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Fruiting and seed production of producer and poor-producer baobab trees and on different land use types in Northern Venda, South Africa.

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A research report submitted to the Faculty of Science, University of the Witwatersrand, in partial fulfilment of the requirements for the Degree of Masters of Environmental Sciences by Coursework and Research Report.

Johannesburg

24 March 2016

Supervisors

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Declaration

I, Ester Nangolo declare that this research report, apart from the contributions mentioned in the acknowledgements, is my own, unaided work. It is submitted for the Degree of Master of Environmental Sciences by coursework and research report to the University of the Witwatersrand. It has not been presented before for any degree or examination to any other University.



(Signature of candidate)

24 Day of March **2016**

Abstract

In southern Africa, the baobab (*Adansonia digitata* L.) is an economically important trees because it contributes significantly to the livelihoods of local people, particularly in northern Limpopo in South Africa (the southern-most edge of the baobab distribution). All parts of the baobab are useful and considered important for subsistence and commercial uses. Understanding factors that affect fruit and seed production is important to better characterize the long-term success of tree populations. Some adult baobab trees have high fruit production (50 −299 fruits per tree, per year) and are subsequently called 'producers' or 'female' trees, while there are other trees that produce fewer fruits (< 5 fruits per tree, per year) that are called 'poor-producers' or 'male' trees.

For this study, baobab fruit dimensions (mass, length, volume and ratio) were, measured and compared between artificially- and naturally-pollinated producer and poor-producer trees. Fruit dimensions were also correlated to the number of seeds per fruit. Using 2D geometric morphometric analysis, baobab fruit shapes were analyzed and compared to determine if fruit shape differs between fruits formed on producer and poor-producer baobab trees. I found no significant difference in fruit shape between producer and poor-producer baobab trees. Although, artificially-pollinated trees produced bigger and more uniform shaped fruits and contained more seeds in comparison to the smaller unevenly shaped fruits produced by naturally-pollinated trees.

Furthermore, I compared fruit and seed production between naturally-pollinated producer/poor-producer and between artificially-pollinated producer/poor-producer trees that occur on different land use types (i.e. nature reserves, rocky outcrops, plains, fields (land use for agricultural purposes) and villages) in Northern Venda, South Africa. There was a significant difference in fruit and seed production between naturally and artificially-pollinated producer and poor producer trees that occur on different land use types. On average, producer trees yielded more seeds than poor-producer trees. The highest fruit and seed production was recorded in fields and villages. Seed mass variation also differed significantly between producer and poor-producer trees and between the different land use types. A very weak negative relationship between baobab seed number and mass was found in both producer and poor-producer baobab trees. Baobab seed viability was estimated using a 0.1% tetrazolium solution, 100% viability was found in all tested seeds from both producer and poor-producer baobab trees.

Result of this study may aid with the identification of land use types where baobabs have the lowest seed production and therefore require additional conservation effort to ensure that fruits are harvested sustainably. The estimation of seed production may improve the ability to estimate the total seed oil that is available in each land use type.

Key words: *Adansonia digitata*, fruits, land uses, pollination, poor-producer, producer, seeds

Dedication

I dedicate my work to my mother (Aili M.Nangolo) and my Aunt (Selma D. Shaanika) for their financial support throughout the year. I also dedicate this to my siblings (Mirjam and Kefas Nangolo) and all my friends especially (Martha Amwaama) for their love and words of encouragements throughout this study.

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Table of contents

Declaration i

Abstract ii

Dedication iv

Acknowledgements v

Table of contents vi

Chapter 1.0 1

1.1 Literature review 1

Fruit and seed production 1

Fruit and seed characteristics 6

Fruit shape analysis 8

Chapter 2.0 11

2.1 Introduction 11

2.2 Aims, objectives and research questions 13

2.3 Materials and methods 15

Study area 15

Study species 16

Data collection and analyses 17

Baobab fruit comparisons 18

Baobab seed viability 19

Fruit shape Analysis 20

Baobab seed comparisons and seed production estimate 22

Chapter 3.0 24

3.1 Results 24

Comparison of fruit ratio (fruit length/fruit width) between artificially-pollinated and naturally-pollinated producers and poor-producer baobab fruits 24

Comparison of fruit mass between artificially-pollinated and naturally-pollinated poor-producer and producer baobab fruits 26

Relationship between baobab fruit volume and the number of seeds per fruit 30

Relationship between baobab fruit length and the number of seeds per fruit 32

Relationship between baobab fruit mass and fruit length 34

Relationship between average baobab seed mass and the number of seeds per fruit 36

Comparison of Coefficient of Variation (CoV) in seed mass (g) between naturally-pollinated and artificially-pollinated 'male' and 'female' baobab fruits 38

Comparison of Coefficient of Variation (CoV) in seed mass from naturally-pollinated baobab fruits collected from different land use types. 39

Comparison of baobab seed viability between artificially-pollinated and naturally-pollinated 'male' and 'female' fruits. 41

Seed production estimate in producer and poor-producer baobab trees 42

Baobab fruit shape analysis 45

Chapter 4.0 47

4.1 Discussion 47

Baobab fruit and seed characteristics 47

Baobab fruit shape analysis 49

Seed number and mass trade off in baobabs 49

Baobab fruit production on different land-use types 51

Estimation of seed production in producer and poor-producer baobab trees 52

Baobab seed viability 54

4.2 Conclusion 56

Reference list 57

Appendices 63

List of Figures

Figure 1.1: Twelve different baobab shapes observed in baobab fruits (*Adansonia digitata*) from Blue Nile and North Kordofah, Sudan. Source: Gurashi and Kordafani (2014). 10
Figure 2.1: Location of study site where fruits were collected, Northern Venda in Limpopo Province, South Africa. Source: Venter and Witkowski (2010).....16
Figure 2.2 Baobab (Adansonia digitata) tree and fruits. Source: Wiehle *et al*. (2014).....17
Figure 2.4: Baobab seeds cut open lengthwise before being placed in a 0.1% tetrazolium solution. Photograph: E. Nangolo.....20
Figure 2.5: Illustration of the 12 landmarks placed on each baobab photograph. Photo: E. Nangolo.....22
Figure 3.2: Boxplot for artificially and naturally-pollinated baobab fruit ratio. Where A_F: artificially-pollinated producer, A_M: artificially-pollinated poor-producer, N_F: naturally-pollinated producer and N_M: naturally-pollinated poor-producer fruits. 26
Figure 3.3: Comparison of mean fruit mass (g) between fruits formed from artificially-pollinated producer (mean = 237.66 g, SE = 61.98 g) and poor-producer (mean = 314.25 g, SE = 20.93 g) and naturally-pollinated producer (mean = 182.24 g, SE = 10.15 g) and poor-producer (mean = 132.88 g, SE = 17.98 g) baobab trees.....27
Figure 3.4: Boxplot for artificially and naturally-pollinated baobab fruits mass (g). Where A_F: artificially-pollinated producer, A_M: artificially-pollinated poor-producer, N_F: naturally-pollinated producer and N_M: naturally-pollinated poor-producer fruits.....28
Figure 3.5: Comparison of the relationship between baobab fruit mass and the number of seeds per fruit between fruits from (a) artificially-pollinated producer and (b) poor-producer trees and between (c) naturally-pollinated producer and (d) poor-producer baobab trees. 29
Figure 3.6: The relationship between baobab fruit mass and the number of seeds per fruit for pooled data of fruits from (a) artificially-pollinated and (b) naturally-pollinated producer and producer baobab trees, and from (c) 'female'/producer trees and (d) 'male'/poor-producer trees. 30
Figure 3.7: Comparison of the relationship between fruit volume and the number of seeds per fruit between fruits from artificially-pollinated (a) poor-producer and (b) producer baobab trees and between naturally-pollinated (c) poor-producer and (d) producer baobab trees. 31

Figure 3.8: The relationship between fruit volume and the number of seeds per fruit for pooled data of fruits from (a) artificially-pollinated and (b) naturally-pollinated trees and between (c) 'female'/producer trees and (d) 'male' /poor-producer trees. 32
Figure 3.9: The relationship between baobab fruit length and the number of seeds per fruit for pooled data of fruits from artificially-pollinated (a) 'male'/poor-producer and (b) ' female'/producer baobab trees and between naturally-pollinated (c) poor-producer and (d) producer baobab trees. 33
Figure 3.10: The relationship between baobab fruit length and the number of seeds per fruit for pooled (a) artificially-pollinated and (b) naturally-pollinated baobab fruits and between (c) 'female' and (d) 'male' baobab fruits. 34
Figure 3.11: Comparison of the relationship between fruit mass and fruit length between fruits from artificially-pollinated (a) poor-producer and (b) producer baobab trees and between (c) naturally-pollinated poor-producer (d) and producer baobab trees. 35
Figure 3.12: The relationship between baobab fruit mass and fruit length for pooled (a) artificially-pollinated and (b) naturally-pollinated baobab fruits and between (c) 'female' and (d) 'male' baobab fruits. 36
Figure 3.13: Comparison of the relationship between seed mass and the number of seeds per fruit between fruits from artificially-pollinated (a) producer and (b) poor-producer and between (c) naturally-pollinated-poor producer and (d) producer fruits. 37
Figure 3.14: The relationship between baobab fruit mass and fruit length for pooled (a) artificially-pollinated and (b) naturally-pollinated baobab fruits and between (c) 'female' and (d) 'male' baobab fruits. 38
Figure 3.16: Comparison of Coefficient of Variation (CoV) in seed size of fruits from naturally-pollinated flowers on trees from different locations. The lowercase letters (a, b, c and d) represent a statistically significant difference between the land use types as revealed by the (LSD) test. 40
Figure 3.17: Comparison of baobab seeds before (A) and after (B) being immersed in the tetrazolium solution. 41
Figure 3.18: The four regression lines (a) Linear (b) Log (c) Polynomial and (d) Power tested to determine the best fit line that was used to estimate the number of seeds per fruit from producer baobab trees. 42
Figure 3.19: The four regression lines tested to determine the best fit line that was used to estimate the number of seeds per fruit from poor-producer baobab trees. 43

Figure 3.20: Comparison of fruit production and seed production estimate between producer and poor-producer baobab trees over a nine year period. 44
Figure 3.22: Two dimensional deformation grid of consensus shape (red) and rotated landmarks (green) from all fruits from both producer and poor-producer trees. 46
Figure 3.23: A plot of the Canonical Variance Analysis (CVA) analysis results for fruit shape of fruits from producer ('female') and poor-producer ('male') baobab trees. The CV scores are presented on the x-axis and the frequency of individuals with that CV score is on the y-axis. 46

Chapter 1.0

1.1 Literature review

Fruit and seed production

Adansonia digitata L. is a tetraploid species (160 chromosomes; Baum and Oginuma, 1994) belonging to the Malvaceae (Sidibe and William, 2002; Gurashi and Kordofani, 2014). *Adansonia digitata* is endemic to mainland Africa and some oceanic islands, such as Comores, occurs in southern Africa, east Africa and parts of western Africa (Wickens, 1983; Baum, 1995). The other seven species of genus *Adansonia* are diploid (88 chromosomes) and are found in different parts of the world. Six species are endemic to Madagascar and one is endemic to Australia. Baobabs are recognised locally and internationally for food and seed production. The study of fruit and seed production is important to better characterize the long-term success and sustainability of tree populations.

Fruit and seed production depends on the effectiveness of pollination. Baobabs have large, spectacular nocturnal flowers with partially separated stigma, anthers and nectars. The flowers are enclosed by five calyx lobes (Baum, 1995). The calyx tissues produce nectar, which in *A. digitata* accumulates on the inner parts of the petals (Baum, 1995). The reproductive structures, both 'male' and 'female' reproductive structures of the baobab are found on the same flower i.e., the flowers are hermaphrodite (Oginuma, 1994). The flowers are visited by large bodied pollinators which are reported to be bats for the African baobab and fruit bats and lemurs for two of the species in Madagascan species while the Australian baobab species and four of the Madagascan species are pollinated by hawkmoths (Baum, 1995). The possibility of baobabs to be wind-pollinated was proposed in 1945, however, this is highly unlikely because baobabs have a small stigmatic area and the pollen is not light enough for this mode of pollination (Baum, 1995).

Humans can manipulate pollination by transferring pollen from one tree to another tree by hand, transferring much more pollen than would likely occur under natural pollination; this type of pollination is called 'artificial pollination'. A study by Bertin (1982) found that hand pollination increases fruit production as well as the number of fruits that reach maturity in

Trumpet creeper flowers (*Campsis radicans* L.) which implies that hand pollination is helpful in fruit and seed production.

Das *et al.* (2013) compared to natural and hand-pollination to determine the best pollination method for passion fruit (*Passiflora edulis* S.)and found that hand pollination produces the highest number of fruits per tree as well as the largest fruits then self and natural pollination. In addition, hand pollination also reduces the "misshape" of fruit and produces near perfect of perfect shaped fruits in Cherimoya fruits (Schroeder, 1941). Hand pollination also reduces the time between flowering and fruit maturity (Das *et al.,* 2013), thus it is a fast and effective pollination method.

Another factor affecting fruits and seed production is flower picking by humans which leads to a reduction in seed production and seed storage per plant (Witkowski and Lamont, 1995). For example in *Banksia hookeriana*, flowers were picked for commercial purposes in Australia. The removal of flowers reduced the number of fertile cones per plant, and as a result, there was a reduction in seed production, seed storage and viable seeds by 50%, 57%, 50% respectively. Although no flower picking has been reported in baobabs, humans and baboons pick baobab fruits. Baboons are reported to bite and drop immature fruits from baobab trees in different land use types in South Africa causing up to 85% reduction in the production of mature fruits within the nature reserves and rocky outcrops habitats (Venter and Witkowiski, 2011). A study on baobabs in the northern–most province of South Africa, Limpopo Province, showed that fruit harvesting decreases seed availability from 100% to 10% (Venter and Witkowski, 2013b). The study concluded that baobab trees are highly tolerant to fruit harvesting only in the absence of livestock. In other words, baobab populations are stable under fruit harvesting but, addition of livestock has influenced population stability and consequently led to less fruit production. It is therefore important for baobabs to be well protected from livestock to ensure continuous economic usage.

Fruit production for many species has been attributed to tree stem diameter and crown volume. However, studies have shown that these dimensions can only be used as indicators for fruit production for species with small fruits, which suggests these are not suitable measures for baobabs (Sidibe and Williams, 2002). Thus, explaining the potential causes behind fruit production has been difficult in baobab trees. For example, Venter and Witkowski (2011) found that there was a poor relationship between baobab trees stem diameter, crown volume/area and fruit production because fruit production is very variable.

Previous work on baobab fruits has shown that the number of fruit produced on a tree is not consistent among baobab trees (Venter and Witkowski, 2011). For instance, (Venter and Witkowski, 2011) found that some adult trees had high fruit production producing 50−299 fruits/tree per year, and subsequently called 'producers' or 'female' trees, while other trees yielded low fruit production (<5 fruits/tree per year), and were called 'poor-producers' or 'male' trees . Venter (2012) studied the phenology, flowering and fruit-set patterns of producer and poor-producer baobabs in northern Venda, South Africa and her data showed no difference in flowering phenology. All trees had the same timing and length of flowering. However, there was a statistical difference in the number of fruits per tree, and mean fruit-set was higher in producer than in poor-producer baobab trees.

Seed production is dependent on fruit production and the information on seed viability is important when studying seed production. Seed germinating can test seed viability. Seed germination is a process where embryo development is activated. This process begins when the seed absorb water causing the embryo to expand and elongate and is completed when the radicle grows out of the seed coat (Miransari and Smith, 2013, Finch-Savage and Leubner-Metzger, 2006). Seed viability and germination may be used to estimate the potential future regenerations of seedlings (Dooley *et al.*, 2013).

Before determining whether seeds are viable or can germinate, sometimes dormancy must be broken. Seed dormancy is a seed property that determines or defines the sets of environmental conditions where seed germination is likely to take pace (Prins and Maghembe, 1994).

Dormant seeds germinate when all germination conditions are favourable (Venter and Witkowski, 2013c; Cousins *et al.*, 2014). Prins and Maghembe (1994) suggested that seed coats of fruit trees have germination inhibitors that only allow germination after heavy rains to successfully deactivate those inhibitors. Regeneration strategies of plant species within their natural environments are understood by studying seed dormancy and germination of stored seeds as well as dispersal and seedling establishment (Cousins *et al.*, 2014).

For example, Prins and Maghembe (1994) optimized a simple technique to germinate seeds of indigenous fruit trees in Malawi. Interestingly, wild fruits are given little research attention because of their slow growth rate and the long time between germination of seeds and the growth of trees until they are large enough for fruit production. In addition, most wild fruits

are inedible and are therefore less studied (Prins and Maghembe, 1994). Prins and Maghembe (1994) found that direct sowing, cleaning, plus soaking techniques yield an 80% germination rate for most species and concluded that different species requires different seed pre-treatments for best germination results. For baobabs, pre-treatment of seeds can be done by soaking seeds in concentrated sulphuric acid for a period of 6 to 12 hours, which has yielded up to 90% germination (Sidibe and Williams, 2002). However soaking seeds in boiling water is safer than using sulphuric acid and works as well (Venter, 2012; Venter and Witkowski, 2013c). Once the dormancy mechanism of a species is understood, the seeds can be treated in a specific manner in order to break dormancy.

Prins and Maghembe (1994) also studied the effects of temperature on seed germination. Their results showed that seeds stored at cold room temperatures had poor germination and that seed viability also decreased with increase in time of storage. Further, seed viability in *Zostera marina* (eelgrass) is correlated to seed age in that seeds stored for about four years showed only 32% viability compared to 77% viability from freshly collected seeds (Dooley *et al.,* 2013). This may be because the seed coat gets a stronger fracture with an increase in age. On the other hand, seed storage did not significantly reduce seed viability in *Kumara plicalitis* tree ('fan aloe'), although seeds stored under high temperatures (ambient and 25 °C) had a higher percentage of germination than seeds stored at lower temperatures. (Cousins *et al.,* 2014).

Similarly, Venter and Witkowski (2013c) tested baobab seed viability of a range of laboratory-stored seeds and found a difference in the number of viable seeds between periods of storage although the viable seeds were found to be heavier than the non-viable seeds. The study also found that the weight of non-viable seeds did not differ between seed ages but that of viable seed weight did differ. This variation in weight of the viable seeds was attributed to the seed source, the size of fruit, and area of fruit collection, not on seed age. This implies that seed viability is dependent on the time of storage.

Another way to test potential for seed germination is by using tetrazolium salt solutions to indicate a living embryo. During the tetrazolium test, malic acid, a dehydrogenase enzyme, transfers H^+ ions released during the citric acid cycle of respiration to the 2,3,5-triphenyltetrazolium chloride (Gimenez *et al.,* 2014). It is this reduction reaction that turns the seed embryo from colourless to red; indicating that mitochondrial activities have taken

place. Thus when the seed embryo turns red, the seed is said to be viable (Gimenez *et al.,* 2014).

It is important that the seed embryo is well exposed before the seed is soaked in the tetrazolium solution. Generally, seeds are imbibed by immersing in water, cut open, and then exposed to different tetrazolium concentrations (0.05%, 0.5% and 1%) for 2−6 hours at room temperature. The 1% tetrazolium solution generally yields the best results (Gimenez *et al.,* 2014; Grzybowski *et al.,* 2012). When seeds are removed from tetrazolium solution, they must be washed with water and kept in ionised water until examination (Gimenez *et al.,* 2014; Grzybowski *et al.,* 2012). Previous work on *Acacia karroo* and *Chromolaena odorata* post-fire viability using tetrazolium showed that *C. odorata* seeds did not survive above 100 °C, while a portion of *A. karroo* seeds could survive extreme temperatures between 150 °C−200 °C for a very short time period (Mbalo and Witkowski *et al.,* 1997). This implies that viability in seeds may depend on the potential of the seed to survive extreme temperatures.

Germinating the seeds can also demonstrate seed viability, but a tetrazolium test is often used to test seed viability (Gimenez *et al.,* 2014) after or instead of germination trials. The tetrazolium test can yield faster results but the effectiveness of the tetrazolium solution is dependent on the time and temperature at which seeds are exposed to it, as well as on the tetrazolium concentration used. It is therefore important to choose the best method for best results. With this knowledge, seed germination trials are standard practice to determine seed viability; however not all seeds will necessarily lose dormancy after treatment, and hence the remaining ungerminated seeds from a trial should be tested with tetrazolium as some of them may still be viable.

Seed production is a trade-off between seed mass and the number of seeds produced by a particular species, individual or even a fruit (Sera and Sery, 2004), and in recent years, the effectiveness of seed production has been a common focus of research. This trade-off implies that species tend to produce either many small seeds or few large seeds (Giorgis *et al.,* 2013). Seed mass is a reflection of the amount of food contained in the embryo that allows plant establishment in the first life stages such as seedling development, maintenance and repair (Quero *et al.,* 2007). Seed size range is known to range from 0.0001 mg (seed size typical for orchids seeds) to 20 kg (seed size typical for *Lodoicea maldivica* commonly known as the ‘double coconut’; Moles *et al.,* 2005).

