Elephant fruit: the dispersal attributes of *Balanites maughamii*

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Abstract

Endozoochorous plant species that have obligate dispersal mutualisms with megafauna are at risk of extinction because of the unique conservation threats associated with large animals. This study examines *Balanites maughamii* which appears to have similar dispersal attributes to *Sclerocarya birrea* and *Balanites wilsoniana*, both of which are considered to be obligate elephant fruits. Here, I emphasize the traits that make *B. maughamii* suitable for elephant dispersal and examine the cues required for seed germination. Large, fleshy fruits with a single, exceptionally hard seed which requires a crushing force of > 2.0 kN to promote germination are characteristic of this species.

Using scent analysis of ripe fruits, the range of volatile that are responsible for the pungent odour were identified as being those which should function in mammal-attraction. Finally I examine the potential for long-distance dispersal by elephants using a gut passage time experiment, which revealed that elephants retain seed for approximately 48 hours, equating to a 4-8 km dispersal distance. Using fruit and seed bait stations to determine which other animals interact with *B. maughamii*, I found that small mammals may play a significant role in seed predation and that there is little evidence to suggest extensive secondary dispersal by rodents. These finding allude to the vulnerability of the long-term maintenance of *B. maughamii* in the absence of elephants, who appear to be their only legitimate disperser.

Keywords: *Balanites maughamii*, dispersal, endozoochory, gut passage time, scent

Introduction

The breakdown of mutualisms, as a consequence of the global decrease in biodiversity has prompted close examination of the intricate ecologies that exist in both pristine and vulnerable ecosystems (e.g. Bond 1994). Knowledge of keystone species, i.e. species upon which many others depend and interact with (*sensu* Bond 1993), is vital to our understanding of the potential vulnerability of these ecologies. Big trees are particularly influential in their local systems, where they provide essential ecosystem services and structural heterogeneity (Belsky *et al.* 1989; Dean *et al.* 1999). This considered, the reduction in tree regeneration, as well as the congruent loss of the majority of the world's large animals (Peres 2001; Peres & Palacios 2007) has become cause for concern.

Mutualisms which exist between plants and animals are integral to the functioning ecology of a system. Among these is the role that animals play as dispersers of plant propagules. Animal-mediated dispersal is a common phenomenon across the globe (Herrera 2002; Donatti *et al.* 2007), which lends support to its importance in shaping a variety of ecosystems. Its influences on plant population dynamics (Jordano & Herrera 1995; Ness *et al.* 2006), community dynamics and structure (Levin *et al.* 2003; Levine & Murrel 2003; Howe & Miriti 2004) and recruitment limitation (Clark *et al.* 2007) are thus important in a conservation context.

Dispersal is a critical event in the life-cycle of a plant, and has major influences on the germination success and ultimate recruitment of new individuals into the system. Plant species may thus be threatened by the extinction of an interaction, when the activity of their mutualists is compromised (Bond 1994). The global trend towards habitat fragmentation does not bode well for the maintenance of disperser mechanisms as fragmented landscapes are known to have decreased tree recruitment and an increase in disperser limitation (Cordiero *et al.* 2009).

Plant dispersal involves the initial movement of a seed away from the parent plant, followed by deposition of the seed in to the final environment where it can germinate. However, this process can often involve multiple steps and a suite of dispersers (Vander Wall & Longman

2004; Vander Wall *et al.* 2005), e.g. scatter hoarding agoutis who cache Guarea glabra seeds found in the bird faeces (Wenny 1999) Unlike in pollination biology, dispersal mutualisms do not exhibit a high degree of specificity, and are characterised rather, by generalist interactions between fruit and frugivore (Herrera 1989; Corlett 2007). As a result of this, tightly co-evolved mutualisms are extremely rare in dispersal biology, but one does find convergence of fruit traits into distinct guilds according to the types of the disperser that they interact with (Herrera 2002).

Fruit guilds are characterised according to several phenotypic traits, which include the presence of a fleshy mesocarp, colour, scent and also size. Fruit size is one of the only traits that are thought to be an adaptation to disperser selection, as plants do not often specialise their fruit according to a disperser (Jordano 1995). Despite this, fruit guilds do appear to reflect specific 'dispersal syndromes', which are a collection of traits, either of the fruit or frugivore, that frequently occur together and which are ecologically suited to the dispersal mutualism that they characterise (Howe 1986; Herrera 1989).

One such dispersal syndrome is associated with endozoochory by frugivorous megafauna. Megaherbivores (animas > 1000 kg *sensu* Owen-Smith 1988; Hanson & Galetti 2009) often represent effective seed dispersers (*sensu* Schupp 1993) as a consequence their extensive ranging behaviour, which scales with body size (Peters 1983). Long distance seed dispersal is highly advantageous to a plant as it ameliorates the density-dependent impacts that are greatest nearer to the parent plant (Janzen-Connell effect: Janzen 1970; Connell 1971; Howe & Smallwood 1982). Large body size also means that megaherbivores are able to disperse substantial quantities of seeds of a wide variety of species (Fragoso & Huffman 2000; Guimaràes *et al.* 2008; Blake *et al.* 2009).

The megafaunal dispersal syndrome is well-documented (Guimarães *et al.* 2008; Campos-Arceiz and Blake 2011), with a con*sensu*s fruits which require dispersal by a megaherbivore should be dull-coloured, odorous, fleshy and large (4-10 cm), and produce a single seed (>2cm) with a hard protective coat. All of these traits act to facilitate attraction of large mammal disperser or enhance its chances of survival during ingestion (Guimarães *et al.*

2008). However, while these traits are appropriate for megafaunal dispersal; they may also act to severely limit the types of animals that the species can depend on (Herrera 2002).

Dispersal effectiveness has implications for species persistence (Wills *et al.* 1997; Harms *et al.* 2000; Nathan & Muller-Landau 2000), particularly when the dispersal mechanism has become specialized. In extreme cases, the fruits of species such as *Sclerocarya* and *Balanites* species have become specialized to the point of having an obligate or near-obligate disperser - the elephant (Cochrane 2003; Campos-Arceiz & Blake 2011). This phenomenon puts these plant 'elephant fruits' at a high risk of dispersal failure, i.e. when recruitment is drastically reduced, if there disperser is extirpated from the system (e.g. Babweteera *et al.* 2007).

Elephants – the iconic megaherbivores - act as significant and highly effective dispersers of many tree species in all of the habitats that they occur in (Blake *et al.* 2009; Campos-Arceiz and Blake 2011). However, the attributes that make them highly effective at seed dispersal (large body size and ranging ability), also put them at risk of habitat fragmentation and human-wildlife conflict (Kerley *et al.* 2008).

