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Phenology and Reproductive Biology of Kenyanendangered Sandalwood (*Osyris lanceolata* Hoscht and steudel)

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Abstract: The Osyris lanceolata Hoscht. and Steudel. is an evergreen drought resistant shrub of the Santalaceae family. It thrives well in the ASALs of tropical Africa and on the margins of forests and bushlands. The mature tree species has dense heart wood that produces essential oils used in perfumery and pharmaceutical industries to make expensive cosmetics and drugs. Trunks and roots of mature preferable female trees are illegally over-extracted from the forest hence threatening the species with extinction to meet the ever-rising international industrial demand. This study therefore aimed at describing the phenology and the reproductive biology of the species in Kenya. Two distinctive populations namely; Kabarnet and Kitui were considered. Onset and end dates of flower bud initiation, flower opening, fruit initiation, fruit maturity and ripening were observed on artificially pollinated flowers, anther emasculation and on control. Fruits that developed in each experiment were assessed for full or empty embryo by cross sectioning of the fruits. Flowering began in early February and September, and flowering season closed in July and December respectively. The species complete its flowering phase in 109±16 days. Assisted pollination increased the reproductive success of the trees by 39.7%.

Keywords: Conservation; Phenology; Osyris lanceolata; Reproductive Biology.

Introduction

The *Osyris lanceolata* is a dioecious Arid and Semi-Arid Lands (ASALs) species that is hemi-parasitic on the roots of host trees such as *Rhus natalensis* (Krauss), *Dodonaea viscose* (L) Jacq, *Tecomaria capensis* (Thunb) Lindl, *Catha edulis* (Vahl.) Endl, *Apodytes dimidiate* (E.Mey. ex Arn), *Brachystegia spiciformis* (Benth.), *Pongamia pinnata* (L.) Pierre, *Casuarin aequisetifolia* (L.) and *Aphloia theiformis* (Vahl) Benn. (Teklehaimanot *et al.*, 2003; Mwang'ingo *et al.*, 2007; Global Plants, 2016). It is highly exploited due to its valuable and diverse products that include essential oils for making perfumes and expensive cosmetics, herbal medicines and religious carvings just like other the sandalwoods in the family (Mwang'ingo *et al.*, 2007; Tamla *et al.*, 2011; Lawless, 2013). It usually achieves a height of 6 to 11 m and diameter at breast height (dbh) of 3 to 7cm when mature (Mwang'ingo *et al.*, 2007; Machua *et al.*, 2009). High international demand for sandalwood products and the reduction in the major world suppliers of sandalwood products (Australia and India) has led to general decline in the sandalwood populations in the world (Tamla *et al.*, 2011; Rashkow, 2014; Peden *et al.*, 2017).

The search for an alternative source of sandalwood has led to a raise in cases of illegal ruthless felling and removal of *O. lanceolata* from the natural forests in Kenya, Tanzania, Uganda and other East Africa countries by smugglers despite governmental restrictions (Teklehaimanot *et al.*, 2003; Mwang'ingo *et al.*, 2007; Kamondo *et al.*, 2009; Kamondo *et al.*, 2012; DA SILVA *et al.*, 2016). The O. lanceolata populations in Kenya are sparsely distributed in western, eastern, coastal and Rift Valley regions (Kamondo *et al.*, 2009; Kamondo *et al.*, 2012). The Kenyan O. lanceolata is protected by Legal Notice No. 3176 of 2007 under the Forests Act, 2005 which also mandates the Kenya Forest Research Institute (KEFRI) to propagate the species in the wild (Kenya Law, 2007; Kamondo *et al.*, 2009). The notice also allow for the development of sustainable harvesting and conservation strategies the species in the country. In spite of the legal notice, the *O. lanceolata* populations has been decreasing since 2002 as a result of sharp rise in the illegal extraction of the species from its natural habitat to meet the international trade demands (Mwang'ingo *et al.*, 2003; Mwang'ingo *et al.*, 2007). This has threatened its existence in the natural and thus Kenya has applied for its inclusion of the species in the IUCN red list of the endangered species in the index page to foster its conservation (Iucn, 2013; Cttes, 2016).

The reduction of the sandalwood species population in the world has been tagged to both overexploitation and the reported poor natural regeneration through seeds

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which may be due to reproductive failure as reported by Mwang'ingo *et al*. (2007). Low levels of artificial regeneration are also reported in *O. lanceolata* populations in Kenya just as is the case with other sandalwood trees (Iucn, 2013). The species is also reported to grow and develop slowly and that its reproductive phenology varies considerably depending on eco-regions (Mwang'ingo *et al*., 2007; Kamondo *et al*., 2012; Mothongoane, 2011; Tamla *et al*., 2011). Seed collection to foster the conservation approaches is also poor due to low seeding of the species. There is therefore needed to understand the reproductive biology and phenology of *O. lanceolata* in order to understand the reproductive challenge so as to design a mitigation strategy to counteract the reproductive failure, low regeneration and recruitment thus improving the mechanism of conservation of the species in the natural.