Variation in seed mass between species may be a result of differences in abiotic and biotic characteristics within habitats (Sera and Sery, 2000), thus seed mass varies from location to location. Variation in seed mass within species may also be caused by the timing and success of pollination. Species that adapt to unpredictable biotic and abiotic factors normally self-pollinate therefore produce a small number of large seeds within a short time, while insect pollinated species often produce many small seeds over a longer time period (Sera and Sery, 2000). It is therefore important to study differences in seed mass versus the number of seeds within populations in different habitats to provide information on ecological characteristics that yield optimum seed production effort (Brancalion and Rodrigues, 2013).

The size of seed has an influence on a plant species' ecology (Moles *et al.,* 2005; Quero *et al.,* 2007). For example, species producing many small seeds stand a better chance of successful seed dispersal because small seeds can be transported over longer distances. On the other hand, small seeds store less food for early plant development which makes them susceptible to harsh environmental conditions (Quero *et al.,* 2007; Sera and Sery, 2000; Giorgis *et al.,* 2015). Seed size also has an influence on germination as large seeds have a greater germination percentage, producing larger and more vigorous seedlings as opposed to smaller seeds because of the amount of food stored in the bigger seeds compared to the smaller seeds (Hendrix, 1984; Quero *et al.,* 2007; Moles *et al.,* 2005).

Fruit and seed characteristics

Baobab trees produce a variety of fruit sizes (all edible; Venter, 2012). Fruit shape can range from globose, ovoid, oblong-cylindrical to irregular (Sidibe and Williams, 2002) and the size varies between 7.5−54 cm long and 7.5−20 cm wide. Venter and Witkowski (2011) measured fruit size based on fruit length (measured top to bottom) and diameter (measured across the middle part of the fruit). Fruit diameter was found to be constant for all seeds. They then categorised the fruits into three size classes according to length: large class (*c.* 20 cm × 8 cm), medium class (*c.* 15 cm × 8 cm) and small class (*c.*10 cm × 8 cm).

Wiehle *et al.* (2014) measured baobab fruit length and girth (widest point) using a measuring tape and calculated fruit-shape ratio (fruit length ÷ fruit diameter). The longest fruit measured was 37.5 cm long, the widest fruit was 16.2 cm thick and the biggest fruit-shape ratio found was 4.9 (Wiehle *et al.,* 2014). Results also showed that there was no significant difference in

fruit characteristics between different locations within Sudan. However, Sanchez (2011) measured fruit and seed length, fruit and seed weight, pulp weight, pulp percentage and counted number of seeds per fruits and found that a significant difference in fruit characteristics among the different locations within Malawi. Interestingly, previous work has also shown that heavier baobab fruits contained more seeds and fruit pulp (Sanchez, 2011; Venter and Witkowski, 2011). Collectively, these results suggest that there is great variation in baobab fruit characteristics. Therefore it is important that whenever fruits are collected for any study, all characteristics should be measured.

Fruit characteristics can also be affected by the availability of water, light and inorganic nutrients as these variables also influence plant reproduction. Plants tend to produce large fruits and seeds when resources are abundant (Fenner and Thompson, 2005), however site and climate also affect plant reproduction. Venter and Witkowski (2011) found no significant difference in baobab fruit production between different land use types, but found a difference in the number of mature fruits. It was suggested that this variation could be attributed to fruit predation. The numbers of mature fruits per tree were highest in villages and lowest in nature reserves, which indicates there is control over trees against fruit predation in villages but not in nature reserves. Thus trees are more protected in villages and they therefore keep their fruits until matured.

Apart from fruit characteristics, people dependent on baobabs characterise mature baobab trees according to size and leaf colour, bark colour, fruit taste, seed colour, fruit pulp colour, and fruit colour (Sidibe and Williams, 2002). For instance, in rural populations in Mali, people differentiated between baobabs trees using the colour of the bark. Black bark is associated with mild tasting fruits, red bark with the most delicious fruits, whereas baobabs with grey/white barks are preferred for fibre rather than fruits (Sidibe and Williams, 2002) In Benin, baobabs are differentiated on the basis of fruit size and fruit shape (Assogbadjo *et al.*, 2008). Local people in western Africa (Benin, Burkina Faso, Ghana, and Senegal) suggested that producer trees in west Africa have sweet acidic and non-slimy fruit pulp compared to slimy pulp of the poor-producer trees (Assogbadjo *et al.*, 2008).
Such indigenous information is needed for conservation purposes as locals may opt to plant trees of preferred traits leading to loss of genetic diversity.

Fruit shape analysis

Trees often produce fruits with different characteristics such as size and shape. In attempt to explain these morphological differences, fruit shape has been analysed. The landmark-based morphometric approach is used to study shape variation and it is an effective method for determining differences in the shape of organisms or structures. This is because the landmarks contain information regarding the space between landmarks on the organism and from these data, differences in morphology can be presented in diagrams which are more informative than numeric tables (Conesa *et al.*, 2012; Webster and Sheets, 2010). Landmarks are the numbers placed on the fruit photograph around the fruit boundary to mark the fruit shape. Webster and Sheets (2010) distinguished between two other types of morphometric methods: traditional morphometrics and outline-based geometric morphologies. 'Traditional morphometrics' is based on the length, ratio and angles measurements, whereas 'outline-based geometric morphologies' does not require landmark placement, but is based on the summary of the shape parameters. The morphometric approach used in this study is a quantitative method that uses landmarks as the primary source of morphological data, assuming that the landmarks placed on the fruit boundary are homologous (Gonzalo *et al.*, 2009). The positioning of the landmarks on photographs, along the fruit boundary, is therefore critical to the end results of the analysis.

The morphometric method can be applied to a variety of organisms. Consequently, various plant structures such as leaf and fruit shape have been studied and factors such as climate and geographical as well as environmental variables to cause the differences in leaf morphology have been discussed (e.g. Conesa *et al.*, 2012; Glennon and Cron, 2015). For example Foster *et al*. (2015) did a comparative study of fish shape variation between *Chirostoma jordani* and *Goodea atripinnis* fishes found in the lentic and lotic habitats in the United States of America (USA). The study based on the geometric morphometric analysis found a significant difference in the body shape variation between the two species. The fish occurring in the lentic habitat appeared to have superior-positioned mouth, deeper bodies, and a wider and longer peduncle as opposed to fish in the lotic habitat. The results did not only show difference in body shape between the two species of fish within the different habitats but also

within the same species. For example, *G. atripinnis* individuals found in lentic habitats had smaller heads compared the lotic *G. atripinnis*. These differences in shape were attributed to phenotypic plasticity and genetic variation among the individuals. Phenotypic plasticity can be explained by the impact of the environment on organisms and their response and adaptation to these changes which causes similar organisms to behave differently and have different characteristics under contrasting environmental conditions (Stearns, 1989). The morphological differences such as the mouth position on the fish could be due to the availability and type of prey within the habitats (Foster *et al.,* 2015).

The classification of morphological characteristics is important to understand agricultural production and market value of plant and animal species. This information can also be used by conservationists in species or location conservation planning (Gurashi and Kordafani, 2014). Assogbadjo *et al*. (2010), observed that both environmental and genetic factors seem to highly affect baobab fruit characteristics when they studied variation in baobab fruit characteristics in Benin using morphological analysis. The fruits were collected from baobab trees in two zones within Benin (Guinean and Sudano-Guinean climatic zone). They measured fruit length, width, ratio (fruit length divided by fruit width) and fruit weight. Very low variation in morphological fruit characteristics was found between and within the two study areas. However, there was high variation (more than 40%) in fruit weight within baobab trees. Similarly, morphological variation in *Adansonia digitata* was studied in Sudan (Gurashi and Kordafani, 2014). Baobab fruits were collected from five different locations within two study areas (North Kordofa and Blue Nile). Fruit dimensions (length and width), fruit weight and ratio were measured and correlated to morphological traits. High variation in baobab fruit shape among baobab trees was found but there was no variation in fruit shape within individual baobab trees, i.e., the fruit shape did not differ among the fruits collected from the same baobab trees as was found by Assogbadjo *et al.* (2010).

A total of twelve baobab fruit shapes was observed in Sudan; crescent shape, ellipsoid, rhomboid, ovate, spheroid–emarginate, obovate, fusiform, oblong pointed, globose, ellipsoid pointed, high spheroid and clavate (Figure 1.1). These shapes varied between baobab trees but remained the same within individual trees. The ellipsoid shape was the most common fruit shape observed. The differences in fruit shape were attributed to differences in environmental conditions and climate between and within the two study areas in Sudan (Gurashi and Kordafani, 2014*).* This indicates that the environmental variables have a major effect on fruit characteristics and explains why fruit production within the same species can vary significantly between individuals in two adjacent environments.

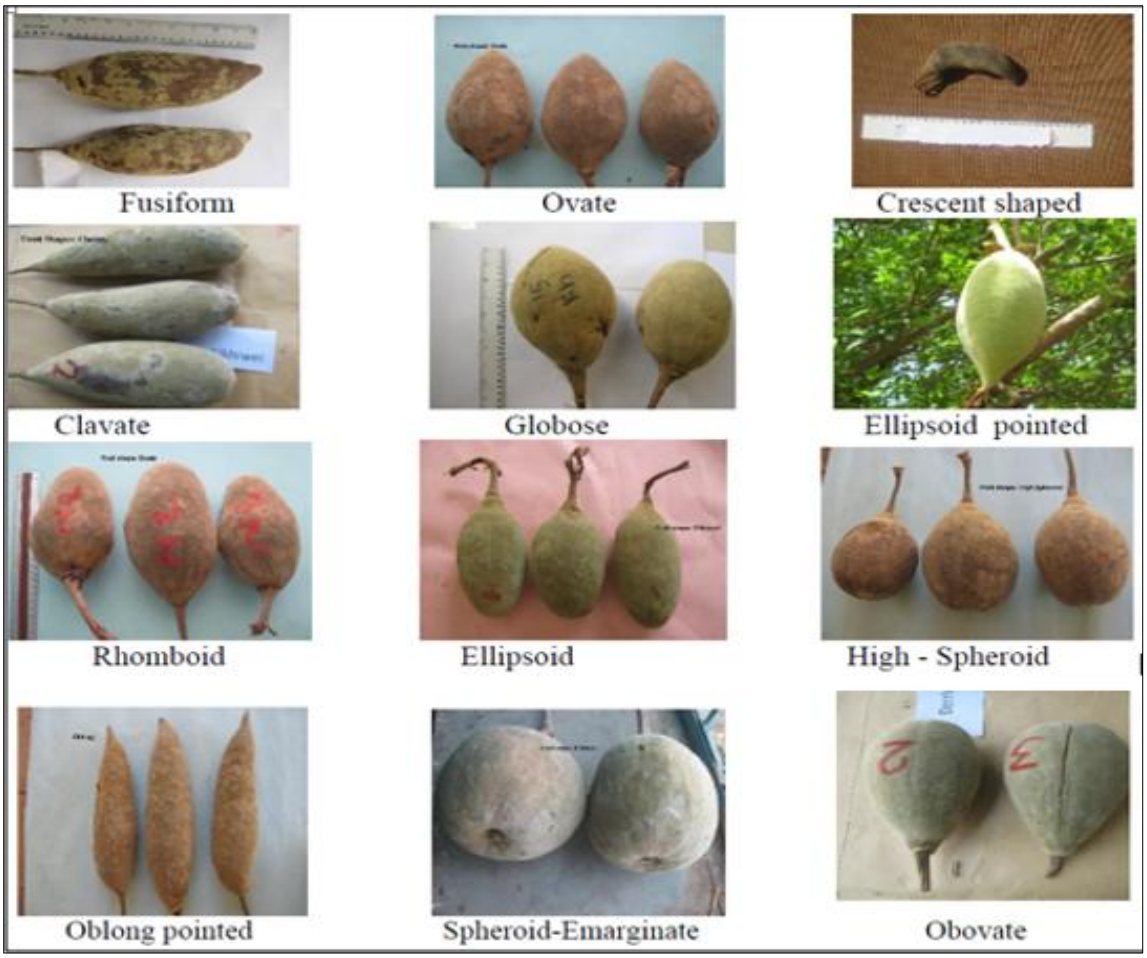


Figure 1.1: Twelve different baobab shapes observed in baobab fruits (*Adansonia digitata*) from Blue Nile and North Kordofah, Sudan. Source: Gurashi and Kordafani (2014).

Although many informative studies have been carried out specifically on baobabs, baobabs fruit and seed production and fruits set characteristics, only little is known about the producers and poor-producer baobab trees. Studies focusing on producer and poor-producer baobab trees will provide valuable information that can be used in baobab population conservation. The information can also be used in baobab economic decisions.

Chapter 2.0

2.1 Introduction

Africa is a developing continent where most people live in poverty. Rural Africans depend on natural resources and generate household income from marketing natural resources derivatives; most of which are plant materials (Anthony, 2007). In southern Africa, the baobab (*Adansonia digitata* L.) is among the most important trees that contribute significantly to the livelihoods of local people (Venter and Witkowski, 2013; Gurashi and Kordofani, 2014). *Adansonia digitata* is a tetraploid species (160 chromosomes; Baum and Oginuma, 1994) belonging to the Malvaceae (Sidibe and William, 2002; Gurashi and Kordofani, 2014). *Adansonia digitata* is endemic to mainland Africa and some oceanic islands such as Comores; occurring in southern Africa, east Africa and parts of western Africa (Wickens, 1983; Baum, 1995). The other seven species of genus *Adansonia* are diploid (88 chromosomes) and are found in different parts of the world. Six species are endemic to Madagascar and two are endemic to Australia.

Northern Limpopo is the southern-most edge of the baobab distribution in South Africa (DAFF, 2012). All parts of the baobab tree are useful and considered important for subsistence and commercial reasons. Most important, are the fruits that are used for consumption and the seeds used to make oil that is sold in cosmetic shops in both local and international markets (Venter and Witkowski, 2011). Baobabs are also reported to provide traditional medicines for humans and livestock (DAFF, 2012) and play an important role in promoting biodiversity through the conservation of water and soil, therefore enhancing crop yield (Gurashi and Kordofani, 2014). The domestication and cultivation of the baobab tree is therefore important to protect its natural survival and provide income to local populations (Sanchez, 2011). Venter and Witkowski (2010) highlighted the importance of baobabs by the local people of the Venda region and the implications of fruit harvesting on the trees. They found that the harvesting did not have a significant impact on the trees, but was a positive experience for the locals. This highlights the strong relationship between baobabs and the people of Africa.

The reproductive life cycle of any flowering plant is largely dependent on pollination. Baobabs are long-lived angiosperm trees and therefore depend on pollination for reproduction (Kehlenbeck *et al.,* 2015). African baobabs are naturally-pollinated by fruit bats (Megachiroptera), nocturnal moths and on very rare occasions by bush babies (Baum, 1995; Sidibe and Williams, 2002 and Kehlenbeck *et al.,* 2015). Natural pollination is the transfer of pollen grains from a male flower to the stigma on a female flower, where the pollen tube travels down to the ovules to fertilize the flower (Kehlenbeck *et al.,* 2015). In baobabs, the dark brown to black seeds are covered by the white fruit pulp and contained within hard and woody shelled fruits that mature 6 months from pollination (Venter and Witkowski, 2011; Chauto-Mellizo *et al.,* 2012 and Kehlenbeck *et al.,* 2015). Despite the importance of natural pollination, pollination systems are reported to be under human-induced threat such as habitat fragmentation and changes in land use (Kearns *et al.,* 1998). Due to the economic and aesthetic value of pollination, it is important to study and identify possible conservation measures via pollination to ensure plants survival and reproduction. One of the pollination conservation efforts is artificial pollination (pollination by hand) which allows plants to reproduce even in absence of pollinator animals. It is therefore important to recognize the keystone role played by animal pollinators (Kehlenbeck *et al.,* 2015).

Insufficient pollination may result in different offspring characteristics and differences in offspring qualities such as germination rate, seed and fruit set, and biomass (Chauto-Mellizo *et al.,* 2012). Insufficient pollination may also result in the reduction of the amount of pollen grains that get deposited on the stigma which further affects the quantity and quality of the seeds and fruits produced (Kehlenbeck *et al.,* 2015). For instance, in baobabs, some adult trees produce many fruits per year (50−299 fruits) and are subsequently called "producers" or "female" trees, while other trees produce very few fruits (< 5 fruits), are called "poor-producers" or "male" trees (Venter and Witkowski, 2011). The difference in the number of fruits produced per baobab tree per year shows that fruit production is not consistent between baobab trees, which may be a result of insufficient pollination. In some flowering plants, offsets of pollination benefits have been recorded, in which environmental variables affect the ability of the plants to develop seeds (Chauto-Mellizo *et al.,* 2012). In most cases there is a trade-off between the number of seeds produced and the size of the seeds. This trade-off implies that species tend to produce either many small seeds or few large seeds (Giorgis *et al.,* 2013).

The study of pollination alone may not answer all scientific questions regarding fruit production because even in successful events of pollination, fruit production may still differ between individuals within a species. Fruit production affects seed viability, the ability of the seeds to geminate. The most accurate method of determining seed viability is by germinating the seeds. However, in some species including baobabs, seed germination can take a long time to complete (from days to months), hence a tetrazolium test may be used as an alternative to estimate seed viability (Gimenez *et al.*, 2014). As noted in chapter 1, viability using tetrazolium solution is estimated by observing a change in the seed embryo colour from white to pink as hydrogen ions (H+) that are released during respiration reduce the 2,3,5-triphenyltetrazolium chloride (Gimenez *et al.*, 2014), indicating that the seeds are alive (viable). The pink colour of the embryo thus indicates that seeds are viable. It is therefore important that the seed embryo is well exposed to the tetrazolium solution to yield good results.

For this study, I compared fruit and seed characteristics between naturally and artificially-pollinated producer and poor-producer baobab fruits and baobabs in different land use types to understand if poor-producer trees can produce viable seeds of the same size and quality as producer trees. The study further determined if artificial pollination can promote baobab fruit and seed production. Comparison of fruit and seed production of trees in areas of different land usage may aid with the identification of land use types where baobabs have the lowest seed production and therefore require additional conservation effort to ensure that fruits are harvested sustainably. In addition, overall estimation of seed production may improve the ability to estimate the total seed oil that is available in each land use type.

2.2 Aims, objectives and research questions

The aim of this study was to compare fruit and seed characteristics between (a) naturally and artificially-pollinated producers and poor-producers baobab trees and (b) between baobabs trees on different land use types (communal land, nature reserve, village, field, plains and rocky outcrops) in Northern Venda, South Africa.

The following objectives need to be meet to achieve the aim outline above:

Objective 1

To compare fruit and seed characteristics (a) between artificially-pollinated (i.e. pollination by hand across different trees) and naturally-pollinated producers and poor-producers baobab trees and (b) between baobab trees on different land use types.

Research question for objective 1

Do seed and fruit characteristics differ between naturally-pollinated and artificially-pollinated producers and poor-producers baobab trees and between baobab trees on different land use types? If so, how do the characteristics differ?

Objective 2

To determine the relationship between fruit size and the number of seeds per fruit

Research question for objective 2

Is there a relationship between fruit size and the number of seeds per fruit? If so, what is the relationship?

Objective 3

To determine and compare seed viability between naturally and artificially-pollinated producer and poor-producer baobab fruits.

Research question for objective 3

Do the percentage and total seed viability differ between naturally and artificially-pollinated baobab fruits?

Objective 4

Use previously obtained estimates of baobab fruit production from the Venda region together with the results from the above objectives to estimate baobab seed production in different land use types.

Research question for objective 4

How does seed production differ between (a) naturally-pollinated and artificially-pollinated producer and poor-producer baobab trees and (b) between baobab trees on different land use types?

2.3 Materials and methods

Study area

Baobab fruits were collected from trees in northern Venda, a remote rural area in Limpopo Province in South Africa (Figure 2.1). Northern Venda is located south of Zimbabwe, west of Botswana and east of the Kruger National Park (25° 50' S and 30° 45' E; Venter and Witkowski, 2013) and is approximately 400 m above sea level (Venter and Witkowski, 2010). This is semi-arid area that receives between 334 and 425 mm of rainfall annually, which makes the area prone to wild fires and low rainfall leads to low yields from subsistence agriculture and animal husbandry, which are the two most common land use activities within the area (Venter and Witkowski, 2010). The most common livestock in this area are cattle, goats and donkeys that browse on the savannah grass. In addition, baboons are spotted around the area but very few other wildlife species such as elephants occur (Venter and Witkowski, 2010).