There has been great difficulty in determining which plants are reliant on dispersal by elephants, or functional equivalents, as only in Africa and parts of Asia are elephants still extant. Ideas on 'gomphothere fruits' (Janzen & Martin 1982) have based assumptions of dispersal anachronisms on mutualism with extinct animals, and as such these ideas are purely speculative (Howe 1985; Guimarães *et al.* 2008). These 'megafaunal anachronisms' have inspired much debate regarding the necessity of dispersers for tree persistence (Howe 1993), and what the trait for the guild of "megafaunal fruit' should be (Guimarães *et al.* 2008). However, there is a need to for these hypotheses to be corroborated using plant species that are known to rely on extant elephant mutualisms for dispersal. This will greatly aid our understanding of the ecological mechanisms that contribute to plant population persistence in the absence of an effective disperser.

Africa is the only continent that is still home a considerable amount of its megafauna and provides the opportunity to study intact disperser mutualisms for large fruited species. All plant species that have been found to rely solely of elephants for dispersal are found in Africa (Campos-Arceiz & Blake 2011). However, the majority of the work done has involved tropical fruit species (Campos-Arceiz & Blake 2011). This study looks at the traits of an African savanna tree species, *Balanites maughamii*, which is thought to have a diplochorous dispersal mechanism that relies on elephants for primary dispersal away from the parent tree and subsequent seed movement by scatter hoarding rodents into favourable microsites for germination. Similar studies have been done on *B. wilsoniana* in Uganda (Babweteera *et al.* 2007) and *Sclerocarya birrea* in Kruger National Park, South Africa (Helm *et al.* 2011, Midgley *et al.* 2012), which shares many of the same fruit traits and indeed often co-occurs with *B. maughamii*, potentially sharing the same dispersers.

In trying to understand the ecology of a species that may be obligately dispersed by elephants, I firstly attempt to characterise an African megafaunal fruit based by assessing traits found in *B. maughamii* fruit, which are considered to be part of the megafaunal dispersal syndrome. These traits include fruit morphology and scent, as determined by the identity and emission rate of volatile scent compounds, in addition to seed size and how well-protected the seed kernel is. Following this, I determine what the potential cues for germination of *B. maughamii* through the application of experimental treatments on seeds, in order to ascertain whether elephants play a critical role in germination. Finally, I look at which animal species are interacting with both the *B. maughamii* fruit and seeds and make inferences about the role of certain animal guilds as either seed predators or dispersers, while also examining the potential for long distance dispersal by elephants.

Methods

Study site

This study was undertaken in the Skukuza region of Kruger National Park, South Africa. The Kruger National park is South Africa’s oldest and largest National Park, spanning 19 633 km^2. The wet season of KNP occurs during the summer months (December to March); with 500-700 mm mean annual precipitation. Most of this rainfall occurs during sporadic convective storms.

KNP falls within a savanna biome, which is divided into a xeric north and mesic south and also longitudinally along a geological boundary. The western side, where Skukuza is located, is characterised by soils derived from granitic substrates with a basement complex. Skukuza exhibits a land form type of moderately undulating plains and distinctive catenal sequences (Venter *et al.* 2003) Uplands have sandy soil with associated broadleaved bushveld (dominated by *Combretum* and *Terminalia* spp.), while bottomlands have complex sodic clays with fine-leaved *Acacia* bushveld (mainly *A. nigrescens*).

The Skukuza district supports abundance variety of wildlife, which includes impala, leopard, lion, hyena, wild dog, kudu, white rhino, giraffe and elephant. This study was conducted both outside and inside the Skukuza staff village - an unfenced residential area with several dense tree stands and a golf course.

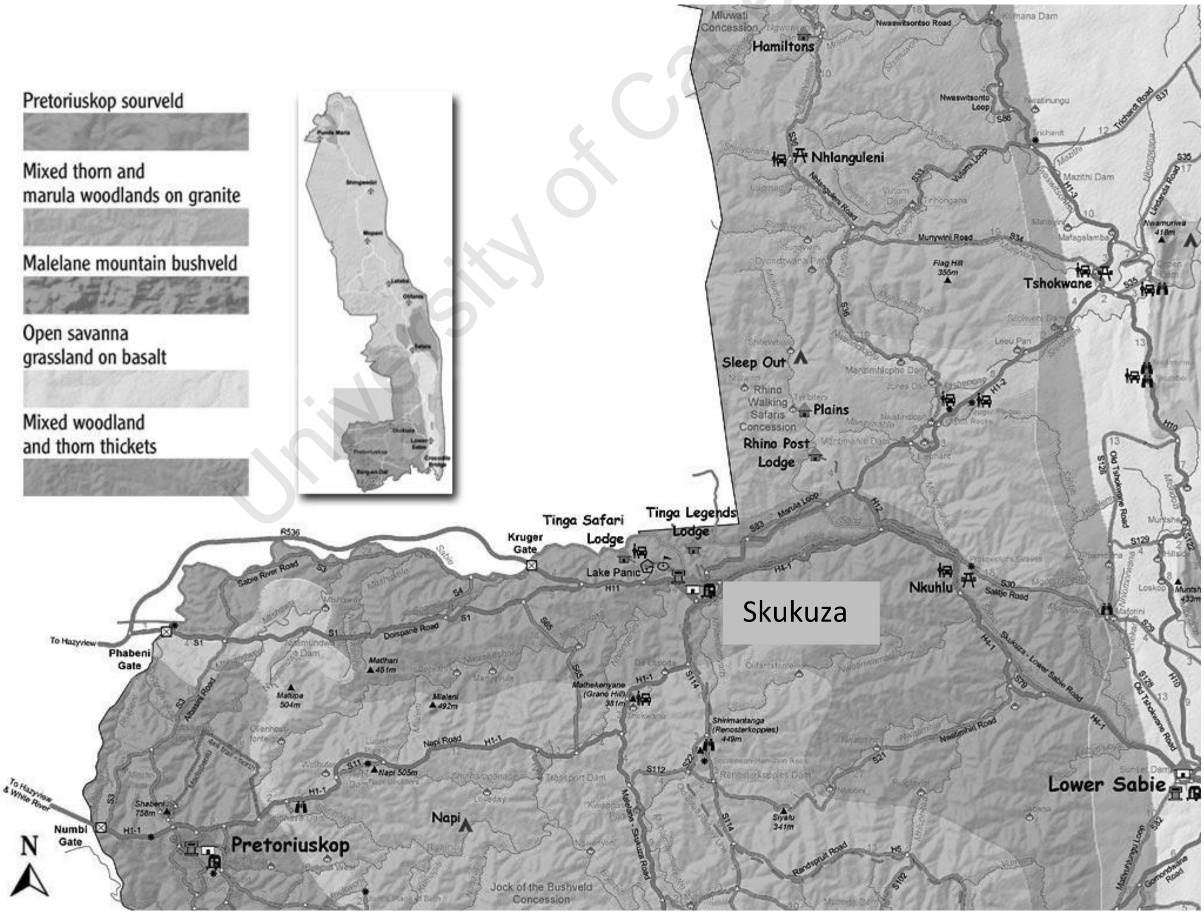

Figure 1: Location of the Skukuza region within Kruger National Park. Skukuza falls within the 'Mixed woodland and thorn thicket' vegetation type Source: http://www.krugerpark.co.za/images/explore-kruger-skukuza-and-surrounds-map.jpg

