South African savanna tree 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

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 superscript letters 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
Comparisons between sites			$F_{3,187}=3.52$, p=0.016		$F_{3,187}=9.86$, p<0.0001		$F_{3,187}=3.10$, p=0.028	$F_{3,187}=5.01$, p=0.0023	$F_{3,187}=5.24$, p=0.017

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

perennial plants must be delayed until the plant is sufficiently fire-resistant (Crawley, 2003), and resistant to other natural disturbances (Silvertown, 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 the size of reproduction and fire resistance.

4.3. Fruit and seed production

In our study, the maximum fruit crop observed was 46.8 kg/tree (2324 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 (1786 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 t/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 the 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 (Koenig and Knopps, 1998; Kozlowski, 1992). 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, particularly those from nutrient-poor environments, 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 the 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.

4.4. 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, 20 m 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 the 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 relatively long distance

secondary dispersal, marula seeds still tend to show some level of dormancy when dispersed from the canopy. Dormancy is usually associated with plant species that have little adaptation to long distance dispersal (Crawley, 2003). Hence, the quiescent period, often observed in 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 vegetation density and hence, protective 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 and 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 the 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.

4.5. Seed bank dynamics

Marula has a viable transient seed bank density of 136 seeds/ha, but a persistent seed bank density of only 20 seeds/ha, both associated with parent trees. 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* (859,004 and 608,856 seeds/ha) and *Dichrostachys cinerea* (10,131 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 (L. Kruger pers. comm.), affording these seeds protection from fire. Burial in the 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 the 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).

4.6. 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 a large proportion of seeds away from 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.

5. 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 the size of resistance to fire, but is further mediated by herbivory and rainfall. Some level of disturbance may be required to ensure effective marula 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 trade-offs with vegetative growth. 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 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. Overall, the results of this study provide a considerable advance in our knowledge of the reproductive phase of the marula lifecycle, and hence more realistic parameterisation for population models.

Acknowledgements

References

Anderson, P.K., 1986. Foraging range in mice and voles: the role of risk. Canadian Journal of Zoology 64, 2645–2653.

Auld, T.D., Denham, A.J., 2006. How much seed remains in the soil after a fire? Plant Ecology 187, 15–24.

Baskin, C.C., Baskin, J.M., 2001. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, London.

Bawa, K.S., Opler, P.A., 1977. Spatial relationships between staminate and pistillate plants of dioecious tropical forest trees. Evolution 31, 64–68.

Biere, A., 1995. Genotypic and plastic variation in plant size-effects on fecundity and allocation patterns in *Lychnis flos cuculi* along a gradient of natural soil fertility. Journal of Ecology 83, 629–642.

Botha, J., Witkowski, E.T.F., Shackleton, C., 2002. A comparison of anthropogenic and elephant disturbance on *Acacia xanthophloea* (fever tree) populations in the Lowveld, South Africa. Koedoe 45, 9–18.

Chidumayo, E.N., 1997. Fruit production and seed predation in two miombo woodland trees in Zambia. Biotropica 29, 452–458.

Clark, J.S., 1991. Disturbance and tree life history on the shifting mosaic landscape. Ecology 72, 1102–1118.

Clark, C.J., Poulsen, J.R., Levey, D.J., Osenberg, C.W., 2007. Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. The American Naturalist 170, 128–142.

Crawley, M.J., 2000. Chapter 7: seed predators and plant population dynamics, In: Fenner, M. (Ed.), The Ecology of Regeneration in Plant Communities, second ed. CABI publishing, London, pp. 167–182.

Crawley, M.J., 2003. Plant Ecology, second ed. Blackwell Science, USA.

De Jong, T.J., Van der Meijden, E., 2004. Sex ratio of some long-lived dioecious plants in a sand dune area. Plant Biology 6, 616–620.

Emanuel, P.L., Shackleton, C.M., Baxter, J.S., 2005. Modelling the sustainable harvest of *Sclerocarya birrea* subsp. *caffra* fruit in the South African lowveld. Forest Ecology and Management 214, 91–103.

Estes, R.D., 1991. The Behaviour Guide to African Mammals: Including Hoofed Mammals, Carnivores, Primates. University of California Press, USA.

Gadd, M.E., 2002. The impact of elephants on the marula tree *Sclerocarya birrea*. African Journal of Ecology 40, 328–336.

Gertenbach, W.P.D., 1983. Landscapes of the Kruger National Park. Koedoe 26, 9–121.

Goodall, V.C., 2006. Statistical analyses of artificial waterpoints: their effect on the herbaceous and woody structure composition within the Kruger National Park. MSc Thesis, Rhodes University, Grahamstown.

Grant, C.C., Davidson, T., Funston, P.J., Pienaar, D.J., 2002. Challenges faced in the conservation of rare antelope: a case study on the northern basalt plains of the Kruger National Park. Koedoe 45, 45–62.

Grossman, D., Grunow, J.O., Theron, G.K., 1980. Biomass cycles, accumulation rates and nutritional characteristics of grass layer plants in canopied and uncanopied subhabitats of *Burkea* savanna. Proceedings of the Grassland Society of Southern Africa 15, 157–161.