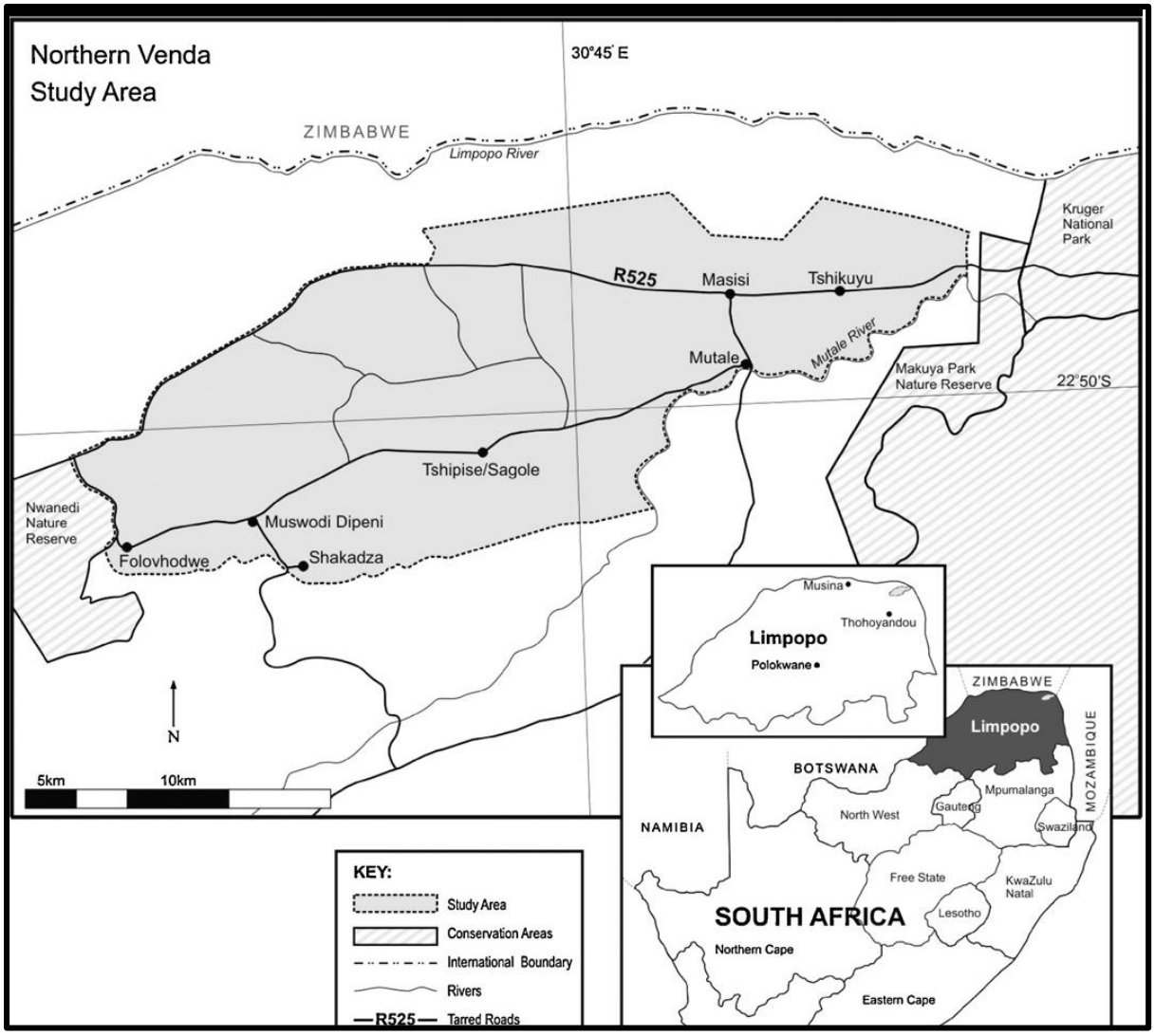


Figure 2.1: Location of study site where fruits were collected, Northern Venda in Limpopo Province, South Africa. Source: Venter and Witkowski (2010).

Study species

Baobab (*Adansonia digitata*) belongs to the Malvaceae, subfamily Bombacoideae and it is widely distributed in west, east and southern Africa (Venter, 2012). In South Africa, *A. digitata'*s distribution is confined to the Limpopo River valley (Wickens and Lowe, 2008). The baobab tree grows best in well-drained soils in dry and hot woodlands in association with *Colophospermum*, *Cordyla*, and *Kigelia* (Venter and Witkowski, 2010).

Baobabs are long-lived deciduous trees that store water in their thick trunks (Venter, 2012). African baobabs are very large and can grow to 18−25 m in height (Sidibe and William 2002). All plant parts of *A. digitata* are important and used widely as sources of food, fibre and medicine. According to Venter and Witkowski (2010), baobab fruits take six months to mature and are harvested in the dry seasons for subsistence purposes and sold to local and international markets to generate household income. Baobab seeds are used as oil in cosmetics and currently the demand for seeds increased in Africa, Europe, and Canada among others. Humans and animals, particularly baboons and elephants are responsible for baobab seeds dispersal.



Figure 2.2 Baobab (*Adansonia digitata*) tree and fruits. Source: Wiehle *et al*. (2014).

Data collection and analyses

A total of 76 (18 poor-producer and 58 producer') baobab fruits from natural pollination events were collected from poor producers and producer baobab trees from villages, fields, rocky outcrops and plains habitats within Northern Venda in South Africa in May 2014. The paternal sources of these fruits are unknown. In the second treatment, a total of 12 baobab fruits (five poor-producer and seven producer) were collected from a controlled cross-pollination experiment, where flowers were artificially-pollinated, and the pollen donors are known (Venter *et al*. in review). Artificially-pollinated fruits were compared with the naturally-pollinated fruits to address these questions associated with Objective 1 and 3.

Baobab fruit comparisons

Using a measuring tape, fruit length and girth were measured in centimeters (cm). Fruit length was measured against the fruit from the top to the bottom of the fruit, whereas fruit girth was the circumference measured at the widest part of the fruit, following the method of (Venter and Witkowski, 2010). Fruit girth was converted to fruit diameter and fruit shape ratio was calculated using a formula used by Wiehle *et al.* (2014), where the fruit-shape ratio is equal to fruit length divided by fruit diameter. Fruit volume was also calculated from these dimensions using the formula: volume = $4/3 * \Pi * r^3$, with three radius dimensions being fruit length divided by two, diameter divided by two for width and for breadth, as width and breadth were generally equal. In addition, a BEL engineering digital balance (0.01 g accuracy) was used to weigh each fruit (Figure 2.1). Using the best fit regression analyses (i.e., the regression model with the highest R^2 value between linear, logarithmic, exponential, polynomial and power), fruit mass and fruit dimensions were compared between producer and poor-producer naturally and artificially-pollinated baobab fruits. Differences in fruit mass and fruit dimensions were tested for significance between naturally and artificially-pollinated baobab fruits using a t-test and Friedman's analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) and Microsoft Excel Data Analysis.



Figure 2.3: Illustration of the BEL engineering digital balance and baobab fruits and seeds. Photographs: E. Nangolo.

Baobab seed viability

Germination trials were undertaken in the insectary at the University of the Witwatersrand, Johannesburg, for a total of 240 baobab seeds. Eight seeds were randomly chosen from each of the ten randomly selected naturally-pollinated 'male' baobab fruits, ten naturally-pollinated 'female' baobab fruits and ten artificially-pollinated baobab fruits. Before germination, the seeds were soaked in boiling water for 12 hours as a pretreatment to break dormancy. The seeds were then placed on moist filter paper in transparent laboratory petri dishes (eight seeds per petri dish). All petri dishes were kept under the same conditions,

temperature (20°C) and light (12 hours). Seed germination was monitored daily and the number of days it took for the root tip to emerge was recorded. However, many of the seeds did not germinate successfully. Only 17 out of the 240 seeds (7%) germinated over a period of 3 months. Instead seed viability was tested using 0.1% tetrazolium solution (Gimenez *et al.,* 2014; Grzybowski *et al.,* 2012). A pair of secateurs was used to cut the seeds open lengthwise to expose the embryo (Figure 2.3).

The opened seeds were placed cut-surface down in a 0.1% tetrazolium solution in a dark room for 14 hours. Seed viability was assessed by observing the change in colour of the seed embryo. Seeds with white embryos after 14 hours of being immersed into a 0.1% tetrazolium solution were considered nonviable. The number of viable and nonviable seeds was compared between naturally-pollinated and artificially-pollinated 'male' and 'female' baobab fruits.

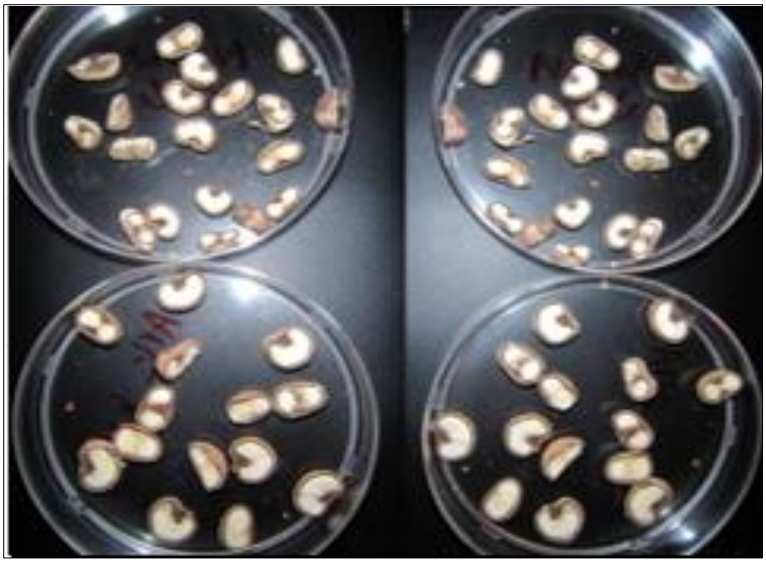


Figure 2.4: Baobab seeds cut open lengthwise before being placed in a 0.1% tetrazolium solution. Photograph: E. Nangolo

Fruit shape Analysis

All baobab fruits used for this study were photographed using a Canon ELS 5D Mark II camera. A total of 80 baobab fruits were photographed (19 poor-producer and 61 producer) to analyse fruit shape. To investigate differences in fruit shape, I used landmark-based morphometrics (Foster *et al.,* 2015; Webster and Sheets, 2010). This method can help distinguish morphological differences between groups using shape (Webster and Sheets, 2010). Using GIMP (General Image Manipulation Program), I placed twelve landmarks (LM) on each baobab fruit photograph according to the methodology outlined by Glennon and Cron (2015). The first two landmarks were placed on the base and the apex of the fruit, and the fruit apex was considered the midpoint between the fruit and the fruit stalk. The remaining ten other landmarks were placed between the first and the second landmarks, maintaining intervals of 10 % between them. The landmarks were placed opposite to each other and this was done consistently for all images. LM 1 and 2 represented type I landmarks for explaining variation in the fruit shape. Landmarks X and Y were type II, which represents the widest and narrowest parts on the fruit, and type III landmarks are the remainder which reduce the space between landmarks for geometric significance (Bookstein, 1991).

I exported the landmarked photographs from GIMP to ImageJ in order to extract the landmark coordinates from the inserted landmarks (Girish and Vijayalakshmi, 2004). Using R, I used the Morpho v.2.0.3-package (Schlager, 2014) to conduct a general Procrustes analysis on the landmark data. Then a relative warps analysis was conducted to reduce dimensionality of partial warp scores (Rohlf *et al.,* 1996). A deformation grid was used to visualize the shape changes that were explained by the relative warps analysis. Lastly, a canonical variate analysis was used to test if the variation showed significant difference between fruits from producer and poor-producer baobab trees.



Figure 2.5: Illustration of the 12 landmarks placed on each baobab photograph. Photo: E. Nangolo

Baobab seed comparisons and seed production estimate

The seeds were extracted from the baobab fruit pulp by washing the pulp off with water. The washed seeds were left to dry and the number of seeds per fruit was counted. From each fruit, ten randomly chosen seeds were weighed individually using a digital weighing scale (0.01 g accuracy). Regression analyses were used to compare the number of seeds per fruit with fruit dimensions (fruit length, and fruit volume) and fruit mass between producer and poor-producer naturally and artificially-pollinated baobab fruits.

I used baobab fruit production data from Venter and Witkowski (2011), Venter (2012), and Venter (unpublished data) to estimate baobab seed production. Venter monitored fruit production of a total of 107 baobab trees for a period of 6 years (2007−2012). After the submission of her PhD thesis, she continued monitoring 40 of her 107 trees for three more years. For this study, I used the fruit production data for the 40 trees over a period of 9 years (2007−2015) to calculate a good prolonged seed production estimate. Venter (2012) categorized fruits into small (± 10 cm × 5 cm), medium (± 15 cm × 8 cm) and large (± 20 cm

× 10 cm) baobab fruits based on fruit size. Venter (2012) based fruit size on fruit length and fruit diameter. For this study, I used the same measurements for fruit classes and I used the following equation to estimate the total number of seeds per tree.

Total seeds/tree = (No. of fruits $_{small}$ x seeds/fruit $_{small}$) + (No. of fruits $_{medium}$ x seeds/fruit $_{medium}$) + (No. of fruits $_{large}$ x seeds/fruit $_{large}$)

Where,

No. of fruits $_{small}$ is the number of small fruits per tree and the number of seed $_{small}$ is the number of seeds per fruit length of the small fruits.

No. of fruits $_{medium}$ is the number of medium fruits per tree and seed $_{medium}$ is the number of seeds per fruit length of the medium fruits.

No. of fruits $_{large}$ is the number of large fruits per tree and seed $_{large}$ is the number of seeds per fruit length of the large fruits.

The seed production estimate was based on the naturally-pollinated fruits. Artificially pollinated fruits were excluded to ensure that the number of seeds per tree is accurately estimated without human manipulation.

A t test was used to test for significant difference in the number of seeds per baobab fruit length between producer and poor-producer trees. This was done to determine whether the same formula can be used to estimate seed production for both producer and poor-producer baobab trees. A significant difference was found between the number of seeds per fruit in producer and poor-producer trees thus, seed estimates were done separately for the producer and poor-producer baobab trees.

Four regression types (linear, logarithmic, polynomial and power) were used to determine the best-fit line that gives the best estimate for the number of seeds per baobab fruit. In fruits from both producer and poor-producer trees, Power curve fit had the R^2 = 53% and R^2 = 31% respectively. This regression line appeared to include the data points equally around the mean and therefore can more accurately be used to extrapolate the number of seeds in relation to baobab fruit length. The results were presented graphically (Figure 3.19)

Chapter 3.0

3.1 Results

Comparison of fruit ratio (fruit length/fruit width) between artificially-pollinated and naturally-pollinated producers and poor-producer baobab fruits

No significant difference was found in mean fruit ratio (fruit length/fruit width) between naturally-pollinated poor-producer (n = 18) and naturally-pollinated producer (n = 58) baobab fruits ($t = -0.069$, $df = 74$, $P = 0.945$). However, there was a significant difference in mean fruit ratio between artificially-pollinated poor-producer (n = 5) and artificially-pollinated producer (n = 7) baobab fruits ($t = -2.85$, $df = 10$, $P = 0.017$); artificially-pollinated poor-producer fruits have a higher mean fruit ratio than artificially-pollinated producer baobab fruits (Figure 3.1).

No significant difference was found in mean fruit ratio (fruit length/fruit width) between naturally-pollinated poor-producer (n = 18) and artificially-pollinated poor-producer (n = 5) baobab fruits ($t = -0.050$, $df = 19$, $P = 0.961$; Figure 3.1). In contrast, mean fruit ratio differed significantly between artificially-pollinated producer (n = 7) and naturally-pollinated producer (n = 58) fruits (t value = 3.679 df = 8, P = 0.010,); naturally-pollinated producer fruits have a higher mean fruit ratio than artificially-pollinated producer fruits (Figure 3.1) The small standard error bars on Figure 3.1 indicate that there is only slight variation in the observed fruit ratio values of fruits between treatments.

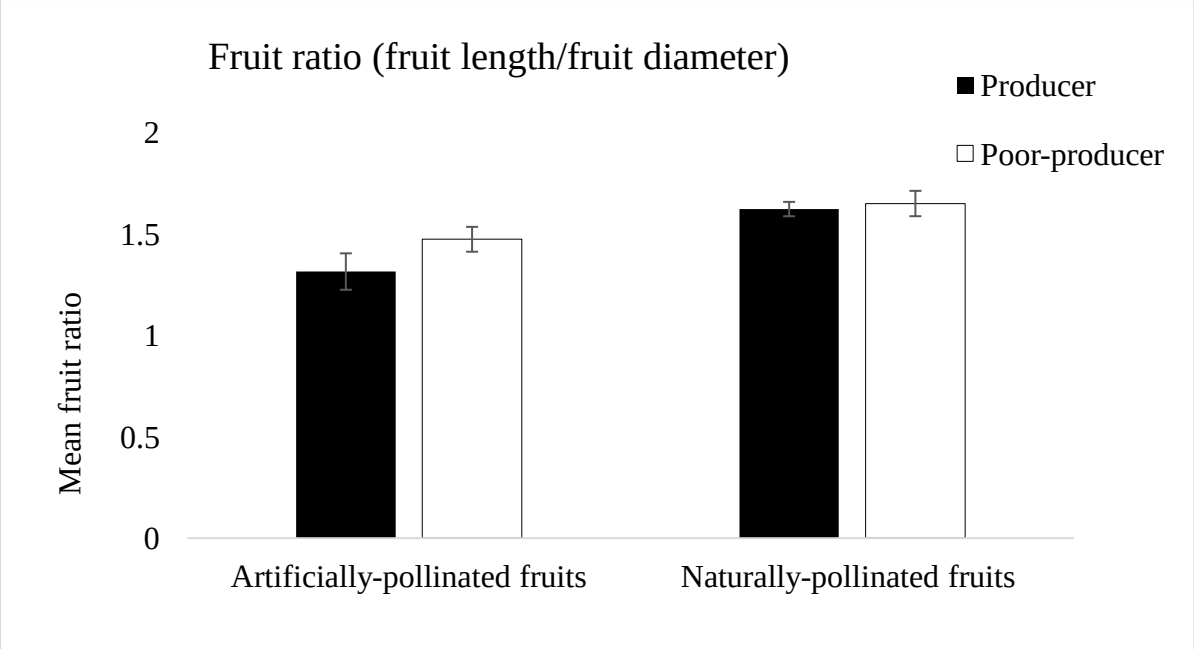


Figure 3.1: Comparison of mean fruit ratio (fruit length/fruit width) between fruits formed on artificially-pollinated producer (mean = 1.32, SE = 0.09) and poor-producer (mean = 1.47, SE = 0.06) and naturally-pollinated producer (mean = 1.62, SE = 0.35) and poor-producer (mean = 1.65, SE = 0.06) baobab trees.

The lines in the box represent the median value, the whisker below and above the box represent the values below the median (minimum values) and above the median (maximum values) respectively. The boxplot (Figure 3.2) shows that fruits from artificially-pollinated producer (A_F) and natural-pollinated poor- producer (N_M) baobab trees have a negatively skewed fruit ratio because more values are concentrated on the lower scale than on the upper scale. Naturally-pollinated producer (N_F) baobab trees showed a symmetrical fruit ratio which implies that the values are equally spread from the median to the lower and upper scale. It was observed that three individual fruits formed on both naturally-pollinated trees showed a fruit ratio that was out of the range; these fruits are outliers as they appear above and below the box.

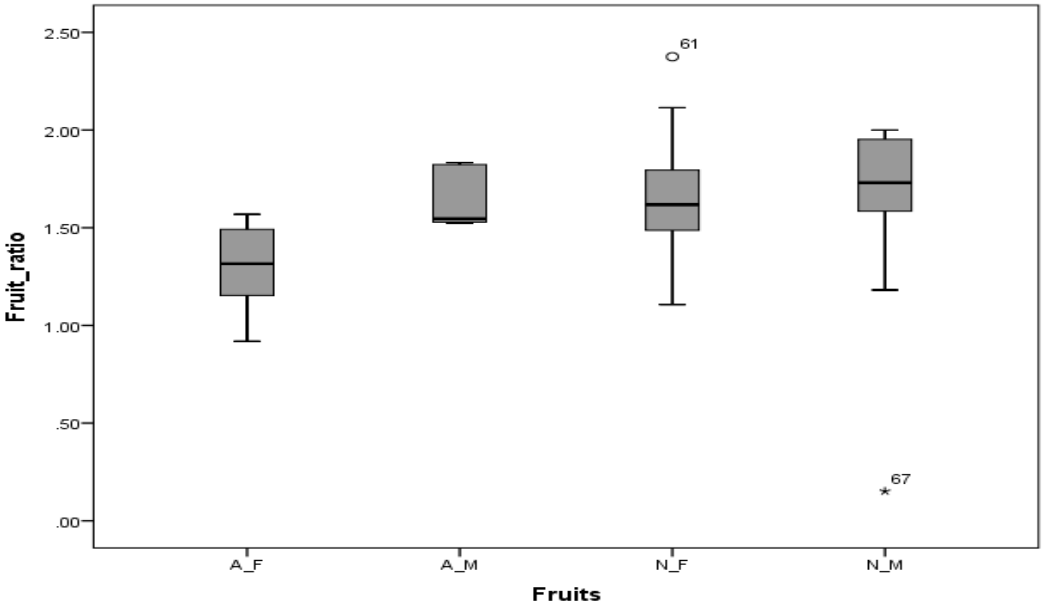


Figure 3.2: Boxplot for artificially and naturally-pollinated baobab fruit ratio. Where A_F: artificially-pollinated producer, A_M: artificially-pollinated poor-producer, N_F: naturally-pollinated producer and N_M: naturally-pollinated poor-producer fruits.

Comparison of fruit mass between artificially-pollinated and naturally-pollinated poor-producer and producer baobab fruits

No statistical difference was found in average fruit mass between fruits formed on artificially-pollinated poor-producer (n = 5) and producer (n = 7) baobab trees (t = − 0.99 df = 10, p = 0.34, F = 0.99 and t value = − 0.99), but there was a significant difference in average fruit mass between fruits from naturally-pollinated poor-producer (n =18) and producer (n = 58) baobab trees (t value = 2.25, df = 74, P = 0.03, F = 5.05). On average, fruits formed from producer trees are heavier than the fruits formed by poor-producer baobab trees (Figure 3.3).