Study species

Balanites maughamii (Sprague) subsp. *maughamii* (Zygophyllaceae) is an upper canopy deciduous savanna tree distributed from KwaZulu-Natal to southern Tanzania and is found in Kruger National Park (Sands 2001, Babweteera *et al.* 2007). Usually found in small, clumped stands, trees occur more frequently on sandy soils in the vicinity of pans, springs or on river banks, but are not limited to such soils (Van Wyk & Van Wyk 1997). These trees can reach heights of up to 25 m, and have a spreading, rounded crown (Sands 2001).

The tree produces odorous, single seeded fruits with a cylindrical to oblong-ellipsoid shape (Figure 2a). Fruits are fleshy and are a brownish green colour when ripe. The seeds consist of a thin exocarp, a fibrous mesocarp, and a characteristically thick woody endocarp (Figure 2b). Fruiting begins around January every second or third year and fruit-fall occurs between May and July (Sands 2001). In addition its reseeding behaviour, *B. maughamii* is known to propagate vegetatively through root suckering (Sands 2001, Bijl 2012).

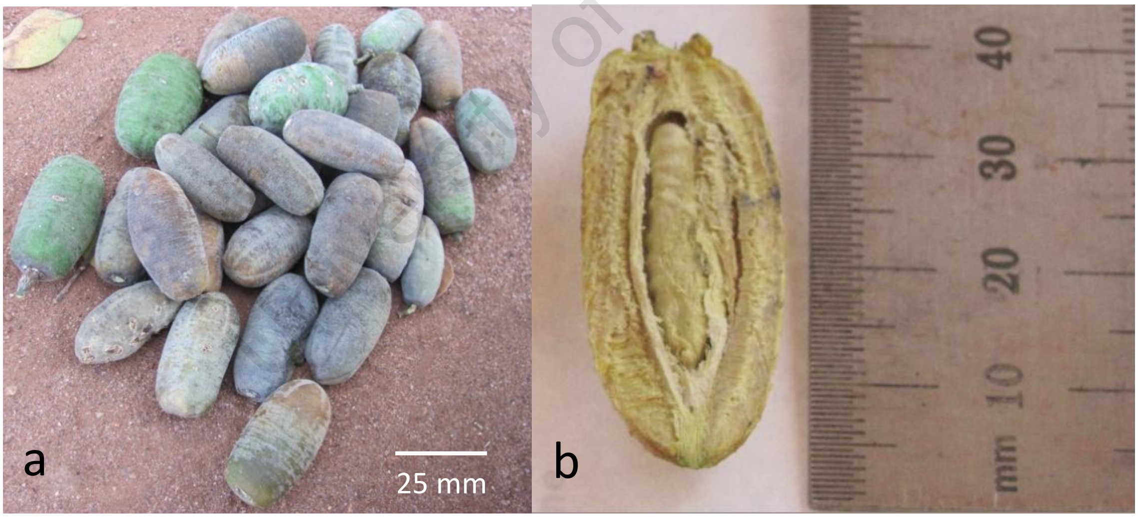

Figure 2. *Balanites maughamii* (a) fruit and (b) cross-section showing the seed.

The wood and fruit of *B. maughamii* are used by humans for medicinal and magical purposes (Netshiluvhi 1996). This heavy utilization makes *B. maughamii* a potentially vulnerable species despite its protection under the National Forests Act of 1988.

Experimental design and protocol

In trying to characterise the fruit syndrome of *B. maughamii*, I examined features of the ripe fruit which may influence selection or limitation of dispersers. Whole fruits of *B. maughamii* were collected from beneath the canopies of adult fruiting trees during June 2013. These fruits were collected stored in brown paper bags and used in bait stations or for fruit volatile scent analysis. Any seeds whose fruit coat had been removed by animals were also collected for use in bait experiments and for germination trials. Seeds found in this way are abundant beneath fruiting parent trees and are easily accessible. Cracked or damaged seeds were ignored, as this damage may influence germination and confound experimental treatment.

Scent Analysis

In order to elucidate which odours may be important in attracting dispersers, I identified the dominant compounds which cause the odour of *B. maughamii* fruits. Scent was extracted from ripe *B. maughamii* fruits, which had been stored in clean paper bags for 3 months.

Fruits, whose flesh had been scored with a clean blade in order to enhance odour emission, were enclosed in polyacetate bags (Kalle, Germany). The dynamic headspace within each bag was sampled for scent by drawing air through a small glass trap containing 1 mg Tenax® and 1 mg Carbotrap®, an activated charcoal (Shuttleworth & Johnson 2010). An air pump was used to produce a constant flow rate of 200ml/min and was run for 4 hours.

The emitted scent was divided by the number of fruits per sample to get the emission rate per fruit per hour. Three samples of five or six fruits were run concurrently in the same vicinity, and for the same time period as a control sample, which was an empty polyacetate bag. Any scents that were in the environment at the time of sampling or those resulting from handling of the traps would be present in the control and thus, eliminated from the analysis. Finally, all fruits, a total of 16, were sampled together, to increase the likelihood of sampling smaller traces of components.

Using a Gas Chromatography –Mass Spectrophotometer (GC-MS), I obtained gas chromatographs for the contents of each scent trap. A Bruker 450 gas chromatographer with a 30 m x 0.25 mm internal diameter (film thickness 0.25 µm) Bruker BR-SWAX column was coupled to a Bruker 300 quadrupole mass spectrometer in electron-impact ionization mode at 70 eV. The detector was set by Extended Dynamic Range (EDR) and for electroantennographic detection (EAD) by splitting the column inside the GC oven using a Gerstel Graphpack® 3D/3 effluent splitter with one end running through to the mass spectrophotometer and the other end exiting the apparatus for EAD. For GC-MS analysis in which EAD was not required, a rubber bung was used to block the EAD column, so as to avoid diluting the sample.