Haig, A.W., 1999. The impact of impala and elephant on the demography and dynamics of *Sclerocarya birrea* subsp. *caffra* (marula) in the Eastern Lowveld of South Africa. BSc (Hons.) in Agriculture. University of Natal, Pietermaritzburg.

Hall, J.B., O'Brien, E.M., Sinclair, F.L., 2002. *Sclerocarya birrea*: a monograph. School of Agricultural and Forest Sciences, Publication Number 19. University of Wales, Bangor, pp. 1–157.

He, T., Krauss, S.L., Lamont, B.B., Miller, B.P., Enright, N.J., 2004. Long-distance seed dispersal in a metapopulation of *Banksia hookeriana* inferred from a population allocation analysis of amplified fragment length polymorphism data. Molecular Ecology 13, 1099–1109.

Helm, C.V., Witkowski, E.T.F., Kruger, L., Hofmeyr, M., Owen-Smith, N., 2009. Mortality and utilisation of *Sclerocarya birrea* subsp. *caffra* between

2001 and 2008 in the Kruger National Park, South Africa. South African Journal of Botany 75, 475–484.

Helm, C.V., Wilson, G., Midgley, J., Kruger, L., Witkowski, E.T.F., (in press) Investigating the vulnerability of an African savanna tree (*Sclerocarya birrea* ssp. *caffra*) to fire and herbivory. Austral Ecology, doi:10.1111/j.1442-9993.2010.02232.x.

Hemborg, A.M., Bond, W.J., 2006. Do browsing elephants damage female trees more? African Journal of Ecology 45, 41–48.

Herrera, C.M., Jordano, P., Guitan, J., Traveset, A., 1998. Annual variability in seed production by woody plants and the masting concept: reassessment of principles and relationship to pollination and seed dispersal. The American Naturalist 152, 576–594.

Holtzhausen, L.C., Swart, E., Van Rensberg, R., 1990. Propagation of the marula (*Sclerocarya birrea* subsp. *caffra*). Acta Horticulturae 275, 323–329.

Jacobs, O.S., Biggs, R., 2002. The status and population structure of the marula in the Kruger National Park. South African Journal of Wildlife Research 32, 1–12.

Jordaan, A., Wessels, D.C.J., Kruger, H., 2002. Structure of the style and wet non-papillate stigma of *Colophospermum mopane*, Caesalpinioideae: Detarieae. Botanical Journal of the Linnean Society 139, 295–304.

Kauffman, M.J., Maron, J.L., 2006. Consumers limit the abundance and dynamics of a perennial shrub with a seed bank. The American Naturalist 168, 454–470.

Kearns, C.A., Inouye, D.W., 1997. Pollinators, flowering plants and conservation biology. Bioscience 47, 297–307.

Koenig, W.D., Knopps, J.M.H., 1998. Scale of mast-seeding and tree-ring growth. Nature 396, 225–226.

Kozlowski, J., 1992. Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. Trends in Ecology & Evolution 7, 15–19.

Lamont, B.B., Wiens, D., 2003. Are seed set and speciation rates always low among species that resprout after fire, and why? Evolutionary Ecology 17, 277–292.

Leakey, R., Pate, K., Lombard, C., 2005. Domestication potential of marula (*Sclerocarya birrea* subsp. *caffra*) in South Africa and Namibia: 2. Phenotypic variation in nut and kernel traits. Agroforestry Systems 64, 37–49.

Levick, S., Rogers, K., 2008. Patch and species specific responses of savanna woody species to browser exclusion. Biological Conservation 181, 489–498.

Lewis, D.M., 1987. Fruiting patterns, seed germination, and distribution of *Sclerocarya caffra* in an elephant-inhabited woodland. Biotropica 19, 50–56.

Luoga, E.J., Witkowski, E.T.F., Balkwill, K., 2002. Harvested and standing wood stocks in protected and communal miombo woodlands of eastern Tanzania. Forest Ecology and Management 164, 15–30.

Midgley, J.J., Lawes, M.J., Chamaille-Jammes, S., 2010. Turner review no. 19: savanna woody plant dynamics: the role of fire and herbivory, separately and synergistically. Australian Journal of Botany 58, 1–11.

Miller, M.F., 1994. The fate of mature African *Acacia* pods and seeds during their passage from the tree to the soil. Journal of Tropical Ecology 10, 183–196.

Moore, R.P., 1985. Handbook on Tetrazolium Testing. The International Seed Testing Association, Zurich.

Nason, J.D., Hamrick, J.L., 1997. Reproductive and genetic consequences of forest fragmentation: two case studies of neotropical canopy trees. The Journal of Heredity 88, 264–276.

Neke, K.S., 2005. Regeneration ecology of savanna woodlands in relation to human impacts. PhD Thesis, University of the Witwatersrand, Johannesburg.

Neke, K.S., Owen-Smith, R.N., Witkowski, E.T.F., 2006. Comparative resprouting response of savanna woody plant species following harvesting: the value of persistence. Forest Ecology and Management 232, 114–123.