There was a significance difference in average fruit mass between fruits formed on naturally-pollinated poor-producer (n = 17) and artificially-pollinated poor-producer (n = 5) baobab fruits (t value = − 5.84, df = 9, P = 0.00025); fruits from artificially-pollinated poor-producer trees have a higher average fruit mass than fruits from naturally-pollinated poor-producer trees (Figure 3.3). In contrast, no significant difference was found in average fruit mass between fruits formed on artificially-pollinated producer (n = 7) and naturally-pollinated producer (n = 58) baobab trees (t value = − 0.88, df = 6, P = 0.41). The standard error in Figure 3.3 indicates that the observed fruit mass values varied most in the fruits formed on

artificially-pollinated producer trees (SE = 61.98 g), whereas artificially-pollinated poor-producer trees showed the smallest variation in fruit mass (SE = 20.93 g).

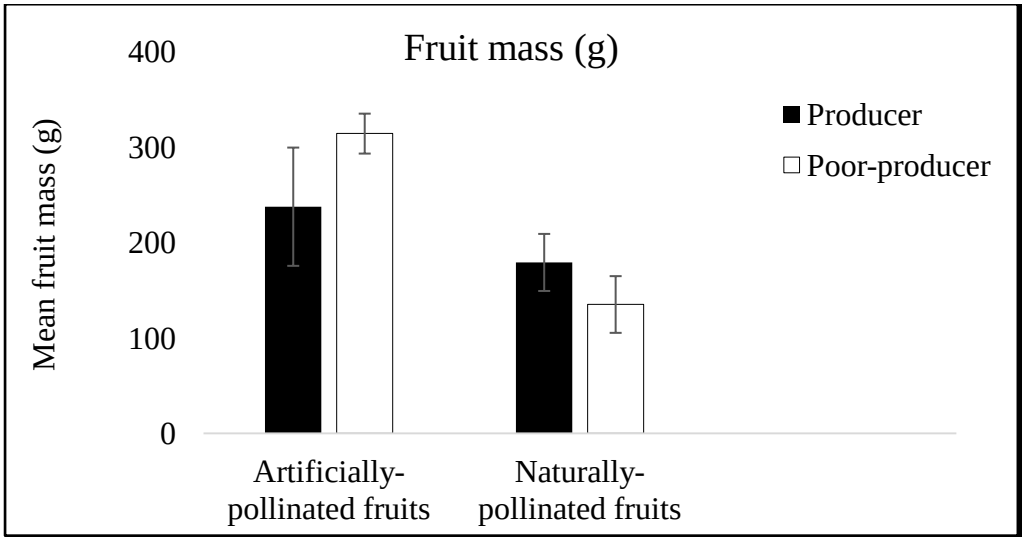


Figure 3.3: Comparison of mean fruit mass (g) between fruits formed from artificially-pollinated producer (mean = 237.66 g, SE = 61.98 g) and poor-producer (mean = 314.25 g, SE = 20.93 g) and naturally-pollinated producer (mean = 182.24 g, SE = 10.15 g) and poor-producer (mean = 132.88 g, SE = 17.98 g) baobab trees.

The line in the box represent the median value, the whiskers below the box represent the values below the median (minimum values) whereas the whisker above the box represent the values above the median (maximum values). The boxplot (Figure 3.4) shows that fruits from artificially-pollinated producer (A_F) trees showed symmetrical fruit mass implying that all observed values are equally spread from the median to the lower and upper scale. On the other hand, poor-producer (A_M) trees showed a positively skewed fruit mass; many values are concentrated on the upper scale than on the lower scale. Observed fruit mass in fruits from naturally-pollinated poor-producer (N_M) trees were negatively skewed because most values were in the lower scale. Naturally-pollinated producer (N_F) baobab trees have a positively skewed fruit ratio because more values are concentrated on the upper scale than on the lower scale. It was observed that three individual fruits formed on naturally-pollinated poor-producer trees showed a fruit mass that was out of the range, these fruits are outliers as they appear above the box.

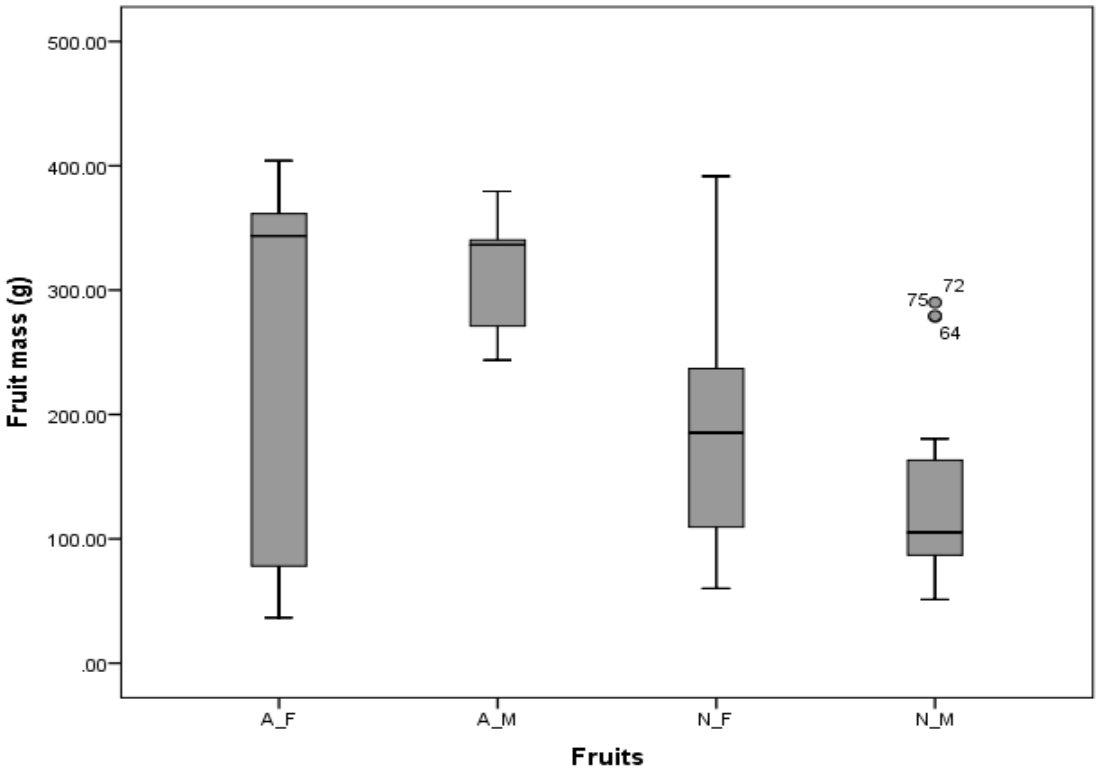


Figure 3.4: Boxplot for artificially and naturally-pollinated baobab fruits mass (g). Where A_F: artificially-pollinated producer, A_M: artificially-pollinated poor-producer, N_F: naturally-pollinated producer and N_M: naturally-pollinated poor-producer fruits.

Relationship between fruit mass and the number of seeds per fruit from artificially and naturally-pollinated baobab trees.

The positive linear relationship between the number of seeds per fruit and the mass of the fruit suggest that heavy fruits contain more seeds than light-weight fruits (Figure 3.5). Fruits from artificially-pollinated producer trees have the strongest positive correlation ($R^2 = 0.99$) and fruits from naturally-pollinated producer trees have the weakest positive correlation ($R^2 = 0.34$) between the number of seeds per fruit and the size of the fruit (Figure 3.5).

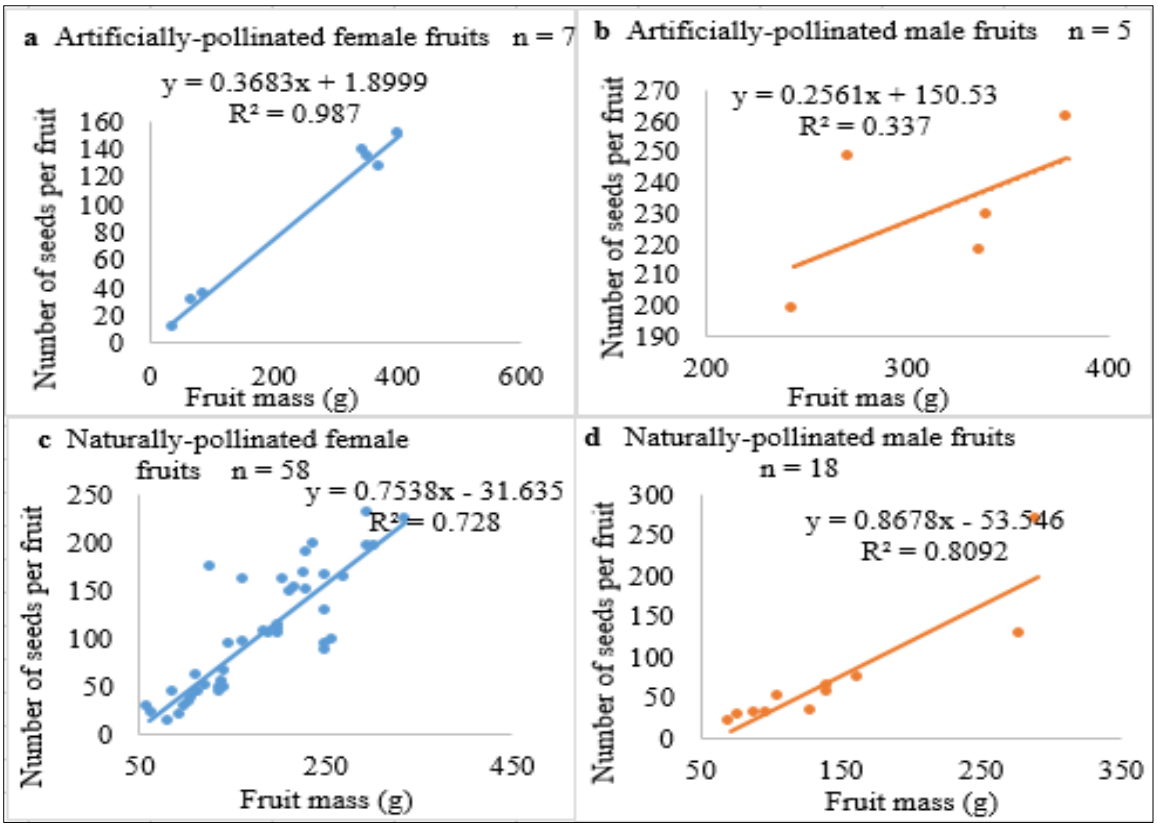


Figure 3.5: Comparison of the relationship between baobab fruit mass and the number of seeds per fruit between fruits from (a) artificially-pollinated producer and (b) poor-producer trees and between (c) naturally-pollinated producer and (d) poor-producer baobab trees.

When all fruits from naturally-pollinated poor-producer and producer baobab trees and all fruits from artificially-pollinated poor-producer and producer baobab trees data are pooled, the relationship between fruit mass and the number of seeds per fruit remains positive (Figure

3.6).

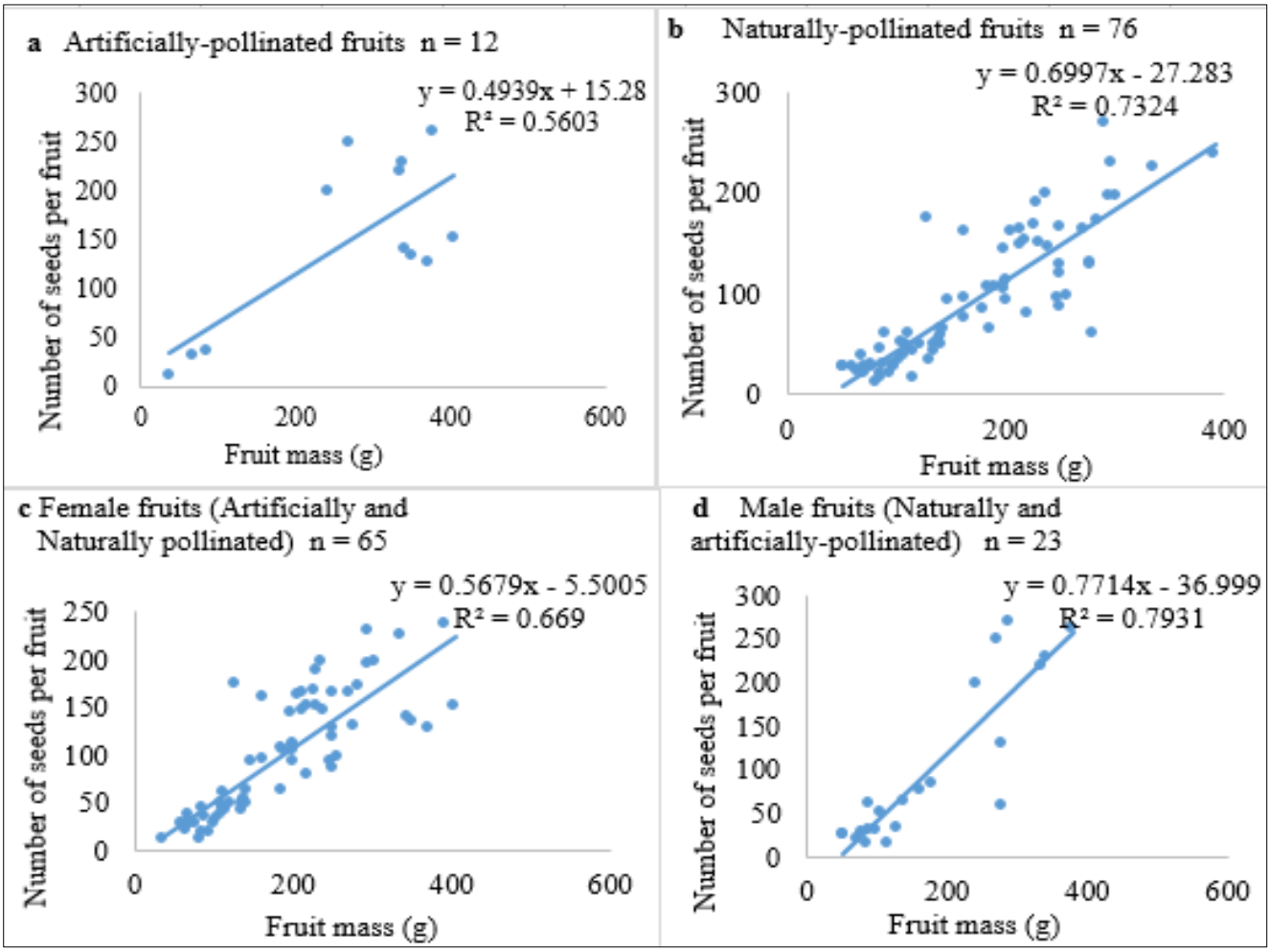


Figure 3.6: The relationship between baobab fruit mass and the number of seeds per fruit for pooled data of fruits from (a) artificially-pollinated and (b) naturally-pollinated producer and producer baobab trees, and from (c) 'female'/producer trees and (d) 'male'/poor-producer trees.

<u>Relationship between baobab fruit volume and the number of seeds per fruit</u>

There is a positive linear relationship between the number of seeds per fruit and the fruit volume; fruits with a larger volume contain more seeds than fruit with a smaller volume (Figure 3.7). Fruits from the artificially-pollinated producer baobab trees have a very strong positive correlation (R^2 = 0.76; Figure 3.7b) as compared to fruits from artificially-pollinated poor-producer fruits, which have the weakest positive correlation (R^2 = 0.28; Figure 3.7a).

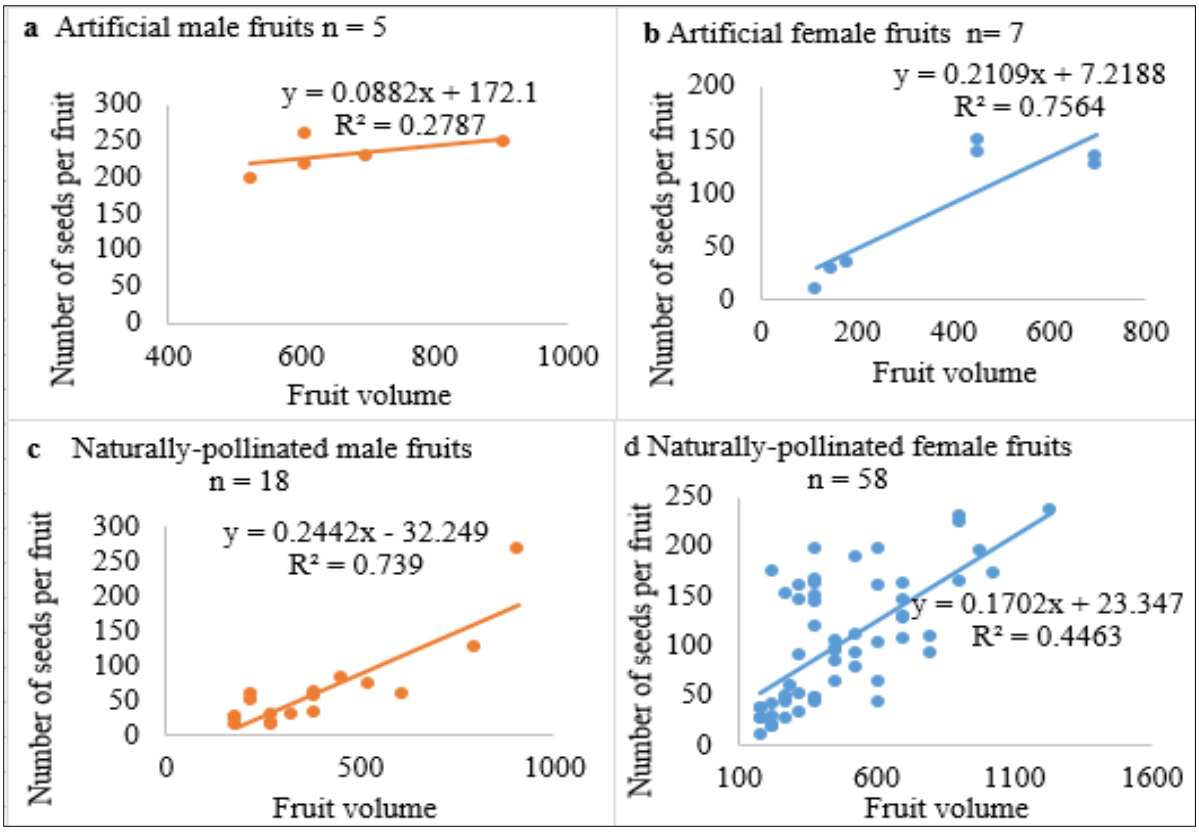


Figure 3.7: Comparison of the relationship between fruit volume and the number of seeds per fruit between fruits from artificially-pollinated (a) poor-producer and (b) producer baobab trees and between naturally-pollinated (c) poor-producer and (d) producer baobab trees.

The relationship between fruit volume and the number of seeds per fruit remains positive when all naturally-pollinated baobab 'male' and 'female' fruits and all artificially pollinated baobab 'male' and 'female' fruits data are pooled (Figure 3.8).

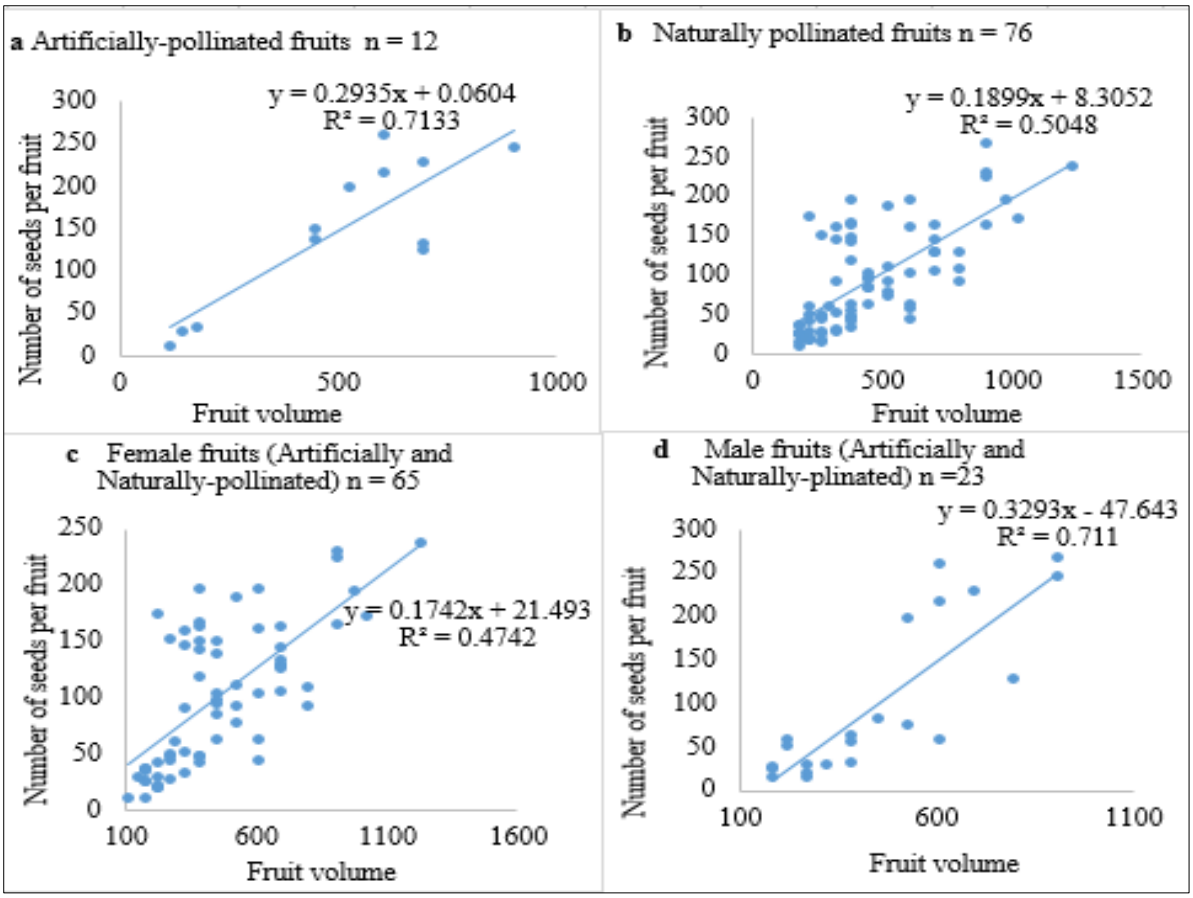


Figure 3.8: The relationship between fruit volume and the number of seeds per fruit for pooled data of fruits from (a) artificially-pollinated and (b) naturally-pollinated trees and between (c) 'female'/producer trees and (d) 'male' /poor-producer trees.