A ChromatoProbe thermal desorption device (both previously produced by Varian, Amirav & Dagan 1997; Dötterl, Wolfe & Jürgens 2005) was equipped to the Bruker 1079 injector in which the scent traps were placed. Helium carrier gas was run through the sample at a flow rate of 1.8 ml min^{-1}. The injector was held at 40 °C for 2 min with a 20:1 split. For the following 10 minutes, the temperature was increased to 200 °C at 200 °C min^{-1}, and then increased to 250 °C at 200 °C min^{-1} and held for a further 10 minutes. After the initial 2 minutes, the split was removed for thermal desorbtion and then a 100:1 split was introduced after 4.2 minutes to flush the injector. The GC oven was held at 40 °C for 3 minutes and then heated up to 240 °C at 10 °C min^{-1} and kept at this temperature for 12 minutes.

To ensure accuracy with quantification of emission rates, known amounts of methyl benzoate were injected into cartridges and thermally desorbed under identical conditions to the samples.

Mechanical Crushing

To determine the maximum force required to break the woody endocarp, seeds were subjected to mechanical crushing using a Zwick (Zwick, Germany) 1484 200-kN load cell machine at the Mechanical Engineering Department, University of Cape Town. Seeds, of a variety of sizes, were measured for their length and width using digital Vernier callipers. A total of 100 seeds were laterally compressed at a speed of 100mm/min. When an audible crack was heard, or there was a rapid decrease in the force required to squash the seed, the maximum force (in kilo Newtons) that the seed withstood was noted. Also noted were the position of the crack and whether the seed inside had been crushed.

The average of the force required to crack all of the seeds was used to calculate 90 % and 70 % of the maximum force. Fifty seeds were squashed at 100mm/mm with a force of 2.43 kN (90 %) and at 1.89 kN (70 %). These seeds were later used in germination experiments.

Germination cues

Seeds were subjected to several experimental treatments that mimic certain influences that a seed may experience, in order to elucidate which of these influences may act as important cues for germination. These treatments included mechanical crushing (seeds that had been cracked and those that had endured 90 % and 70 % of the average maximum force) as a proxy for animal bite force, removal of the woody endocarp, and providing a small hole (ca. 1-2 mm diameter) through which air and water could pass, which was achieved by sanding down tip of the seed to reveal the kernel. Both ‘naked’ and ‘air hole’ seeds were soaked in water for 24 hours, along with a control group. Approximately 20 seeds were used for each treatment, with the exception of those that underwent mechanical crushing, which had a sample size of 48 seeds per treatment.

In addition to the artificial treatments, seeds located in elephants dung were also used in germination trials. Elephant dung was searched for *B. maughamii* seeds during June 2013. Any whole or crushed seeds were collected and stored in paper bags and taken to the University of Cape Town. A control group consisted of seeds that were collected from beneath their parent tree.

All seeds were sown at a greenhouse at the University of Cape Town during August 2013, using river sand in plastic punnets containing 8 seeds each. Punnets were watered every second day and checked for signs of germination weekly. Splitting of the longitudinal flanges and maturation of the embryonic plumule were considered as a germination success.

Elephant gut passage time

In order to determine the potential extent of a long distance dispersal event by an elephant, whole fruits were fed to elephants and then the time taken for these to emerge in the dung was noted. This feeding experiment was conducted at Elephant Whispers at R40 Hazyview, Mpumalanga (S 25˚01,686' E 031˚07,430'), an elephant sanctuary, which is home to six tame elephants, rescued from local reserves, including Kruger National Park (Appendix I). There are no *B. maughamii* trees at the sanctuary.

Each elephant was fed 30 *B. maughamii* fruit that had been collected in Skukuza, KNP. The fruits were scattered in front the elephants, in order to simulate how an elephant might find them in the wild. These elephants were representative of both sexes, as well as a range of ages and sizes. To determine how long the seeds were in the gut before they were excreted, dung was collected in the morning from the stables and throughout the day over a period of 48 hours. It was impossible to match the exact times of the dung that came from the stables, so these were assigned a 12 hour interval. For each elephant, the time and date of each bolus was noted, in addition to the number and condition of seeds recovered.

Bait stations

Fruit and seed cafeteria sites were placed under *B. maughamii* trees at 11 locations around the Skukuza staff village (Appendix II). Six of these were located on or near the Skukuza golf course, while the others were placed in dense vegetation off the roadside in the village. This experiment ran from 17-23 April 2013 when only the *B. maughamii* trees located on the golf course were fruiting.

I placed ten fruit beneath the canopies of the three *B. maughamii* trees, fitted with motion-detection camera traps (Bushnell ® model number 119446 and 119476). Cameras were set to take videos of 30 second duration, with one second intervals between clips. Each day, I counted and replaced fruit that was eaten, moved or missing. We also attempted to recover any consumed fruits that were abandoned beyond the cafeteria. The distances of recovered fruit were recorded and any missing or eaten fruit were replaced. This was done in order to gain insight into the consumption and potential dispersal of *B. maughamii* fruit by other mammals.

To understand the predation and dispersal of *B. maughamii* seeds, I repeated the protocol used in the fruit camera trap and cafeteria experiments. At each of ten cafeteria sites, I placed ten tagged seeds to facilitate their recovery if dispersed and buried. These seeds were tagged with a 10 cm fluorescent string attached with odourless, inert epoxy. I also inserted small rare earth magnets in the endocarp of the seeds, in case the rodents or other animals removed the string during dispersal. Unfortunately, no efforts were made to actively search for seeds beyond each site.

During August 2013, seven additional seed bait station were set up and monitored for two weeks in the same areas as previously. Each of these seeds was tagged with reverse-wound cotton bobbins. The end of a bobbin was secured to the seed using epoxy glue, while the actual bobbin was buried where the seed was placed.

Data analysis

Compounds present in the scent emission of *B. maughamii* were identified using Varian Workstation software with the NIST2011 mass spectral library and verified, where possible, using retention times of authentic standards and published Kovats indices. Each chromatogram gives the retention times for each compound and, based on the area beneath the curve, the percentage contribution of each compound to the overall scent and emission rate could be calculated.

To determine the force required to crack the woody endocarp of *B. maughamii* seeds, the mean ± S.D. force that was calculated by averaging the maximum force achieved before visible deformation of the seed was observed, as indicated by cracks in the endocarp. The length and width of each seed were used as predictors in a linear model to test how well these could predict the force required to crack the seed (R core team 2013). No correlation was found, and so the mean was used to calculate 70 % and 90 % of the force required to break seeds as 2.43 kN and 1.89 kN.

The influences of different treatments on germination success of *B. maughamii* were tested using a Chi-squared test in STATISTICA® (Statsoft 2012). Expected germination frequencies were calculated by dividing the total germination success frequency equally among the different treatments, and then multiplying this proportion by the sample size of each treatment.