Nghitoolwa, E., Hall, J.B., Sinclair, F.L., 2003. Population status and gender imbalance of the marula tree, *Sclerocarya birrea* subsp. *caffra* in northern Namibia. Agroforestry Systems 59, 289–294.

Opler, P.A., Bawa, K.S., 1978. Sex ratios in tropical forest trees. Evolution 32, 812–821.

Palmer, E., Pitman, N., 1972. Trees of Southern Africa. A.A. Balkema, Cape Town.

Prins, H.H.T., Van der Jeugd, H.P., 1993. Herbivore population crashes and woodland structure in East Africa. Journal of Ecology 81, 305–314.

Shackleton, C.M., 1999. Rainfall and topo-edaphic influences on woody community phenology in a South African savanna. Global Ecology and Biogeography 8, 125–136.

Shackleton, C., 2002a. Growth and fruit production of *Sclerocarya birrea* in the South African lowveld. Agroforestry Systems 55, 175–180.

Shackleton, C.M., 2002b. Growth patterns of *Pterocarpus angolensis* in savannas of the South African Lowveld. Forest Ecology and Management 166, 85–97.

Shackleton, C.M., 2007. The effects of fire on post-fire seed germination of selected savanna woody species. African Journal of Ecology 45, 545–549.

Shackleton, S.E., Shackleton, C.M., Cunningham, T., Lombard, C., Sullivan, A.A., Netshiluvhi, T.R., 2002. Knowledge on *Sclerocarya birrea* subsp. *caffra* with emphasis on its importance as a non-timber forest product in South and southern Africa: a summary. Part 1: taxonomy, ecology and role in rural livelihoods. Southern African Forestry Journal 194, 27–41.

Shackleton, C.M., Botha, J., Emanuel, P.L., 2003. Productivity and abundance of *Sclerocarya birrea* subsp. *caffra* in and around rural settlements and protected areas of the Bushbuckridge savanna, South Africa. Forests Trees and Livelihoods 13, 217–232.

Shackleton, C.M., Guthrie, G., Main, R., 2005. Estimating the potential role of commercial over-harvesting in resource availability: a case study of five useful tree species in South Africa. Land Degradation and Development 16, 273–286.

Shone, A.K., 1979. Notes on the Marula. Bulletin 58. Department of Forestry, Pretoria.

Silvertown, J., 1991. Modularity, reproductive thresholds and plant population dynamics. Functional Ecology 5, 577–580.

Symes, C.T., Perrin, M.R., 2003. Feeding biology of the grey headed parrot, *Poicephalus fuscicollis suahelicus* (Reichenow), in Northern Province, South Africa. Emu 103, 49–58.

Takahashi, K., Sato, K., Washitani, I., 2007. Acorn dispersal and predation patterns of four tree species by wood mice in abandoned cut-over land. Forest Ecology and Management 250, 187–195.

Thompson, K., Band, S.R., Hodgson, J.G., 1993. Seed size and shape predict persistence in soil. Functional Ecology 7, 236–241.

Todd, C.B., 2002. A survey of fruit production in *Sclerocarya birrea* (marula). Unpublished technical Report 3. Commercial Products from the Wild Programme, Pretoria.

Van Wyk, B., Van Wyk, P., 2007. Field Guide to Trees of Southern Africa. Struik, Cape Town.

Vander Wall, S.B., Kuh, K.M., Beck, M.J., 2005. Seed removal, seed predation, and secondary dispersal. Ecology 86, 801–806.

Von Teichman, I., Small, J.G.C., Robbertse, P.J., 1986. A preliminary study on the germination of *Sclerocarya birrea* subsp. *caffra*. South African Journal of Botany 52, 145–148.

Walker, B.H., Stone, L., Henderson, L., Vernede, M., 1986. Size structure analysis of dominant trees in South African savanna. South African Journal of Botany 52, 397–402.

Wassie, A., Bekele, T., Sterck, F., Teketay, D., Bongers, F., 2009. Postdispersal seed predation and seed viability in forest soil: implications for the regeneration of tree species in Ethiopian church forests. African Journal of Ecology 48, 461–471.

Wheelwright, N.T., 1986. Chapter 2: a seven-year study of individual variation in fruit production in tropical bird-dispersed tree species in the family Lauraceae. In: Estrada, A., Fleming, T.H. (Eds.), Frugivores and Seed Dispersal. Dr. W. Junk Publishers, Dordrecht.

Wilson, B.G., Witkowski, E.T.F., 2003. Seed banks, bark thickness and change in age and size structure (1978–1999) of the African savanna tree, *Burkea africana*. Plant Ecology 167, 151–162.

Witkowski, E.T.F., Garner, R.D., 2000. Spatial distribution of soil seed banks of three African savanna woody species at two contrasting sites. Plant Ecology 149, 91–106.

Witkowski, E.T.F., Lamont, B.B., 1996. Disproportionate allocation of mineral nutrients and carbon between vegetative and reproductive structures in *Banksia hookeriana*. Oecologia 105, 38–42.

Yasumura, Y., Hikosaka, K., Hirose, T., 2006. Resource allocation to vegetative and reproductive growth in relation to mast seeding in *Fagus crenata*. Forest Ecology and Management 229, 228–233.

Edited by RI Yeaton