Relationship between baobab fruit length and the number of seeds per fruit

There is a positive linear relationship between baobab fruit length and the number of seeds per fruit; shorter fruits contain fewer seeds than longer fruits (Figure 3.9). However, fruits from naturally-pollinated producer and poor-producer baobab trees showed a weak relationship (Figure 3.9a, b). Fruits from artificially-pollinated producer trees have the best fit (R^2 = 0.96; Figure 3.9b), while fruits from artificially-pollinated poor-producer trees have the weakest fit (R^2 = 0.20; Figure 3.9a).

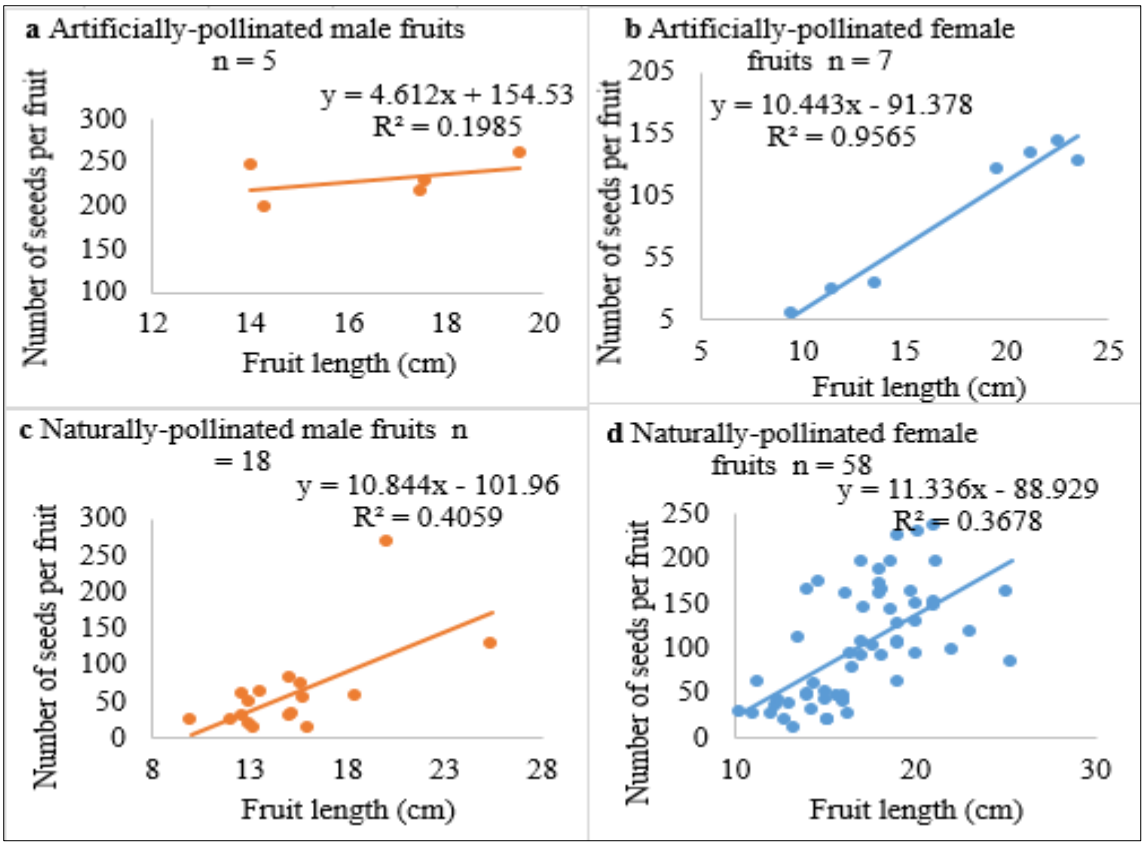


Figure 3.9: The relationship between baobab fruit length and the number of seeds per fruit for pooled data of fruits from artificially-pollinated (a) 'male'/poor-producer and (b) 'female'/producer baobab trees and between naturally-pollinated (c) poor-producer and (d) producer baobab trees.

When all fruits from naturally-pollinated producer and poor-producer baobab trees and all fruits from artificially-pollinated producer and poor-producer baobab trees data are pooled, the relationship between fruit length and the number of seeds per fruit remains weakly positive (Figure 3.10).

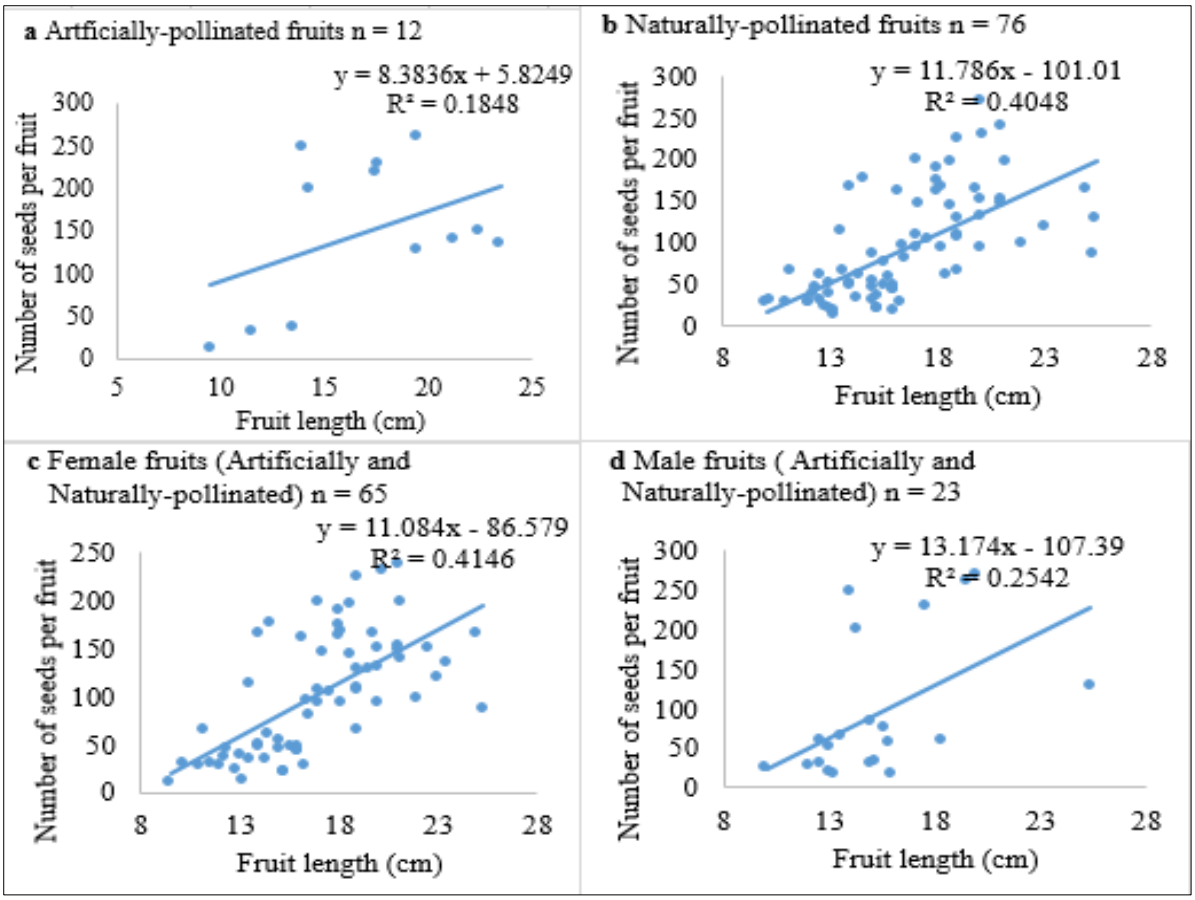


Figure 3.10: The relationship between baobab fruit length and the number of seeds per fruit for pooled (a) artificially-pollinated and (b) naturally-pollinated baobab fruits and between (c) 'female' and (d) 'male' baobab fruits.

Relationship between baobab fruit mass and fruit length

There is a strong positive linear relationship between fruit mass and fruit length in all baobab fruits; long fruits are heavier than short fruits. The artificially-pollinated 'male' baobab fruits have the strongest positive relationship (R^2 = 0.99; Figure 3.11).

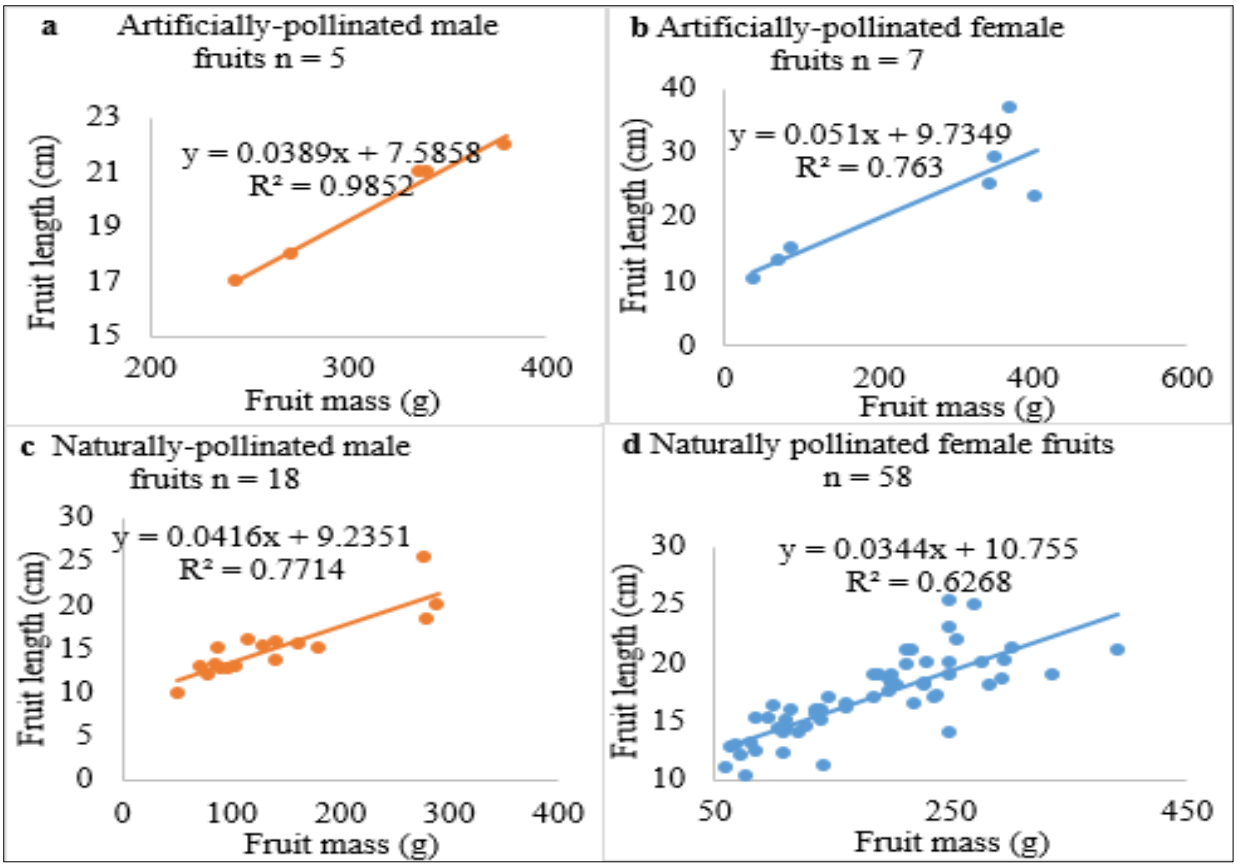


Figure 3.11: Comparison of the relationship between fruit mass and fruit length between fruits from artificially-pollinated (a) poor-producer and (b) producer baobab trees and between (c) naturally-pollinated poor-producer (d) and producer baobab trees.

The relationship between fruit mass and fruit length remains strongly positive when all naturally-pollinated baobab ‘male’ and ‘female’ fruits and all artificially pollinated baobab ‘male’ and ‘female’ fruits data are pooled (Figure 3.12).

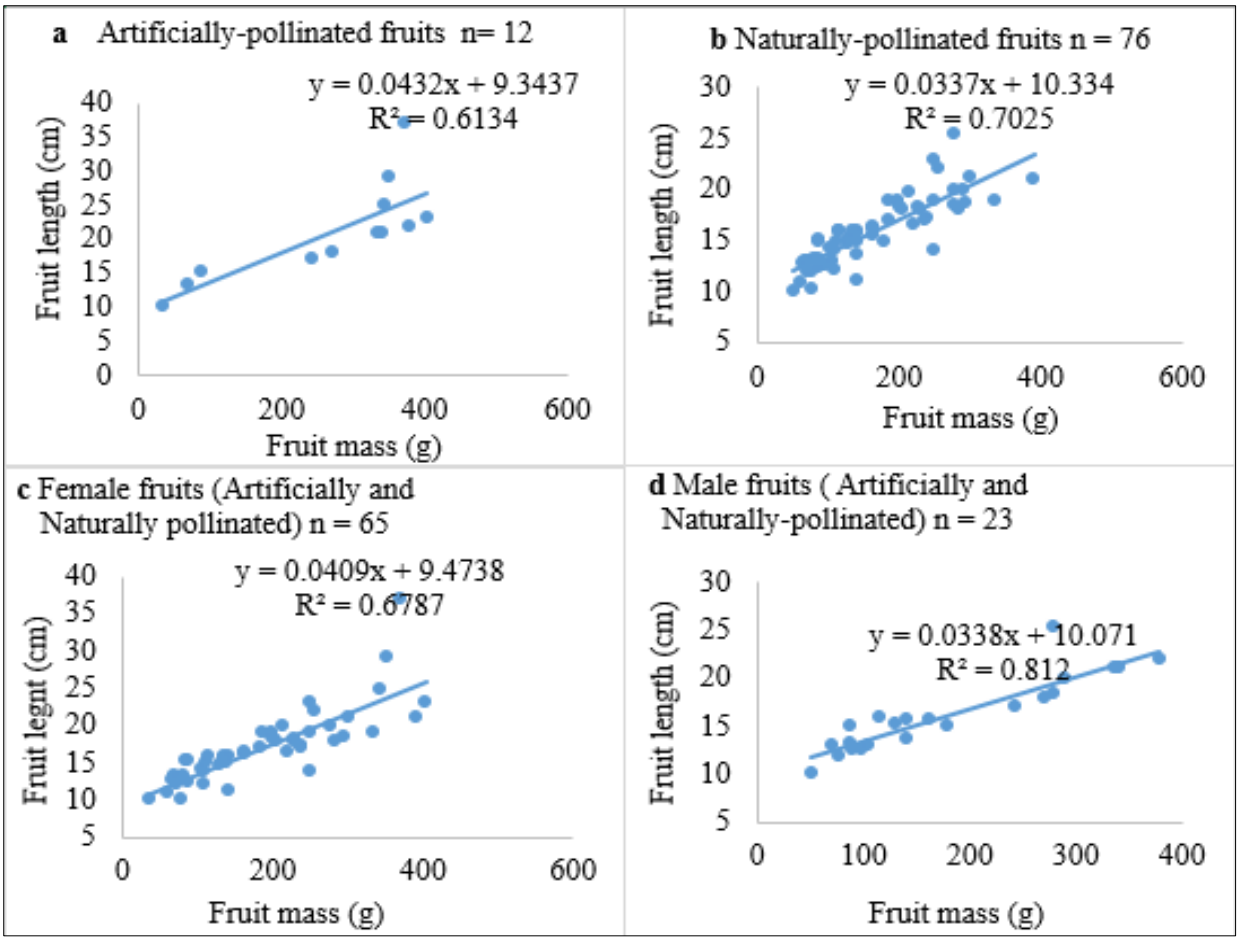


Figure 3.12: The relationship between baobab fruit mass and fruit length for pooled (a) artificially-pollinated and (b) naturally-pollinated baobab fruits and between (c) 'female' and (d) 'male' baobab fruits.

Relationship between average baobab seed mass and the number of seeds per fruit

Artificially-pollinated baobab 'female' fruits show the strongest positive relationship between the average seed mass and the number of seeds per fruit (R^2 = 0.86; Figure 3.13a). This relationship is weak in artificially-pollinated 'male' baobab fruits (R^2 = 0.02; Figure 3.3b). On the other hand, naturally pollinated 'male' and 'female' baobab fruits show a negative

relationship between the average seed mass and the number of seeds per fruit.

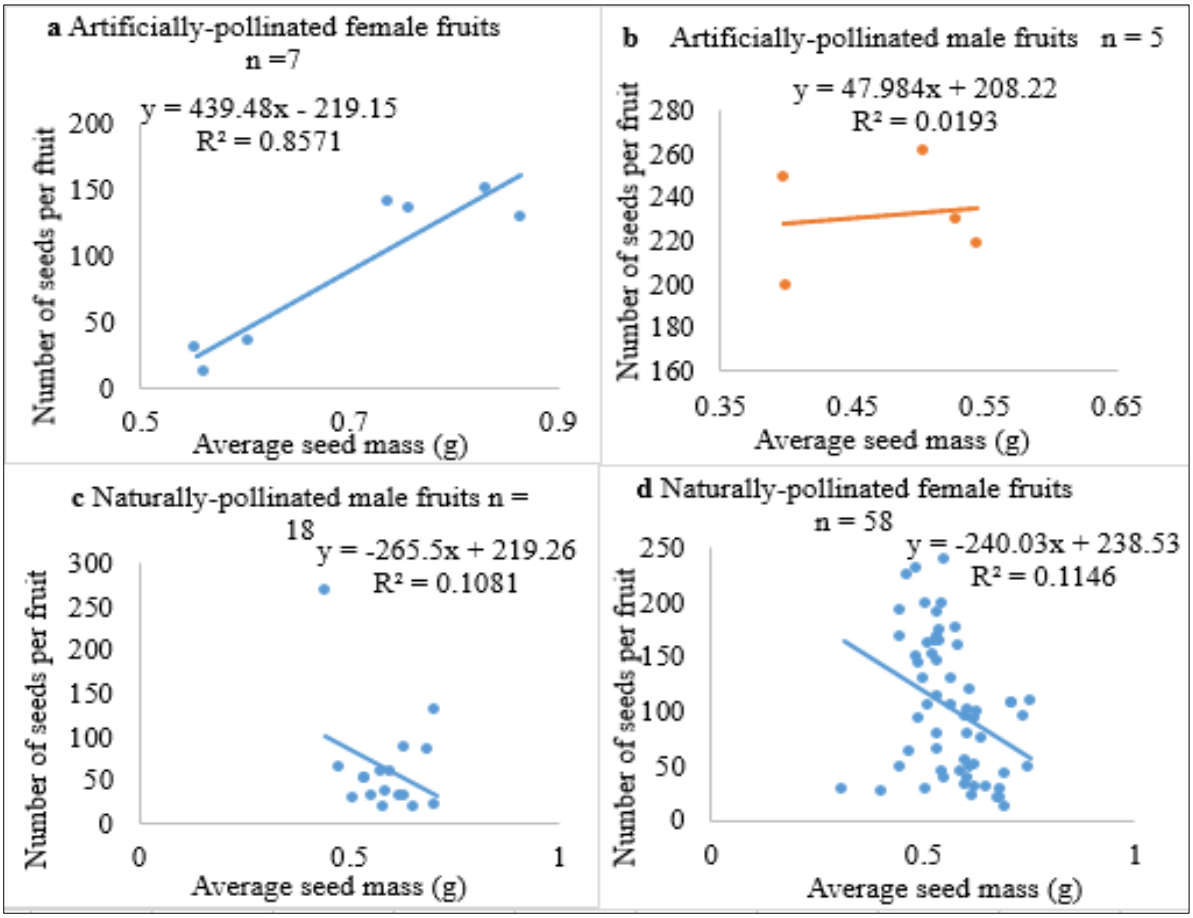


Figure 3.13: Comparison of the relationship between seed mass and the number of seeds per fruit between fruits from artificially-pollinated (a) producer and (b) poor-producer and between (c) naturally-pollinated-poor producer and (d) producer fruits.