A frequency histogram representing the number of seeds that emerged for each time interval was constructed (SAS 2010). The time of first seed emergence was recorded for each elephant and used to calculate the potential dispersal distance based on the daily range of African elephants. Gut retention time was compared to elephant shoulder height in meters, which was used as a proxy for size.

I used camera trap data to identify mammal species that interacted with our cafeterias. Interaction was defined by an animal having physical contact with the fruits or seeds, either through sniffing, chewing, swallowing or accidental kicking. For fruit, each incident was categorized as a consumption (fruit coat removed and seed left *in situ*) or dispersal event. The frequency of these incidents was calculated for each species. Due to the limitation of camera trap footage, these data were combined with cafeteria results in order to assess the distances fruits were moved and the extent of consumption and removal. Any missing fruit was assumed to have been eaten. Seeds were considered predated if the endocarp was opened and the endosperm destroyed.

Results

B. maughamii trees produce large, dull-coloured fruits carrying a single large seed. The endocarp of the seed is thick, woody and extremely hard. Both the fruit and seed are cylindrical to oblong in shape. Fruit size and shape appears to vary slightly between seasons (*pers. obs.*). Seed size measurements taken from the most recent fruiting season yield a mean length of 37.3 ± 4.1 mm and width is 18.1 ± 2.4 mm (mean ± s.d.).

Seed length and/or width were very poor predictors of the maximum crushing force that the seed could endure before deforming. . The force required to crack the woody endocarp of a *B. maughamii* seed is 2.7 ± 0.7 kN, when applied as a compressive force at a speed of 100mm/min. The position of the crack was perpendicular to applied force, and not along the seed coat flanges in 72% (n=50) of instances. Damage to the soft seed endosperm did occur, although not frequently (n=5).

Scent analysis

The scent of ripe *B. maughamii* fruit is detectable by the human nose and has a pungent, sour character. Up to 48 compounds were identified, most of which are aliphatic compounds, including acids, alcohols and ketones (Table 1). Minute traces of cyclic and sulphur-containing compounds were also identified. Making up the greatest proportion was Hexanoic acid (40.44 ± 12.95 % (± S.E.)). This acid, also known as caproic acid, has a strong odour which resembles sweat and goats. Other dominant compounds include isovaleric acid (15.73 ± 3.57 %), and hexanal (14.04 ± 12.12 %), both of which have smells that are rancid and similar to sweat. A volatile scent emission rate of 593.59 ± 128.48 ng/fruit/hour was observed.

Table 1. The chemical composition of scent emitted from scarred fruits of *Balanites maughamii*. Scent compounds are listed according to compound class and Kovats retention indices (KRI). Data presented are average relative proportions per fruit over 3 samples of a total of sixteen fruits. Compound identification criteria and notes: a = comparison of MS with published data; b = comparison of MS and retention time with published data (e.g. http://webbook.nist.gov (Linstrom and Mallard, 2010) and references therein); c = comparison of MS and retention time with published data and authentic standard. Most common compounds are bolded.

Compound class	Compound name	KRI	CAS	*Balanites* fruit volatiles (% ± SE)		
Aliphatic acid	Butanoic acid^{c}	1667	107-92-6	0.42	±	0.10
	4-Methylpentanoic acid^{a}	1699	646-07-1	0.02	±	0.02
	Isovaleric acid^{b}	1704	503-74-2	**15.73**	±	**3.57**
	Valeric acid^{c}	1779	109-52-4	0.85	±	0.18
	Hexanoic acid^{c}	1903	142-62-1	**40.44**	±	**12.95**
	(*E*)-3-Hexenoic acid^{a}	1988	1577-18-0	**1.35**	±	**0.62**
	Heptanoic acid^{c}	2002	111-14-8	0.29	±	0.13
	Octanoic acid^{c}	2109	124-07-2	0.26	±	0.14
	2-Heptenoic acid^{a}	2134	10352-88-2	0.12	±	0.07

	(*Z*)-2-Octenoic acid[a]	2177	1577-96-4	0.01	±	0.00
	Nonanoic acid[b]	2217	112-05-0	0.14	±	0.07
	Dodecanoic acid[b]	2529	143-07-7	0.00	±	0.00
	Tetradecanoic acid[b]	2790	544-63-8	0.01	±	0.00
	Pentadecanoic acid[a]	2974	1002-84-2	0.01	±	0.00
	n-Hexadecanoic acid[a]	3193	1957/10/03	0.15	±	0.11
	(*Z*)-9-Octadecenoic acid[b]	3296	112-80-1	0.01	±	0.01
	Octadecanoic acid[a]	3939	57-11-4	0.02	±	0.01
Aliphatic alcohol	Oct-1-en-3-ol[c]	1487	3391-86-4	**3.91**	**±**	**0.62**
	Octan-1-ol[b]	1603	111-87-5	**1.99**	**±**	**0.85**
	(*E*)-Oct-2-en-1-ol[b]	1661	18409-17-1	0.20	±	0.16
Aliphatic aldehyde	Hexanal[b]	1145	66-25-1	**14.04**	**±**	**12.12**
	Octanal[b]	1339	124-13-0	**1.88**	**±**	**0.24**
	Nonanal[b]	1454	124-19-6	**8.39**	**±**	**3.26**
	2-Butyl-2-octenal[a]	1754	13019-16-4	0.00	±	0.00
	(*Z*)-4-Decenal[a]	2146	21662-09-9	0.20	±	0.11
Aliphatic ester	Methyl caproate[a]	1237	106-70-7	**2.96**	**±**	**1.90**
	Ethyl caproate[b]	1285	123-66-0	**2.31**	**±**	**0.38**
	Propyl hexanoate[a]	1373	626-77-7	0.05	±	0.02
	Butyl Hexanoate[a]	1475	626-82-4	0.24	±	0.10
	n-Butyl hexadecanoate[a]	2535	111-06-8	0.01	±	0.00
Aliphatic ketone	Octan-3-one[b]	1305	106-68-3	0.46	±	0.12
	Nonan-2-one[b]	1442	821-55-6	0.50	±	0.00
	(*E*)-3-Octen-2-one[a]	1460	18402-82-9	1.02	±	0.10
	3,4-Epoxy-2-octanone[a]	1587	17257-80-6	0.35	±	0.10
Benzenoid compound	Methyl salicylate[c]	1842	119-36-8	0.01	±	0.00
Miscellaneous cyclic compound	1-(2-Methyl-1-cyclopenten-1-yl)-ethanone[a]	1649	3168-90-9	0.01	±	0.00
	τ-Caprolactone[a]	1767	502-44-3	0.61	±	0.04
	Dimethylmaleic acid anhydride[a]	1793	766-39-6	0.05	±	0.01
	5-Ethyl-2(5H)-furanone[b]	1820	698-10-2	0.03	±	0.01
	δ-Caprolactone[a]	1862	823-22-3	0.61	±	0.17
	Bicyclo[3.3.1]nonane-2,7-dione[a]	2075	199723-73-4	0.05	±	0.02
	γ-n-Amylbutyrolactone[a]	2101	104-61-0	0.02	±	0.01
	γ-Ethoxy-butyrolactone[a]	2384	96-48-0	0.04	±	0.02

Sulfur-containing compound	Dimethyl trisulfide[c]	1433	3658-80-8	0.21	±	0.18
	S-Methyl methanethiosulfonate[a]	2040	2949-92-0	0.01	±	0.00
Unknown	Unknown (ms: 41,54,82,109,152,154)	2168		0.00	±	0.00
	Unknown (ms: 42,45,57,58,85,86)	2433		0.01	±	0.01
	Unknown (ms: 42,43,57,69,97,115)	2503		0.00	±	0.00
Emission rate (ng/fruit/hr)				593.59	±	128.48
Number of compounds				48		

Germination cues

Germination trials for seeds that underwent experimental treatments suggest that the physical barrier imposed by the woody endocarp is what limits the ability of the seed to germinate. The greatest deviation from the expected germination success frequency was for those seeds which had had their seed coat removed (χ^2= 102.07, d.f. = 8, p < 0.001), thus alleviating any mechanical barrier that a geminating embryo would have to break through. Those seeds which had a small area of their embryo exposed to the air, also showed substantial enhancement of germination success. Not shown in Table 2 is the time taken to germinate. Both the naked seeds and those having an air hole germinated within a week of being sown. For these, germination success was only recorded for 3 weeks, as opposed to all other treatments which only yielded germinants after 6 weeks.

1.5 cm

Figure 3: Seeds of *Balanites maughamii* that have germinated in a greenhouse. Note the splitting of the woody endocarp along the flanges running along the length of the seed.

Although both the naked seeds and those having an air hole were soaked in water prior to sowing; however, the control soaked seeds showed no enhancement. This suggests that it is not moisture which enhances germination success or time taken to germinate.

Mechanical crushing of seeds produced poor germination success. Those that had been cracked ('crush') as well as those that were treated with a compressive force of 2.43 kN (90 % of the mean force required) showed very low germination success. However, the 1.89 kN treatment yielded a slightly higher germination success than was expected. Many of these seeds were not crushed, but perhaps sustained small cracks in the seed coat, which could have weakened its structure and allowed the embryo to open the flanges more easily.

Seeds that had been through the gut of an elephant did not exhibit enhanced germination success. In particular, none of those which had been crushed germinated as it appears as though the embryo was severely damaged. Seeds that were not manipulated in any way had very low germination success, which alludes to the necessity of a disperser interaction to facilitate germination.

Table 2: Chi-squared statistics for germination trials of *Balanites maughamii* seeds under various experimental treatments ($\chi^2 = 102.07$, d.f. = 8, $p < 0.001$)

Treatment	**n**	**Observed**	**Expected**	**O-E**	**(O-E)^2/E**
Control	48	2	9.3	-7.32862	5.7574
Control soak	20	0	3.9	-3.88693	3.8869
Compressive force					
1.89N	48	12	9.3	2.67138	0.7650
2.43N	48	1	9.3	-8.32862	7.4358
Crush	48	1	9.3	-8.32862	7.4358
Elephant dung					
Whole	14	2	2.7	-0.72085	0.1910
Crushed	6	0	1.2	-1.16608	1.1661
Other					
Naked	25	20	4.9	15.14134	47.1859
Air hole	26	17	5.1	11.94700	28.2467
Sum	283	55.0	55.0	0.00000	102.0707

Elephant gut passage time

Of 180 fruit, 34% (n=62) were recovered from elephant dung collected in the 48 hour period after ingestion. 15% of the 62 recovered seeds were intact (n=9). The number of partial seeds recovered may be exaggerated as several fragments may have originated from the same seed. A time series of seed emergence for all of the elephants is represented in Figure 2. The first emergence of seeds occurred between 12-24 hours with a peak in gut passage time at 28-30 hours. Data for the time between 31-36 hours could not be obtained. A large proportion of the seeds were still appearing in the 36-48 hour interval as well as one which appeared after 48 hours (Figure 3). Some seeds may have been retained longer than the sampling period.

There appears to be a relationship between elephant size and emergence time. The two smallest elephants had an earlier emergence time of 12-24 hours. The largest elephant had its first seed emerge at 25 and the majority of seeds were found between 36-48 hours.