When all naturally-pollinated baobab ‘male’ and ‘female’ fruits and all artificially pollinated baobab ‘male’ and ‘female’ fruits data are pooled, the relationship remains negative for artificially and naturally-pollinated ‘male’ baobab fruits (Figure 3.14).

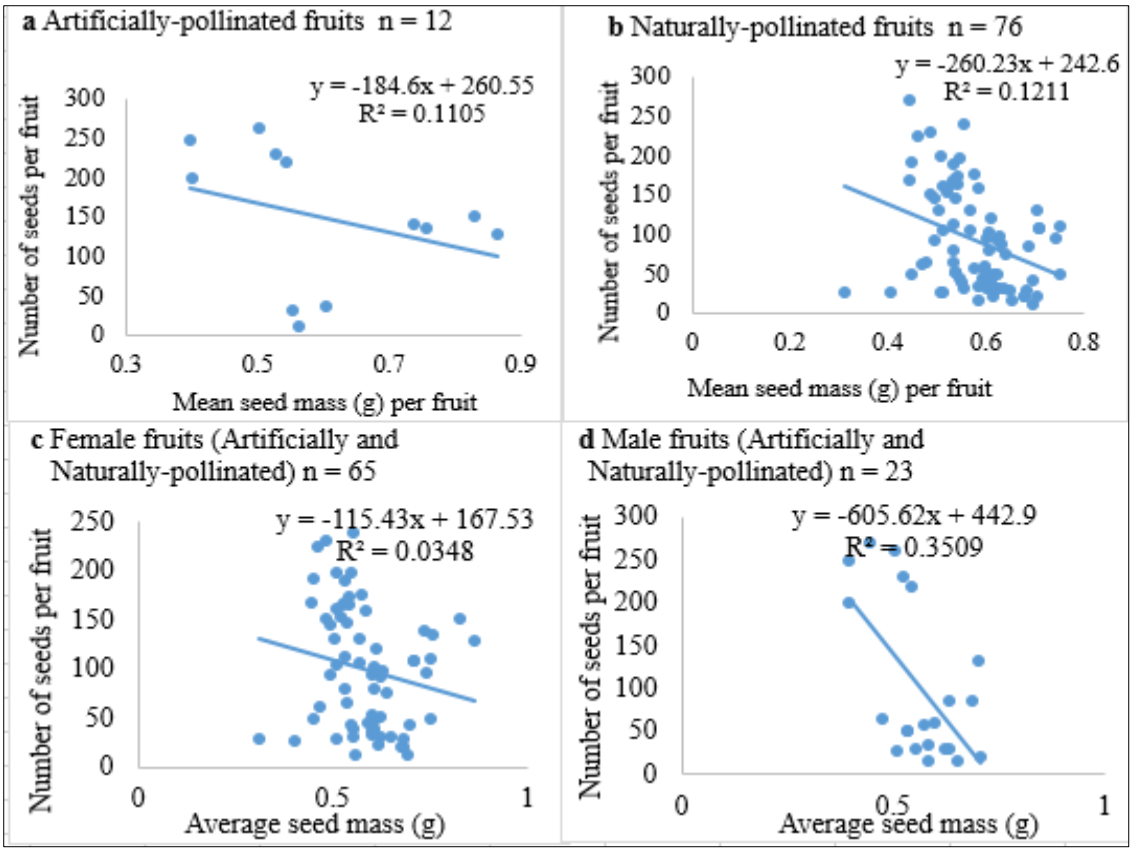


Figure 3.14: The relationship between baobab fruit mass and fruit length for pooled (a) artificially-pollinated and (b) naturally-pollinated baobab fruits and between (c) 'female' and (d) 'male' baobab fruits.

<u>Comparison of Coefficient of Variation (CoV) in seed mass (g) between naturally-pollinated and artificially-pollinated 'male' and 'female' baobab fruits</u>

Seeds extracted from fruits formed on artificially-pollinated and naturally-pollinated producer and poor-producer baobab trees have a relatively low size variation; all below 15% (Figure 3.15). However, this variation in seed size is significantly different among all baobab fruits (df = 75, F = 1.034, P = 0.38). The highest CoV was recorded in the seeds extracted from fruits formed on artificially-pollinated producer trees (11%) and lowest CoV was recorded in the seeds extracted from fruits formed on artificially-pollinated poor-producer trees (6%; Figure 3.15).

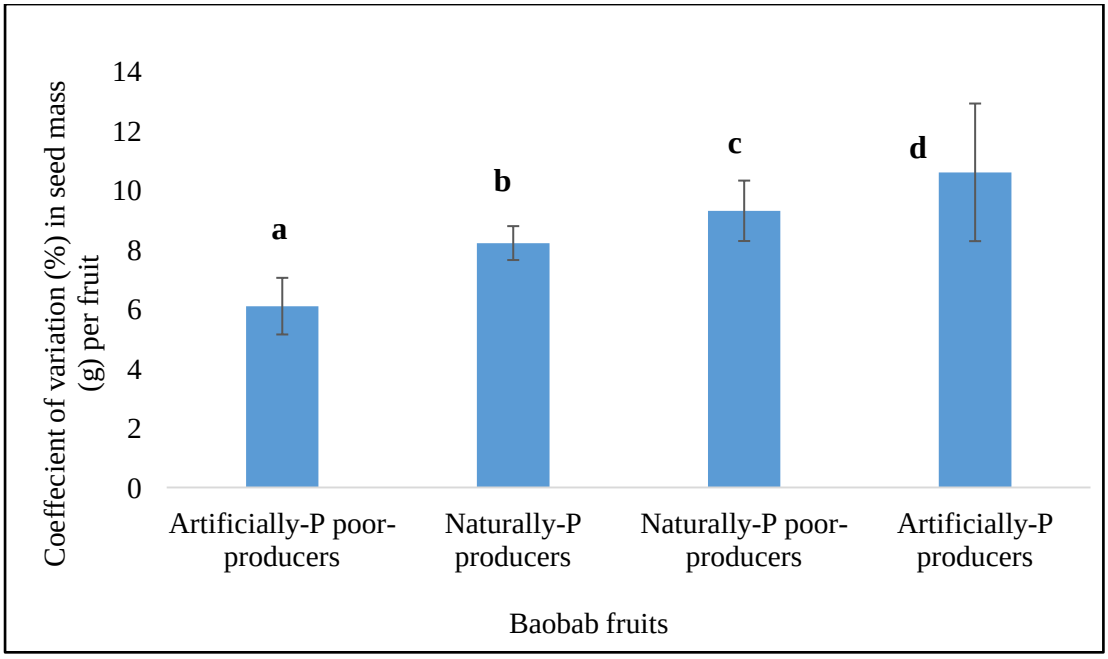


Figure 3.15: Comparison of Coefficient of Variation (CoV) in seed mass of fruits formed on artificially and naturally-pollinated producer and poor-producer trees. The lowercase letters (a, b, c and d) represent the significant difference between baobab fruits as revealed by the Fisher's Least Significant Difference (LSD) test.

Comparison of Coefficient of Variation (CoV) in seed mass from naturally-pollinated baobab fruits collected from different land use types.

Friedman's ANOVA and Fisher's Least Significant Difference (LSD) tests found a significant difference in CoV between all land use types (df = 62, F = 0.549, P = 0.65). Baobab fruits collected from the fields recorded the highest CoV in seed size (9%; Figure 3.16) and the lowest CoV was recorded in baobab fruits collected from the plains (7%).

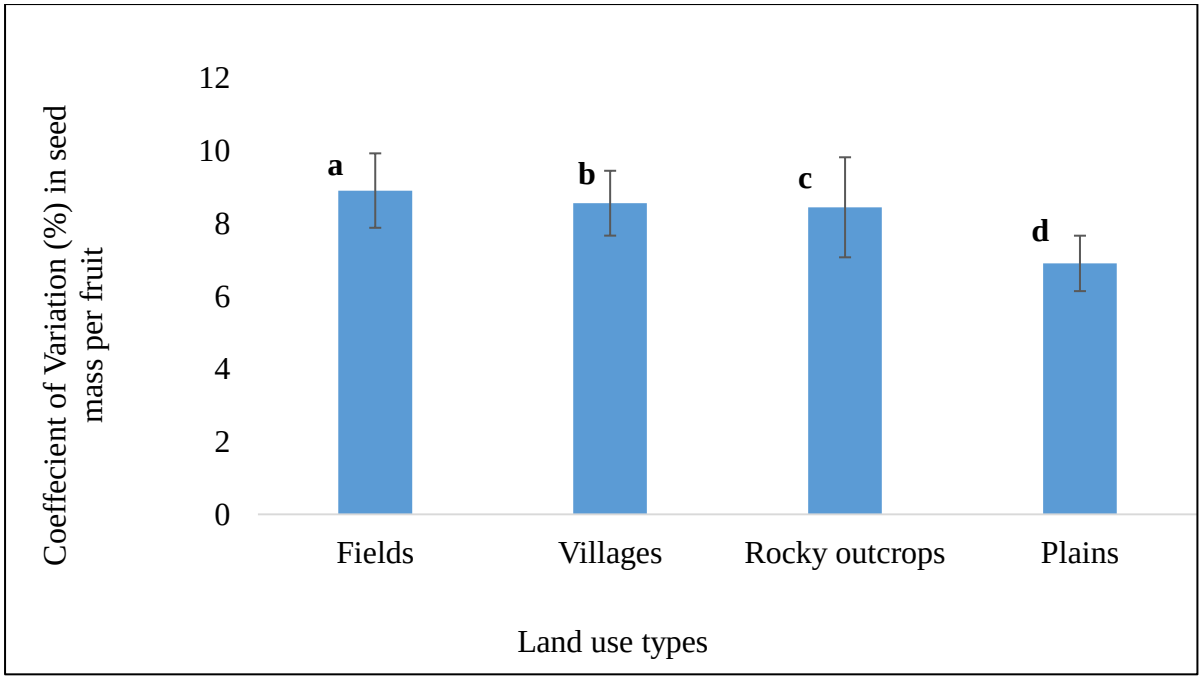


Figure 3.16: Comparison of Coefficient of Variation (CoV) in seed size of fruits from naturally-pollinated flowers on trees from different locations. The lowercase letters (a, b, c and d) represent a statistically significant difference between the land use types as revealed by the (LSD) test.

Comparison of baobab seed viability between artificially-pollinated and naturally-pollinated 'male' and 'female' fruits.

All embryos in the 10 seeds selected randomly from both the artificially-pollinated and naturally-pollinated producer and poor-producer baobab trees and tested for viability by being immersed in a 0.1 % tetrazolium solution for 14 hours turned from white (Figure 3.17a) to pink (Figure 3.17b), suggesting viability.



Image A. White seeds before tetrazolium test.	**Image B**. Pink seeds after tetrazolium test.

Figure 3.17: Comparison of baobab seeds before (A) and after (B) being immersed in the tetrazolium solution.

Seed production estimate in producer and poor-producer baobab trees

A t test revealed a significant difference in the number of seeds per fruit length between fruits from producer and poor-producer baobab trees (t = 0.09, df = 28, P = 0.93). Five regression lines were used to determine the best fit line to estimate the number of seeds per fruit. Both in the producer (Figure 3.18) and poor-producer (Figure 3.19), the power regression line shows the highest R^2 value (R^2 = 0.53 in producer trees and R^2 = 0.31 in poor-producer trees) compared to the linear, log and polynomial regression lines.

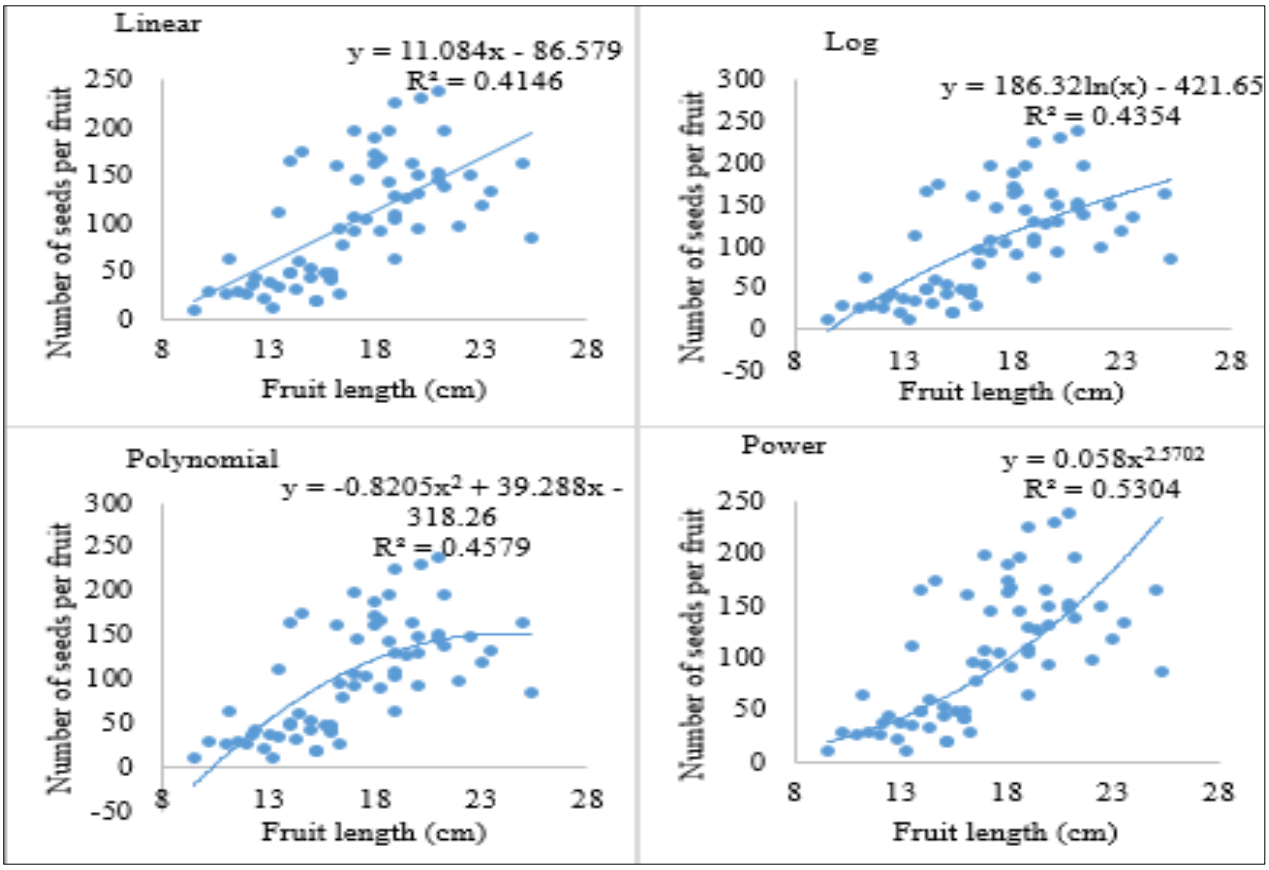


Figure 3.18: The four regression lines (a) Linear (b) Log (c) Polynomial and (d) Power tested to determine the best fit line that was used to estimate the number of seeds per fruit from producer baobab trees.

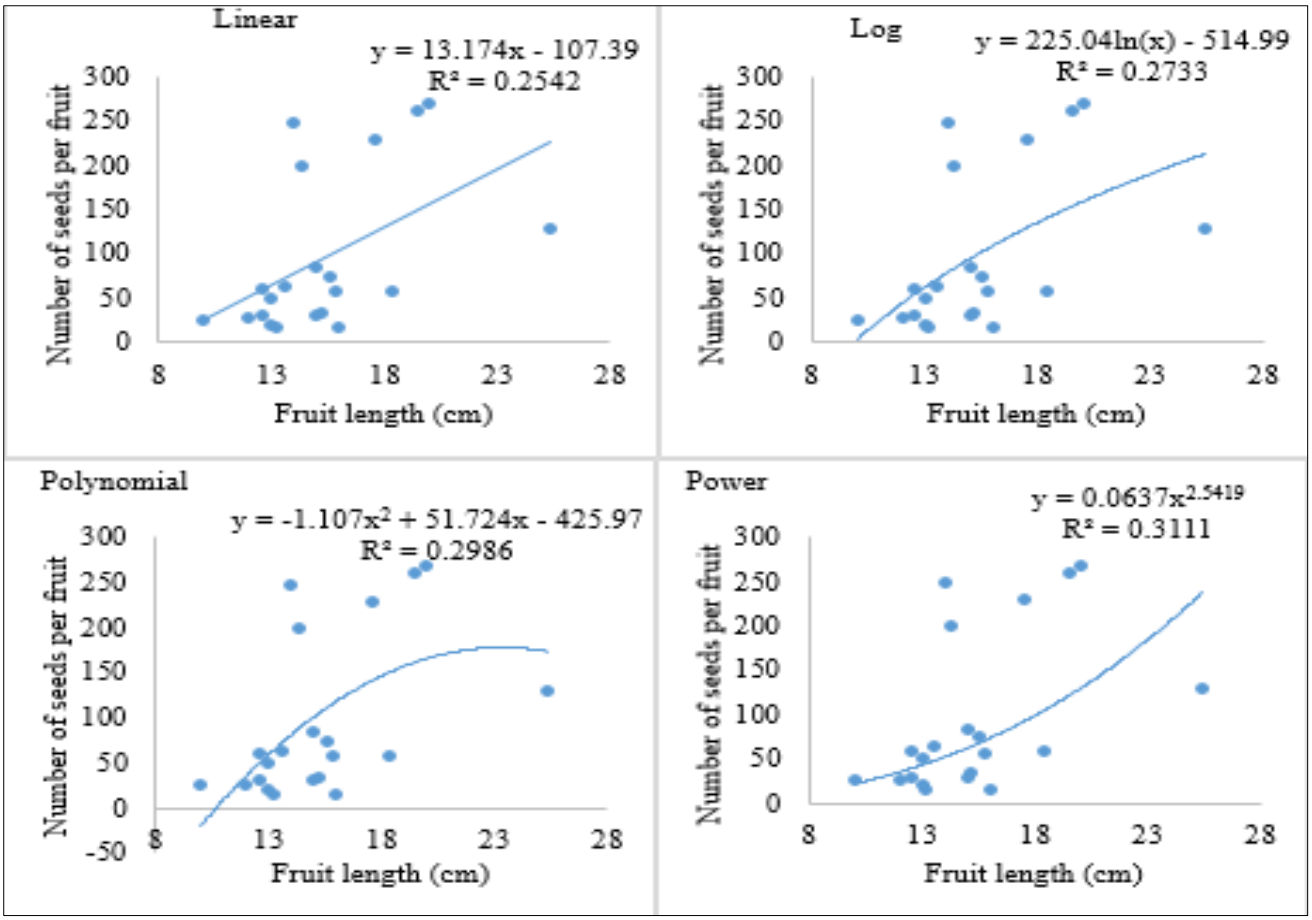


Figure 3.19: The four regression lines tested to determine the best fit line that was used to estimate the number of seeds per fruit from poor-producer baobab trees.

Over the study period of nine years, ‘male’ trees produced fewer fruits compared to the ‘female’ trees (Figure 3.20). Fruit production was the lowest in year 2008 for both ‘male’ (zero fruits) and ‘female’ trees (less than 50 fruits). On average, more medium fruits are produced per year by both ‘male’ and ‘female’ compared to small and large fruits.

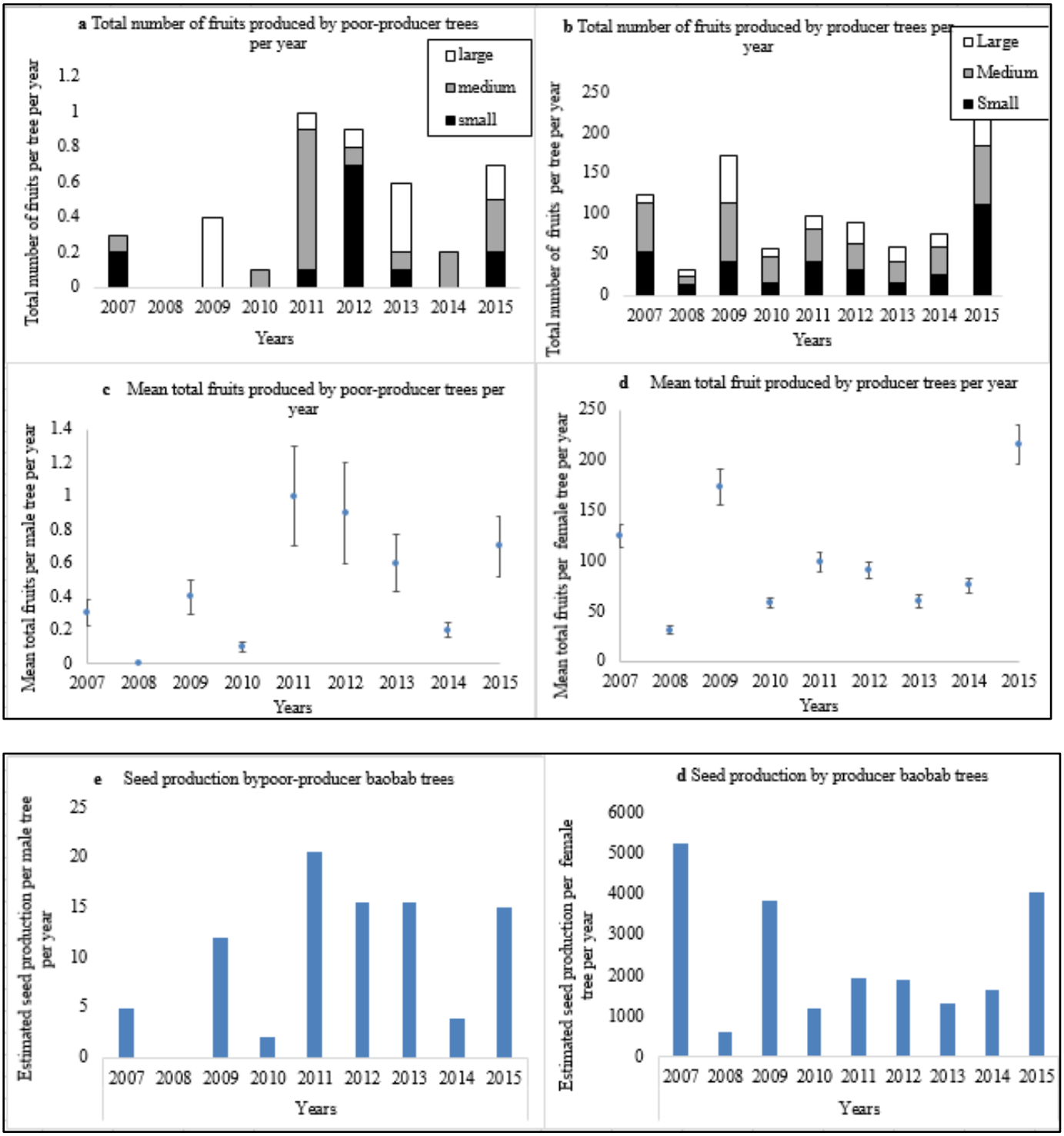


Figure 3.20: Comparison of fruit production and seed production estimate between producer and poor-producer baobab trees over a nine year period.