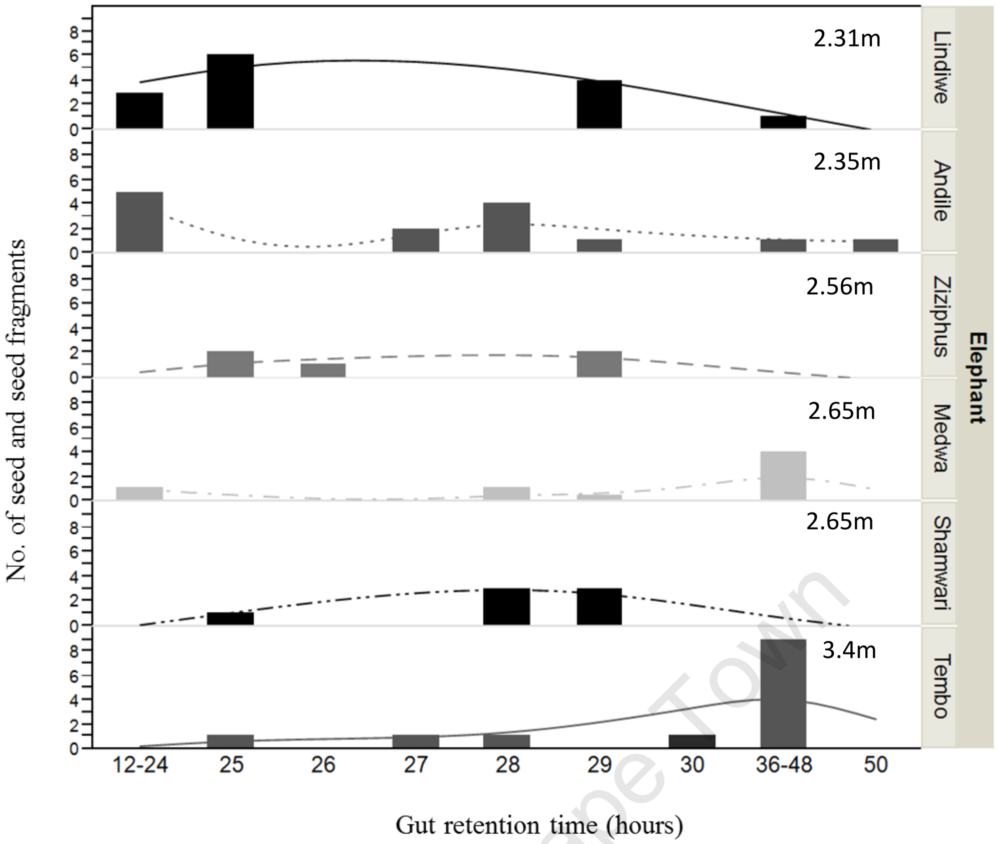

Figure 3. Gut retention time for 180 *B. maughamii* seeds fed to six elephants. No seeds were collected for the period between 31-36 hours. Elephant size is indicated by shoulder height measurements.

Fruit and seed bait stations

Camera traps showed that the mammals which feed on *B. maughamii* fruit, include bushbuck (*Tragelaphus scriptus)*, impala (*Aepyceros melapus)*, and porcupine (*Hystrix africaeaustralis)*. Impala and were responsible for approximately 50% of the consumption events of fruit (n=7), with bushbuck having the same incidence of consumption. Only one instance of a porcupine consuming fruit was observed. .At the fruit cafeterias, we found a 78.9 % consumption rate over three consecutive days. Of the 90 fruit we placed: 36.7 % (n=33) could not be located, 30.0 % (n=27) were eaten with the seeds found *in situ* and 12.2% (n=11) had their seeds dispersed 0.4-3.8 m away (mean distance = 1.9m).

Of the 100 tagged *B. maughamii* seeds we placed under camera traps, 10 were removed. We were unable to recover any of these. No evidence of hoarding behaviour by rodents was found in this study; however, camera footage did reveal one instance of a bushveld gerbil, *Tatera leucogaster*, carrying a seed out of the field of view. Bushbuck, impala and porcupine were all observed either sniffing or chewing the *B. maughamii* seeds without predating them. No movement of seeds was recorded for those placed during August (n = 70), which were tagged with reverse-wound bobbins.

Discussion

The fruits of *Balanites maughamii* seem tailor made for megafaunal dispersal on account their size, colour and the force required tocrack their woody endocarp. *Balanites maughamii* exhibits a fruit syndrome that is characteristic not only of dispersal by mammals (Herrera *et al.* 1989), but more specifically by megafauna (Janzen & Martin 1982; Guimarães *et al.* 2008). The large size of *B. maughamii* fruit and seed has serious implications for the type of animal that can disperse their seed by endozoochory. This trait essentially limits *B. maughamii* to having African elephants as one of their only legitimate dispersers.

The green to yellow-brown colour of *B. maughamii* suggests that its disperser is most likely not an animal that relies heavily on visual stimulus, as these fruit tend to be well camouflaged in their environment (*pers. obs.*). Debussche & Isenmann (1989) propose that olfaction is more important than visual cues for detection in mammal-dispersed species, which fits characteristics of *B. maughamii* fruit which have a pungent odour and high rate of of emission. Dominance of volatile scent compounds that resemble animal smells, such a hexanoic acid and isovaleric acid, should be associated with mammal attraction (Borges *et al.* 2008). Little work has been done in attempting to quantify the contribution that fruit odour makes to dispersal dynamics (Herrera 1989, 2002; Dudavera *et al.* 2006), but it appears as though it may play a significant role in elephant attraction, who use their trunks to locate fallen fruits and who will actively seek fruits that are out of their sight (*pers. obs.*). These data are a novel look at the specific compounds that facilitate elephant attraction. Further study is required to make inferences about which volatiles scent compounds are common among elephant fruits.

The seeds of *B. maughamii* are exceptionally hard, requiring a crushing force of 2.7 ± 0. 7 kN (mean ± s.d.). Having such hard seeds is another megafaunal- disperser mutualism trait that has been identified in many other fruits that are known to be dispersed by elephants, including *B. wilsoniana* and *Sclerocarya birrea* (Babweteera *et al.* 2007; Midgley *et al.* 2012). This adaptation is a crucial element of the megafaunal dispersal syndrome as it is necessary for increasing the probability of seed survival during mastication by elephants. It could also be influential in providing protection from seed predators, including smaller mammals, such as impala and bushbuck, and even from microbial attack (Herrera 2002).

While protection of the soft endosperm and embryo is a necessity if these seeds are to be dispersed by elephant endozoochory, having such an impenetrable endocarp imposes a significant obstacle to the germinating embryo that has to escape from it. The woody endocarp represents an obstacle to the recruitment of *B. maughamii*, as those seeds for which the endocarp was removed germinated had a significantly higher proportion of successful germinants. Additionally, the drastically reduced time taken to germinate when the endocarp was removed indicates further advantage to the seed when the endocarp is compromised. Relatively poor germination success of seeds that had been soaked or those which in which a small hole had been placed to allow oxygen and water to reach the embryo directly, indicated that it is less likely to be a chemical or moisture cue which promotes germination.

Further support for mechanical limitation is evident when one considers that seeds that had been treated with a compressive force if 1.89 kN had better germination success than those which had not been compressed. The addition of this mild compressive force is equivalent to structural weakening of the endocarp, which should reduce the energy required for the embryo to break through. The low success rate of seeds to which a 2.43 kN force was applied, or which were compressed until they had deformed, suggests that if a seed is crushed, damage to the embryo reduces the probability of successful germination.

In the context of elephant fruit consumption, these findings indicate that chewing by elephants acts to weaken to integrity the woody endocarp, making it easier for the seed to swell and open the longitudinal flanges. Reduced time to germination provides a critical advantage to seeds, as they are extremely vulnerable during these early stages of their life cycle (Howe 1993). This is an important function that explains why seeds in the genus, *Balanites*, that have been through the gut of an elephant generally show much improved germination success (Dudley 2000; Cochrane 2003). Although not clear from the small sample size and quality of the seeds found during this study, *B. maughamii* seeds that have been through the gut of an elephant may still have a greater germination success.