Baobab fruit shape analysis

The relative warps analysis showed that of 18 relative warps described, the first two relative warps explained 91.76% of the variation between fruits from producer and poor-producer baobab trees and Relative Warp 1 explained 57.74% of the variation (Figure 3.21). A deformation grid (Figure 3. 22) showed the mean landmark shape and the rotated landmark shape of fruits from producer and poor-producer baobab trees. The mean shape and rotated shape are very close and at some point are attached which implies that there is no difference in the two variables for all fruits. The CVA showed that there is an overlap in the canonical variance scores between the fruits from producer and poor-producer baobab trees (Figure 3.23).

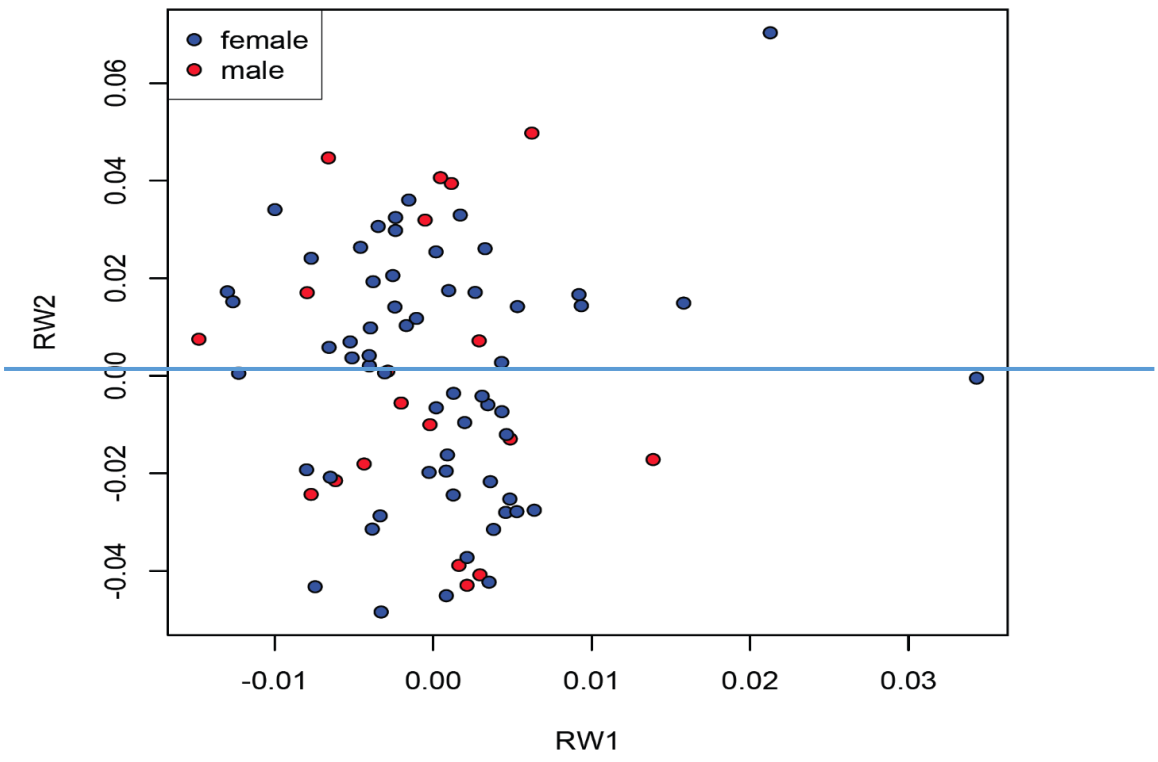


Figure 3.21: Scatterplot for the relative warps using all landmarks for all fruits from both producer poor-producer baobab trees.

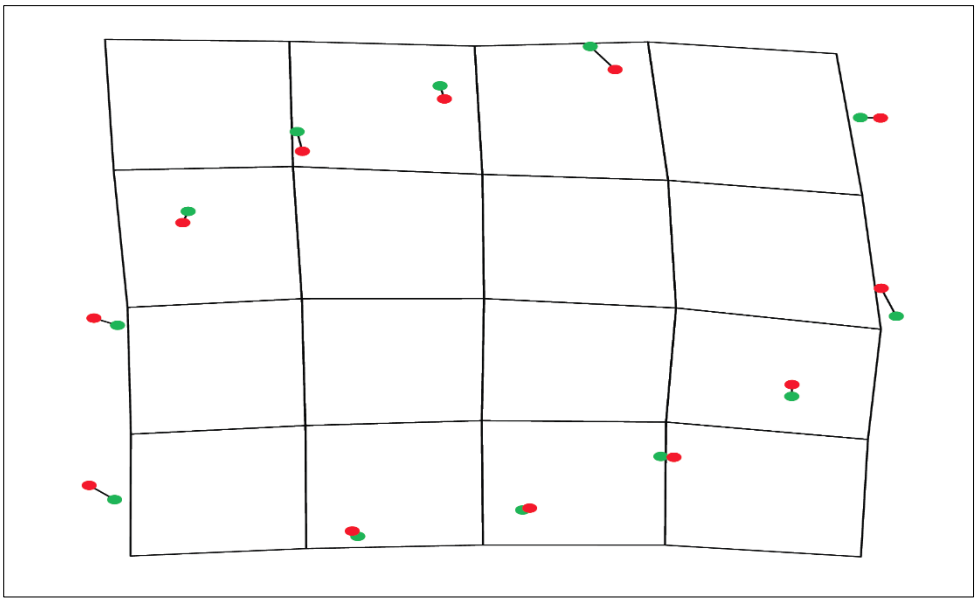


Figure 3.22: Two dimensional deformation grid of consensus shape (red) and rotated landmarks (green) from all fruits from both producer and poor-producer trees.

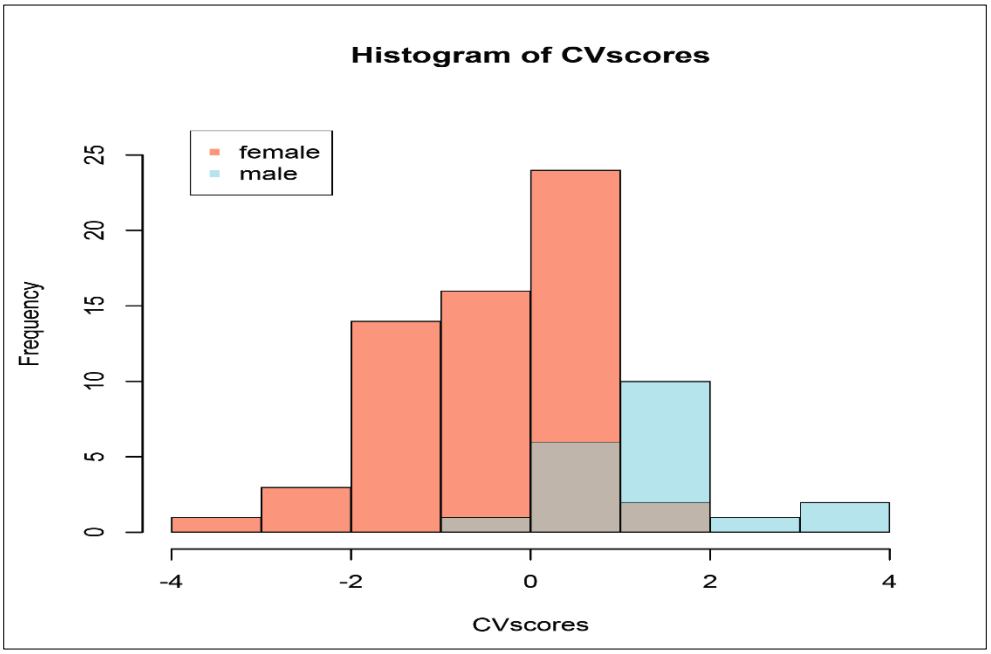


Figure 3.23: A plot of the Canonical Variance Analysis (CVA) analysis results for fruit shape of fruits from producer ('female') and poor-producer ('male') baobab trees. The CV scores are presented on the x-axis and the frequency of individuals with that CV score is on the y-axis.

Chapter 4.0

4.1 Discussion

Baobab fruit and seed characteristics

Fruit and seed characteristic differed significantly between fruits formed on artificially and naturally-pollinated producer and poor-producer trees. Although under natural pollination conditions mean fruit mass varied significantly between producer and poor-producer baobab trees, this difference was not significant in fruits formed from artificially-pollinated producer and poor-producer trees. Many studies concluded that artificial pollination is the most effective mode of pollination for many plants including passion fruit and *Annona cherimola* (commonly known as Cheyrimoya from southern California) and it is regarded as the easiest way of improving and increasing quality fruit set in many fruit trees (Schroeder, 1941; Das *et al.,* 2013). The results of my study support the conclusion that artificial pollination is the most effective mode of pollination, because, both producer and poor-producer artificially-pollinated trees produced fruits of better quality in terms of fruit size (mass and length) compared to fruits from naturally-pollinated trees. In addition, fruits from artificially-pollinated trees contained more fruit pulp than fruits from naturally-pollinated trees. My results are in line with those of Das *et al*. (2013) and Schroeder (1941). They compared fruit and seed characteristics between self, hand and natural pollination and found that hand pollination yielded the highest fruit set per tree. Fruit mass, ratio and the number of seeds per tree was the highest in fruits formed after hand pollination. Hand pollination may also reduce the time required for the flowers to develop into matured fruits and thus plants that have been artificially pollinated can maximize the use of resources within a short period to produce many good fruits. (Das *et al.,* 2013).

This study found a low mean fruit mass in producer trees. This can be explained using the ideas by Das *et al*. (2013) who suggested that fruit trees can only withstand a certain total mass of fruits. Baobab producer trees yielded between 50 and 299 fruits per year (Venter and Witkowiski, 2011), thus can possibly only hold smaller-sized fruits compared to poor producer trees that produce less than five fruits per year. This trade-off between fruit size and the number of fruits produced per tree implies that a tree may either have many small fruits or few big fruits. The number of size fruit may determine the number of seeds per fruit. Baobab

fruit mass and fruit length with the number of seeds per fruit showed here a strong linear relationship which suggests that heavy and long fruits contain more seeds per fruit as opposed to light-weight, short fruits. On the other hand, I found that seeds from naturally-pollinated trees were generally small and had a consistent individual seed mass ranging between 0.4 g and 0.8 g, whereas, seeds from fruits formed on artificially-pollinated trees showed a wide range of individual seed mass ranging from 0.35 g to 1.0 g. Seed mass can be correlated with the altitude at which the species is found (Li *et al.,* 2015). Species growing at lower altitude tend to produce larger seeds than species in high altitudes because high altitudes are characterised by low temperatures which slow down pollen tube growth; thus growth seasons are shortened. High temperatures (30 −35 °C) may also promoting self-incompatibility (Spinardi and Bassi, 2012). Due to this, seeds only reach a certain mass before the growing season ends. In contrast, very cold temperatures that occur soon after flowers opening may affect the ovules and consequently, hinder fertilization. This affects the total number of fruits and seeds produced (Way, 1978). Northern Venda falls within an average altitude of 400 meters above sea level, which is a relatively low altitude and temperature ranges between 8.5 °C and 39.7 °C (Venter and Witkowiski, 2013 c). The temperature is warm enough for pollen growth.

Fruit and seed characteristics are affected by many other factors such as the local water temperature affecting the seed mass (Li *et al.,* 2015). In a warm habitat where water will naturally have a high temperature and thus plants produce larger seeds because the increase in temperature speeds up seed maturation (Li *et al.,* 2015). Trees competition for resources such as water and minerals and the removal of flowers may also cause differences in seed and fruit set characteristics (Trueman and Turnbull, 1993; Spinardi and Bassi, 2012). Differences in fruit set and fruit characteristics may also be due to the source-sink mechanism in plants, which arises when plants have to balance the inorganic material needed for photosynthesis and the allocation of the end products to different parts of the plant. (Spinardi and Bassi, 2012).

Baobab fruit shape analysis

Differences in fruit shape may result from many factors including genetics, climatic and environmental factors, the position of pollen on the stigma may also affect the fruit shape (Way, 1978; Gurashi and Kordafani, 2014). Results from this study revealed that both producer and poor-producer trees in northern Venda yield similarly shaped fruits.

Similarly, Wiehle *et al*. (2014), found a very low fruit shape difference within baobab trees in Sudan; this study suggested that these morphological characteristics are a result of genetics and that young baobab trees inherit these traits from their parental trees. In addition to the genetic variation, variation in fruit morphology between trees can be a result of environmental and climatic factors (Gurashi and Kordafani, 2014). Furthermore, artificially-pollinated fruits had more uniform shapes and according to Way (1978), fruits with uniform shapes tend to have more seeds. My results support this observation because artificially-pollinated fruits contained more seeds per fruit than those pollinated by hand.

Understanding differences in fruit morphologies and attributing such variation to environmental factors and other factors assist in the protection of the species' growing and reproductive needs. This information and knowledge can also be used for improving the species reproduction output by breeding trees with the most favoured and good traits and grow them in the most favourable environment (Gurashi and Kordafani, 2014). From this study, data suggested that artificial pollination may be the good for baobabs because it yielded more uniform shaped and large fruits that contained more seeds compared to natural pollination.

Seed number and mass trade off in baobabs

Reproductive output in plants varies in number and mass and from season to season (Li *et al.*, 2015). The compromise between the output number and mass is known as a trade-off. Trade-offs can therefore be seen as a result of natural selection in which the fittest individual survive and reproduce even better competitors (Messina and Fox, 2001).

In plants, the trade-off between the seed number and mass per fruit and the mass evolved because of limited resources (Messani and Fox, 2001). Studying trade-offs helps to understand how seed mass differs within and between species in plant communities (Paul-Victor and Turnbull, 2009). Seed mass in any plant is controlled by a variety of means, such as genetics and the availability of resources (Gambin and Borras, 2010). On the other hand, seed number is dependent on the availability of resources, where there is more resources during the time of seed production, more seeds will be produced (Gambin and Borras, 2010).

Data presented here did not show a clear relationship between seed mass and seed number in baobab fruits. All fruits formed from both artificially and naturally-pollinated producer and poor-producer baobab trees showed a weak negative linear relationship between seed mass and the number of seeds per fruit, i.e. all fruits contain many big seeds which is the opposite of what is implied by the trade-off. Hence, this did not support the implication of seed number and mass trade off in baobab fruits. The results of this study corroborate those of Gambin and Borras (2010) who found that the seed number and mass trade-off is not as clearly observed in individuals of the same species as it is observed among species. This weak negative relationship between seed mass and the number of seeds can be explained by the differences in species ecological niches. Within a species, individuals compete and adjust their reproductive output according to the availability of the resources and thus may not follow the same productive trend every-year as resource availability varies. Therefore, the trade-off is more visible across species because different species have different resource requirements, survival and reproductive abilities (Messina and Fox, 2001).

The outcome of the seed number and mass trade-off may benefit or risk the survival of the offspring. For example large seeds survive better after germination by being better competitors with the ability to withstand environmental stresses but small seeds have a dispersal advantage. Small seeds are carried over longer distances allowing individuals to spread and establish in different areas. According to Messina and Fox (2001), small seeds are mostly produced in parental plants that compete for establishment with their offspring.

Seed mass can also be influenced by the type of environment in which the parental tree grows. Trees that grow in poor soil and low nutrient environments tend to produce small seeds that will germinate and grow into individuals that are not too successful in resource competition to allow others (including parents) to survive under these conditions (Paul-Victor and Turnbull, 2009). That being the case, the life cycle of particular plants especially plants

with edible fruits should be understood and related to human usage of these products in order to improve fruiting and seed production, and consequently, plant survival.

Baobab fruit production on different land-use types

Resarch focus on different uses of any particular area was initiated because of the concerning and dramatic increase in the human population which seems to threaten species natural production in the different land uses (Teka and Haftu, 2012). Studying fruit and seed production in different land use types is important to identify land use types that yield quality fruit to encourage the local stakeholders to increase fruit production in those areas to meet the high food demand (Teka and Haftu, 2012).

My data showed significant difference in fruit production and Coefficient of Variation (CoV) in seed mass between land use types. Human influenced land use types (fields and villages) produced more baobab seeds then natural lands (nature reserves, plains and rocky outcrops; Venter and Witkowski, 2011). These results were attributed to different factors such as tree-size, life-stage, and predation and site characteristics (Venter and Witkowski, 2011).

Generally, people tend to protect species of their personal interests especially if such trees have useful parts or provide them with edible fruits. Knowing that baobabs are important trees, local people protect these trees to increase the output and this could possibly be the reason why fruit production was high production in the villages. The fields are mostly used for agricultural purposes. Although it is very unlikely for this study, agricultural irrigation and the use of fertilizers that reduces soil pH could lead to a deficiency of macronutrients such as iron and zinc (Teka and Haftu, 2012). The presence of taps in villages may also contribute to soil moisture as a form of artificial watering through burst water pipes or leaking taps may inadvertently increase production is increased (Venter and Witkowiski, 2011).

Low production in rocky outcrops can be attributed to nutrients leaching out of the soil. Rocky areas contain very little soil to retain nutrients for plant growth and reproduce especially in events of high rainfall. In a comparative study of crop production in different land use types in Ethiopia, soil samples were collected from the different land use types and analysed for physicochemicals properties to identify the nutrient rich land which is more

suitable for crop production. The study suggested that cultivated land is the most suitable for most crops in Ethiopia (Teka and Haftu, 2012).

Animals may also contribute to low fruit production. Low fruit production as observed in land uses such as nature reserves can also be attributed to the type of animals present in the reserve. Venter and Witkowski (2011) mentioned that a number of elephants can found in these areas and can destroy trees. A study on baobabs in Zimbabwe noted that elephants feed by striking barks from trees and this has reduced the number of fruits per baobab tree in Zambezi valley (Swanepole, 1993). Browser animals can also reduce the total production by browsing on the seedlings and therefore reducing the number of trees that could grow into mature individuals and successfully reproduce.

Estimation of seed production in producer and poor-producer baobab trees

Fruit and seed sets are the determinants of plant reproduction and hence have a great influence on the size and distribution of plant populations (Steffan-Dewenter *et al.,* 2001). Plants have adapted to produce edible fruits with the main reason of attracting animals for seed dispersal (Lashley, 2014). Plant reproduction output is thus heavily dependent on pollination, herbivory, seed predation and climatic conditions such as temperature and rainfall (Steffan-Dewenter *et al.,* 2001; Lashley, 2014).

The study of fruit production and the estimation of seed production is necessary in economically important species as this can assist in conservation planning as well as businesses in determining their market value from seed products. For example, if the number of baobab trees in each land use type is known and the number of fruits each baobab tree can produces is also known, the total baobab oil production can estimated per land use type and this will help with baobab conservation plans and ease the estimation of local income to be generated from processing baobab seed oil. Estimating seed production may assist with the assessment of animal (frugivores) behaviour and with the regulation of these animal in vulnerable plant communities (Lashley, 2014). The results can also be used by land managers as the monitoring tool to their plant management practice (Lashley, 2014). On that account, the accuracy of fruit and seed estimate is very important.

Seed estimation in this study was based on the three baobab fruit size classes as classified by Venter (2012). The results revealed that there was a difference in the fluctuation of seed production between producer and poor-producer baobab trees over the nine years period. In addition, producer trees produced more seeds and consequently more seeds per year for all the years compared to poor-producer trees. The highest seed production recorded for the poor-producer trees was recorded in 2011 and in 2015 for the producer trees. These results can be attributed to climatic factors such as rainfall and temperature and the availability of other plant growth and reproduction requirements. Rainfall may have been the highest in the years where highest fruit and seed were recorded. On the other hand, excess rainfall water at flowering time may wash away pollen grains and this reduces the fruit and seed set (Ortega *et al.*, 2007).