Elephant dispersal appears to come at a high risk of predation for *B. maughamii* seeds, with 85% of the recovered seeds having been crushed and therefore not capable of germination. However, elephants are still important vectors for the small portion of seeds that survive ingestion. Since breeding herds of elephants can move within Kruger National Park an average of 4 km/daily (Thomas *et al.* 2008), they can potentially disperse seeds more than 4-8 km from the parent tree, which is within the range of what is considered as 'long distance dispersal (Nathan *et al.* 2008). Bulls travel longer distances so they may be able to disperse seeds further across the landscape (Kruger *pers. comm.*). The variable age structure in a herd of elephants could also mean that fruit from the crop of a single tree could be distributed over a variable range, which would has positive consequences for gene flow, meta-population dynamics and colonisation potential for *B. maughamii* (Nathan & Muller-Landau 2000.

Elephants appear to play a highly significant role in the dispersal of *B. maughamii*, but they are not the only animals whose activity *B. maughamii* is exposed to. A variety of medium-sized mammals, including impala, bushbuck and porcupine, will consume the flesh of the fruit, leaving the seed *in situ*. These seeds are often beneath the canopy of the parent tree, well-within the zone of Janzen-Connell effects (Janzen 1970; Connell 1971; Howe & Smallwood 1982). By removing the fruit flesh, non-dispersers greatly reduce the attractiveness of the seed to elephants, which prevents legitimate dispersal. The high abundance of the medium-sized mammals relative to elephants alludes to the substantial loss of dispersal potential that *B. maughamii* seeds face.

Once the seeds are exposed, it is proposed that they become attractive to scatter hoarding rodents who are thought to act as secondary dispersers to some megafaunal fruits (Gautier-Hion *et al.* 1985; Vander Wall *et al.* 2005 Midgley *et al.* 2012). Rodents are also known to detect seeds by olfaction (Vander Wall 1993, 1995), which could mean that strong scent could also be an attractant for secondary dispersers or seed predators – both of which influence the proportion of successful germination. Bijl (2012) observed larder hoarding of

B. maughamii seeds by *T. leucogaster*, which suggests these rodents potentially be notable secondary dispersers, although this is inherently difficult to quantify (see Wang & Smith 2002; Dennis & Westcott 2006).

Many of the traits that make *B. maughamii* fruit suitable for megafaunal dispersal, are also considered to be adaptations to scatter hoarding, including their large endosperm covered by a durable mesocarp, and a sweet, fleshy mesocarp (Smythe 1989). This considered, it seems likely that a diplochorous dispersal system involving both elephants and rodents is what these fruits have adapted to. The relative contribution of rodents as dispersers in a diplochorous system, which also has elephants as the primary dispersers, has important implications for species which have lost their megafaunal mutualists.

This strategy means that although seeds are well-protected from most non-dispersers and have a good chance of valuable long-distance dispersal, there is a substantial risk if elephants are removed from the system. The more specialized species become to this megafaunal dispersal strategy, the vulnerable they are to significant reductions in recruitment in the event that their mutualistic is eliminated. Cochrane (2003) demonstrated a high cost associated with non-dispersal of *Balanites wilsoniana*. Based on the similarity in structure and ecology of this species to *B. maughamii*, it is not unreasonable to assume that in the absence of elephants the same cost would be found.

For *B. maughamii*, the consequences of a breakdown of their mutualism with elephants are two-fold. The large bite force required to facilitate quick germination could only realistically be provided by en elephant (Wroe *et al.* 2005). This ‘large bite force hypothesis” was also supported by the findings of Midgley *et al.* (2012) in the germination facilitation of *Sclerocarya birrea*, which suggests that this may be yet another trait adopted by megafaunal fruits. Thus, elephants impose a selective pressure on these fruits (Fleming & Estrada 1993, Howe 1993, Jordano 1995), causing adaptations that are common account large-fruited African trees on account of the long evolutionary history of elephants in Africa (Haynes 1993).

Regeneration is not impossible without elephants, and their absence would be detrimental to *B. maughamii* (Bijl 2012), and likely result in patchy distribution of weak, slow-to-germinate individuals who are less robust during their recruit and thus more vulnerable in this critical stage. Maintenance in the absence of dispersal has been noted in many ecosystems in which fruits appear to be restricted to megafaunal dispersal (Janzen & Martin 1982; Guimarães *et al.* 2008). However, in the long term, one may fine a clumped distribution of genetically similar individuals, which would increase the vulnerability of the population to environmental stochasticity. A lack of colonisation potential, as a consequence of limited long distance dispersal is also particularly concerning in the face of global climate, where latitudinal shifts in range may be necessary for species survival (Schupp 1993; Clark *et al.* 1998).

The vulnerability of *B. maughamii* as an African megafaunal fruit needs to be considered in the both its ecological and cultural context. Human exploitation of this tree species is considerable (Netshiluvhi 1996). Additionally, the increase in habitat fragmentation as well as human-elephant conflict, could limit the movement of elephants, and thus of *B. maughamii* dispersal. Conservation of this keystone tree species requires a better understanding of its dispersal ecology, including its limitations, germination requirements, and the stability of its dispersal mutualisms.

Conclusion

The traits displayed by the fruit of *B. maughamii* follow the typical megafaunal dispersal syndrome of large, fleshy, strongly scented fruits that carry a single exceptionally hard seed. Elephants appear to be one of the only legitimate dispersers of *B. maughamii*, and are necessary not only for providing valuable long distance dispersal, but also for enhancing germination through the weakening of the woody seed endocarp by mastication. Having these fruit traits are crucial for facilitating successful dispersal by elephants, but which also serve to severely limit the disperser range of *B. maughamii*. This limitation drastically increases the vulnerability of this tree species, in the event of a breakdown of this dispersal mutualism.

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Appendices

Appendix I. Elephant information for the six residential elephants at Elephant Whispers, Hazyview. Height and age valid as of the end of 2012.

Elephant	Height (m)	Sex	Age (years)	First emergence (hours)
Lindiwe	2.31	female	13	12-24
Andile	2.35	female	20	12-24
Medwa	2.65	male	18	12-24
Ziziphus	2.56	male	18	25
Shamwari	2.65	male	19	25
Tembo	3.4	male	30	25

Appendix II. GPS coordinates and locations for the sites with camera traps and tagged *B. maughamii* seeds.

Site	Location	GPS location
Bal1	Behind golf course	S24.9874 E31.5741
Bal2	Behind golf course	S24.9880 E21.5744
Bal3	Skukuza village	S24.9825 E31.5807
Bal4	By water tower, near golf course	S24.9891 E31.5784
Bal5	Skukuza village	S24.9963 E31.5894
Bal6	Skukuza village	S25.0000 E31.5746
Bal7	Skukuza village	S24.9996 E31.5815
Golf1	Golf course	S24.9880 E31.5765
Golf2	Golf course	S24.9878 E31.5771
Golf3	Golf course	S24.9884 E31.5783
Fruit1	c/o Kudu and Elephant Street	S24.9839 E31.5815