The results of this study further showed that poor-producer baobab trees produced a variety of fruit sizes especially the year 2011 and 2012. On the contrary, producer baobab trees produced a more constant fruit size over the study period of nine years. On average, many small and medium fruits were produced compared to large fruits in both producer and poor-producer baobab trees. This can be explained in terms of resource availability. When resources are limited interspecific competition arises among trees leading to differences in output levels. Plants that tend to produce large fruits are those found resource abundant areas. Connolly *et al.* (2001) argued that species distribution may also affect fruit set. Often there is interspecific competition in species that grow in dense patches, competing mostly for nutrients and water and in some cases for space. This competition causes reduction in plant size and consequently a reduction in fruit and seed sets.

The effect of plant size on fruit and seed production was shown by Venter and Witkowski (2011), who found that adult baobab trees produce about 8 times more fruits than the semi-adult trees. Similarly, taller and bigger trees tend to have lower chances of being attacked by fruit predators and better chances of absorbing sunlight for reproduction (Lashley, 2014; Sletvold, 2002). It may also be the case that bigger plants attract more pollinators, resulting in effective fertilization increasing reproduction and improving fruit set quality. Plant size can also be used to predict both current and future fruit and seed output (Sletvold, 2002).

During the assessment of baobab trees for fruit production by Venter and Witkowski (2011), they discovered that baboons contribute to the loss of fruits per tree. Baboons opt for the newly formed fruits that are still soft and immature which I believe is because the baobab

fruit develops a protective hard shell that is difficult to break, but which is generally soft in newly developed fruits. Even though only immature fruits are selected and predated on, this still has an effect on the final seed production per tree. Venter and Witkowski (2011) reported a 58% to 85% loss in baobab fruit production in areas where baboons were present. The study thus suggested that when presence of baboons in baobab occupied areas has to be noted when planning fruit harvesting.

Habitat fragmentation is another factor identified to have an impact on plant reproduction (Diekmann and Tsaliki, 2010). Northern Limpopo in South Africa is a highly fragmented area with different land use types and because most people in this province are unemployed, agriculture is the most practiced human activity. Habitat fragmentation results in the isolation and distancing of plant populations away from each other.

This habitat fragmentation has a consequence of disrupting plant interactions and consequently the pollinators do not reach all populations for cross pollination and hence decrease reproductive success. In cross pollination, isolation of habitat patches affects the movement of pollinators between patches (Diekmann and Tsaliki, 2010). Sunlight on the other hand influences plant reproductive success indirectly by determining how much of the floral surface area is displayed to attract pollinators (Kilkenny and Galloway 2008) but this does not seem to have much of an effect on baobabs because they are pollinated by a nocturnal mammal, the bat, unless other possible pollinators such as moths are present. In natural pollination, flowers in the sun receive about seven times more visitors (pollinators) than the flowers in the shade and hence producing more seeds (Kilkenny and Galloway 2008). Although baobabs do not rely on sunlight to attract pollinators, their floral morphology, the large and white flowers that open at night produce enough nectar for the bats to allow the transfer of pollen.

To understand and be able to estimate seed yield in a particular plant, the environmental conditions should be studied because this information can assist in the prediction of future plant production. In case of future production concern for any particular plant, a strategic forward approach can be applied to save plants and consequently ecosystem services.

Baobab seed viability

The ability of plants to regenerate is dependent on the viability of their seeds. Seed viability can be tested by germinating the seeds or by carrying out a chemical test (e.g. tetrazolium;

Gimenez *et al.,* 2014). For this study, it was proposed to use both methods but unfortunately germination trials were not successful and thus seed viability was based on the chemical test.

Baobab seeds have a hard seed coat that require high temperatures to break. The air temperature (27 ° C) set for the germination trial for this study was probably not high enough or the pre-treatment (soaking seeds in boiling water) was not effective. On that note, dormancy on baobab seeds by the hard seed coat prohibits the baobab seeds to germinate soon after removal from fruits (Niang *et al.,* 2015). A 90−96 % germination rate was recorded in Senegal in baobabs seeds that were soaked in 96% sulphuric acid for seven hours and 0% germination was recorded in seeds that not did not undergo a pre-treatment before the germination trial (Niang *et al.,* 2015). The study thus suggested that 96% sulphuric acid is an effective solution in destroying the hard seed coat in baobabs, consequently speeding up germination. However, Niang *et al.* (2015), suggested that small seeds germinate faster than large seeds because of their ability to enter the soil, but in *ex situ* germination trials, large seeds may show a higher germination rate due to the high amount of stored food.

Seed viability is affected by many factors. The ability of plants to produce viable seeds, animal-seed predation and the time of seed storage after harvest are some of the known factors affecting seed viability (Shaban, 2013). In this study, the tetrazolium test revealed that all seeds tested for viability were viable and thus viability did not differ between producer and poor producer baobab trees. Knowing that baboons predate on baobab fruits, the fruits have a protective coat, thus the seeds cannot be damaged. As baboons only eat the soft immature fruits, this does not count as a contributing factor to the loss of seed viability in developed baobab seeds. Baobab seed viability may thus be affected by other factors such as poor permeability of water and oxygen into the seed due to the hard seed coat that then inhibits embryo development (Niang *et al.,* 2015). Fruit harvesting timing may also affect seed viability because seeds may not be matured at the time of harvesting, baobab fruits should thus be harvested after six months when they are fully mature. This is particularly important if fruits are harvested for studies of this kind.

One of the conservation efforts to improve seed viability in plants is the collection and storage of seeds. However, if conditions such as temperature and relative humidity under which seeds are stored are not controlled, the seeds can lose vigour, consequently viability is also lost (Shaban, 2013). It is also important to note that the effects of temperature and moisture on seed viability differs between and among species (Shaban, 2013). This implies

that seeds of different species cannot all be stored under the same pre-set conditions and expected to retain viability. On that account, seed research studies are therefore important in different species conservation planning.

4.2 Conclusion

Baobab fruit and seed production differed significantly between naturally and artificially-pollinated producer and poor producer trees and between different land use types. Artificially-pollinated trees produced bigger uniform-shaped fruits and hence more seeds in comparison to smaller and uneven shaped fruits produced by naturally-pollinated trees, but the difference was not significant. Producer trees produced more fruits and hence more seeds than poor-producer trees. In addition, mature fruit production and the estimated seed production differed significantly between the different land use types. Human influenced land use types (fields and villages) produced more matured fruits and hence more baobab seeds then natural lands (nature reserves, plains and rocky outcrops). As predicted, there was a strong correlation between fruit length, mass and volume and the number of seeds per fruit; longer and heavier fruits contain more seeds. This study did not find a clear relationship between seed mass and seed number in baobab fruits. Only artificially-pollinated producer trees showed a strong linear relationship between seed mass and the number of seeds per fruit while artificially-pollinated poor producers and all naturally pollinated trees showed a very weak correlation ($R^2 < 0.2$). Although the proposed germination trial to test seed viability was not successful, the randomly picked seeds from fruits of producer and poor producer trees all tested viable under the chemical test (tetrazolium salts solution) thus seed viability did not differ between baobab trees.

I recommend that germination trials should be compared between producer and poor producer baobab trees but seeds must first be treated with 96 % sulphuric acid for seven hours to improve results germination results. Environmental and climatic factors such as rainfall should also be correlated to fruit production to determine what effects such factors have on baobab fruit set production in this region.

This study showed that there is a significant difference in fruit and seed production and fruits characteristics between artificially and naturally-pollinated producer and poor-producer baobab trees and among different land use types in northern Venda.

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Appendices

Table 1: Fruit dimension data for fruit from artificially-pollinated producer ('female') and poor-producer ('male') baobab trees.

Baobab identity	Male/female	Length with tape(cm)	Length with ruler(cm)	Width with tape (cm)	width with ruler (cm)	Weight (g)	Number of seeds
Artificially-pollinated fruits							
A-6-C	Female	25	21.2	30.1	9.5	343.6	139
A-8-C	Female	37	19.5	34	11	371.95	127
A-11-C	Female	23.2	22.5	34.4	9.5	404.11	150
A-26-C	Female	29.2	23.5	32.2	11	351.08	134
B-4-C	Female	10.2	9.5	16	6	36.55	11
B-7-C	Female	15.2	13.5	20	7	87.51	35
B-10-C	Female	13.2	11.5	19.8	6.5	68.83	30
C-4-C	Male	17	14.3	31	10	243.73	199
C-12-C	Male	18	14	33	12	271.18	248
E-1-C	Male	22	19.5	34	10.5	379.49	261
E-4-C	Male	21	17.6	32.1	11	340.3	229
E-7-C	Male	21	17.5	32	10.5	336.55	218

Table 2: Fruit dimension data for fruit from naturally-pollinated producer (‘female’) and poor-producer (‘male’) baobab trees.

Baobab identity	Male/female	Length with tape(cm)	Length with ruler(cm)	Width with tape (cm)	width with ruler (cm)	Weight (g)	Number of seeds
Naturally-pollinated fruits							
BF3	Female	21	19.5	26.2	8.5	213.91	147
BF5	Female	25.3	21	28	9.5	250.24	86
BF3	Female	21	20	25.2	8	218.1	152
BF3	Female	20	18	27	9	230.69	150
BR1	Female	15.2	13.5	20.2	7.5	95.74	20
BR5	Female	14	11.5	21.3	8	122.11	50
BR1	Female	16.3	13.5	23.2	8	99.65	28
BR5	Female	12.2	10	21.9	7	108.15	37
BR1	Female	14.3	12	20.5	8.5	104.14	33
BR5	Female	13.2	9.5	20	7	81.66	12
BP3	Male	10	6.5	20	7	51.28	26
CV6	Female	21.2	18	32.4	10.5	302.29	197
CP6	Female	17.2	11.3	32	11	238.8	146
CP6	Female	18	13.5	35	12.5	283.58	173
CV3	Female	21	14.3	39	13.3	391.51	238
CV5	Female	23	17.5	25.8	9	249.55	119
CP2	Female	18.6	16.5	36	9	198.28	144
CV5	Female	18.2	15	26.4	8.5	201.25	92
CP5	Female	15.2	10	22.6	7.5	85.77	20
CV6	Female	20	16.5	32	11	277.83	131
CV4	Female	10.2	9	21.2	7.5	77.56	29
CP5	Female	19	14.4	29.2	9.5	186.19	64
CV4	Female	14	9.5	23.6	8	109.43	48
CV5	Female	13	10	20	7	69.48	38
BP5	Female	19.8	15	27.4	9	213.26	164
BV4	Female	12	9	21	7	72.18	27
BP6	Female	16.5	18.6	28.2	10	219.81	79
BV3	Female	16	14.5	23	7.5	136.1	42
BP5	Female	17	14	29	9	236.97	198
BF6	Female	19	12	36	11	250.33	129
BV4	Female	12.8	11	21.2	7.5	65.14	22
BF5	Male	25.4	16.5	30	11.5	278.59	129
BF6	Female	16	12.3	27.2	9	141.22	48
BV4	Female	15	11	24	8	111.79	44
BV6	Male	16	10.5	25	8	115.73	16
BV5	Male	18.4	14	36	10.5	279.35	59
BV3	Female	14.6	11.5	25	7.5	128.19	175
BF4	Female	18	13.5	30.4	10	229.2	189
BV3	Female	15.6	12.6	25	9	136.19	48
BP6	Female	22	18.5	27.6	9.5	256.7	98
AF3	Female	16.2	14.3	25.6	8.5	162.59	161
AF3	Female	18.2	15.2	27.4	9	227.89	167
AF5	Male	132	11	20	7	86.79	16
AV5	Female	18.6	13.5	33.6	12.3	294.84	196
AV1	Female	19	15.2	29.2	11.5	200.11	109
AV1	Female	16.4	13.4	27	9.5	162.99	95
AV5	Female	14	18	29.6	12	250.46	165
AV6	Male	15	11.8	28	9.5	180.36	84
AF6	Female	18	13.5	29.4	10.5	205.66	162
AV5	Female	19	15	34.6	12	335.72	225
AV6	Male	15.6	11	28	10	163.27	75
AF5	Male	13	10.5	22.4	7.5	105.24	51
AV6	Male	12	9.5	21.2	7	78.14	27
AV6	Male	20	11.4	33.4	12	290.07	269
AV6	Male	12.6	10.7	21.8	7.5	89.99	60
AV1	Female	17	12.5	29.2	11	184.65	107
Kilima	Male	12.6	9	24.6	8.5	97.81	30
AV6	Male	15	11	22	8	88.37	30
AV1	Female	12.4	8	22.6	10.5	86.3	44
Kilima	Male	15.2	11	26	9	129.92	34
AP4	Female	15	13.5	24.6	8.5	140.09	53
AV6	Male	13	10.5	20.6	8	71.22	20
AV4	Female	11	8	20.2	7	60.12	27
AP4	Female	11.2	15	26.6	10.5	142.78	64
AP4	Female	16	11	24.4	9	115.48	43
kilima	Male	13.6	10.5	26.6	9	140.75	64

Table 3: Statistical summary of fruit ratio for artificially-pollinated and naturally-pollinated baobab fruits.

	Baobab fruits	Mean ± Standard Error	p value
	Artificially-pollinated ‘male’	1.47 ± 0.06	0.017
	Artificially-pollinated ‘female’	1.32 ± 0.09	
	Naturally-pollinated ‘male’	1.65 ± 0.06	0.945
	Naturally -pollinated ‘female’	1.62 ± 0.35	
	Artificially-pollinated ‘male’	1.47 ± 0.06	0.961
	Naturally-pollinated ‘male’	1.65 ± 0.06	
	Artificially-pollinated ‘female’	1.32 ± 0.09	0.010
Fruit ratio	Naturally -pollinated ‘female’	1.62 ± 0.35	

Table 4: Statistical summary of fruit mass for naturally-pollinated and artificially-pollinated baobab fruits

	Baobab fruits	Mean ± Standard Error	p value
	Artificially-pollinated ‘male’	314.25 ± 20.93	0.3442
	Artificially-pollinated ‘female’	237.66 ± 61.98	
	Naturally-pollinated ‘male’	132.88 ± 17.98	0.028
	Naturally-pollinated ‘female’	182.24 ± 10.15	
	Artificially-pollinated ‘male’	314.25 ± 20.93	0.0003
	Naturally-pollinated ‘male’	132.88 ± 17.98	
	Artificially-pollinated ‘female’	237.66 ± 61.98	0.41
Fruit mass(g)	Naturally-pollinated ‘female’	182.24 ± 10.15	

Table 5: Statistical comparison of coefficient variation in seed mass between fruits from artificially and naturally-pollinated producer and poor-producer baobab trees

ANOVA

CoV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	63.582	3	21.194	1.034	.383
Within Groups	1537.219	75	20.496		
Total	1600.801	78			

Multiple Comparisons

Dependent Variable: CoV FRUITS

	(I) Fruits	(J) Fruits	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	4.50222	2.65090	.332	-2.4632	11.4677
		3.00	2.23421	1.82701	.614	-2.5664	7.0348
		4.00	1.67367	2.03315	.843	-3.6686	7.0159
	2.00	1.00	-4.50222	2.65090	.332	-11.4677	2.4632
		3.00	-2.26801	2.12348	.710	-7.8476	3.3116
		4.00	-2.82855	2.30324	.611	-8.8805	3.2234
	3.00	1.00	-2.23421	1.82701	.614	-7.0348	2.5664
		2.00	2.26801	2.12348	.710	-3.3116	7.8476
		4.00	-.56054	1.27106	.971	-3.9003	2.7793
	4.00	1.00	-1.67367	2.03315	.843	-7.0159	3.6686
		2.00	2.82855	2.30324	.611	-3.2234	8.8805
		3.00	.56054	1.27106	.971	-2.7793	3.9003
LSD	1.00	2.00	4.50222	2.65090	.094	-.7787	9.7831
		3.00	2.23421	1.82701	.225	-1.4054	5.8738
		4.00	1.67367	2.03315	.413	-2.3766	5.7239
	2.00	1.00	-4.50222	2.65090	.094	-9.7831	.7787
		3.00	-2.26801	2.12348	.289	-6.4982	1.9622
		4.00	-2.82855	2.30324	.223	-7.4168	1.7597
	3.00	1.00	-2.23421	1.82701	.225	-5.8738	1.4054
		2.00	2.26801	2.12348	.289	-1.9622	6.4982
		4.00	-.56054	1.27106	.660	-3.0926	1.9715
	4.00	1.00	-1.67367	2.03315	.413	-5.7239	2.3766
		2.00	2.82855	2.30324	.223	-1.7597	7.4168
		3.00	.56054	1.27106	.660	-1.9715	3.0926
Bonferroni	1.00	2.00	4.50222	2.65090	.561	-2.6818	11.6863

		3.00	2.23421	1.82701	1.000	-2.7171	7.1855
		4.00	1.67367	2.03315	1.000	-3.8362	7.1836
	2.00	1.00	-4.50222	2.65090	.561	-11.6863	2.6818
		3.00	-2.26801	2.12348	1.000	-8.0227	3.4867
		4.00	-2.82855	2.30324	1.000	-9.0704	3.4133
	3.00	1.00	-2.23421	1.82701	1.000	-7.1855	2.7171
		2.00	2.26801	2.12348	1.000	-3.4867	8.0227
		4.00	-.56054	1.27106	1.000	-4.0052	2.8841
	4.00	1.00	-1.67367	2.03315	1.000	-7.1836	3.8362
		2.00	2.82855	2.30324	1.000	-3.4133	9.0704
		3.00	.56054	1.27106	1.000	-2.8841	4.0052

CoV

			Subset for alpha = 0.05
	Fruits	N	1
Tukey HSD[a,b]	2.00	5	6.1033
	3.00	50	8.3713
	4.00	17	8.9318
	1.00	7	10.6055
	Sig.		.142

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.486.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 5: Statistical comparison of coefficient variation in seed mass between fruits from artificially and naturally-pollinated producer and poor-producer baobab trees on different land use types.

ANOVA

CoV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.106	3	10.369	.549	.651
Within Groups	1171.181	62	18.890		
Total	1202.286	65			

Multiple Comparisons

Dependent Variable: CoV

	(I) Land_use_types	(J) Land_use_types	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	1.90421	1.67403	.668	-2.5154	6.3238
		3.00	.49398	2.26435	.996	-5.4841	6.4721
		4.00	.25205	1.38017	.998	-3.3917	3.8958
	2.00	1.00	-1.90421	1.67403	.668	-6.3238	2.5154
		3.00	-1.41022	2.28716	.926	-7.4485	4.6281
		4.00	-1.65216	1.41727	.651	-5.3939	2.0896
	3.00	1.00	-.49398	2.26435	.996	-6.4721	5.4841
		2.00	1.41022	2.28716	.926	-4.6281	7.4485
		4.00	-.24194	2.08173	.999	-5.7379	5.2540
	4.00	1.00	-.25205	1.38017	.998	-3.8958	3.3917
		2.00	1.65216	1.41727	.651	-2.0896	5.3939
		3.00	.24194	2.08173	.999	-5.2540	5.7379
LSD	1.00	2.00	1.90421	1.67403	.260	-1.4421	5.2505
		3.00	.49398	2.26435	.828	-4.0324	5.0204
		4.00	.25205	1.38017	.856	-2.5069	3.0110
	2.00	1.00	-1.90421	1.67403	.260	-5.2505	1.4421
		3.00	-1.41022	2.28716	.540	-5.9822	3.1617
		4.00	-1.65216	1.41727	.248	-4.4852	1.1809
	3.00	1.00	-.49398	2.26435	.828	-5.0204	4.0324
		2.00	1.41022	2.28716	.540	-3.1617	5.9822
		4.00	-.24194	2.08173	.908	-4.4032	3.9194
	4.00	1.00	-.25205	1.38017	.856	-3.0110	2.5069

		2.00	1.65216	1.41727	.248	-1.1809	4.4852
		3.00	.24194	2.08173	.908	-3.9194	4.4032
Bonferroni	1.00	2.00	1.90421	1.67403	1.000	-2.6584	6.4668
		3.00	.49398	2.26435	1.000	-5.6776	6.6656
		4.00	.25205	1.38017	1.000	-3.5097	4.0138
	2.00	1.00	-1.90421	1.67403	1.000	-6.4668	2.6584
		3.00	-1.41022	2.28716	1.000	-7.6440	4.8235
		4.00	-1.65216	1.41727	1.000	-5.5150	2.2107
	3.00	1.00	-.49398	2.26435	1.000	-6.6656	5.6776
		2.00	1.41022	2.28716	1.000	-4.8235	7.6440
		4.00	-.24194	2.08173	1.000	-5.9158	5.4319
	4.00	1.00	-.25205	1.38017	1.000	-4.0138	3.5097
		2.00	1.65216	1.41727	1.000	-2.2107	5.5150
		3.00	.24194	2.08173	1.000	-5.4319	5.9158

CoV

			Subset for alpha = 0.05
	Land use types	N	1
Tukey HSD[a,b]	2.00	13	7.0240
	3.00	5	8.4342
	4.00	34	8.6762
	1.00	14	8.9282
	Sig.		.745

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.589.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.