Materials and methods

Populations' Geographic Description

Table 1 shows the description of the populations sampled for the entire study. Kabarnet lays in the Kenyan Rift Valley and has a higher elevation and with a lower mean annual rainfall compared to Kitui which in in the Eastern region of Kenya.

Phenology of O. lanceolata

The assessment of phenology of *O. lanceolata* started on the onset of the flower buds initiation. Five mature trees showing evidence of budding were identified in each sex (male and female trees). They were tagged and assessed during the entire flowering season. The time when the first and last flower bud appeared was recorded. The onset and end dates of budding, flowering, fruiting and fruit ripening stages were monitored on each marked tree and recorded. The duration in days that each stage took to complete were also noted for the entire reproductive cycle.

Reproductive Biology of O. lanceolata

A total of 10 randomly selected healthy, large and mature trees of at least 3.0 cm dbh and with evidence of budding were selected from each sex in the natural populations in Kabarnet and Kitui (5 trees from each sex). Each tree selected was large enough to accommodate various treatments for the study of reproductive biology. Three healthy branches on each tree were selected randomly and were marked A (for Anther removal), P (for assisted pollination) and C (for control) respectively.

On the first branch marked C, 10 flower buds were marked and covered with muslin cloth and were monitored through the entire fruit development stages (all newly emerging flower buds were removed using razor blade).

On the second branch marked A, 10 flower buds were marked and covered with muslin cloth and allowed to open into flowers (female trees only). After opening into flowers, all anthers from each flower were removed using scalpel and the flower covered again. The entire fruit development stages were then monitored (all newly emerging flower buds were removed using razor blade).

On the third branch marked P, 10 flower buds were covered using muslin cloth and allowed to open into flowers. Pollen grains from male flowers were applied on the receptive stigmas of female flowers for three consecutive days using a fine brush according to Ngulube (1996) guidelines at each day, the flowers were covered and then monitored over entire stages of flower development till mature ripe fruits were formed (all newly emerging flower buds were removed using razor blade).

The total number of flower buds that develop into flowers, flowers to fruits and then fruits to maturity and ripening on each tree and in each setup were recorded. The duration in days each developmental stage took was recorded. The mature ripe fruits that developed on each branch were collected and seeds extracted. The seeds were cross-sectioned and inspected for viability by determining whether the embryo was fully developed or empty. Number of seeds that had full embryo and empty embryo were recorded separately.

Data Analysis

In phenological events, the total duration of the populations' onset and end dates of flowering cycle was observed and recorded in days. The ranges, means and standard deviation of days in phenology in each stage were determined using MS Excel. The sex ratio of *O. lanceolata* in the natural populations was obtained by comparing the number of male to female trees on MS Office Excel spreadsheet 2007. The reproductive variations in the flowers that formed and developed into a viable fruit were determined in each treatment and fed on MS Excel spreadsheet. The data set obtained was subjected to ANOVA in IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA).

Results

Phenology of *O. Lanceolata* in Kenya.

Generally, *O. lanceolata* flower bud initiation started in January and September in the 1st and 2nd flowering cycles respectively. Flower bud initiation in Kitui was 3 to 4 days earlier than the Kabarnet population. New bud initiation continued up to late June in the first cycle, although late flowers that developed into viable fruits reduced considerably. Fruit maturation started in late

March and November in the first and second cycle respectively (Table 2). The flowering cycle of male trees ended with flower buds withering while the female trees cycle ended when the fully developed fruits had ripened. To complete one flowering cycle, *O. lanceolata* took 109±16 days in both Kitui and Kabarnet populations. Flower bud initiation to active flower formation took 5±1 and 7±3 days in male and female trees respectively.

The floral active parts that had formed withered within 12 to 17 days in females and 14 to 20 days in males. Flower withering took place between 19 to 24 days after flower bud initiation in both sexes. This marked the beginning of fruit initiation in female trees and the end of flowering activity in males. Fruits that formed achieved maturity (unripe fruits) after a mean of 41±6 days, mean of 41±6 days, which were 61±6 days since bud nitiation.

Fifteen days later, fruit ripening phase started. This was 76±3 days since flower bud initiation. All fruits under study started ripened fully within mean of 33 days since the phase started, being 109 days since flower buds were initiated (Table 3).

Table 1: Geographical profiles of the study areas

Population	Longitude	Latitude	Altitude (m)	[1]M.A. Rainfall (mm)	M.A. Temperature (˚C)
Kabarnet	35˚ 68'E	00˚ 50' N	2040.0	635.0	25.0
Kitui	38˚ 00'E	01˚ 21' S	1185.4	775.0	25.0

[1] M.A= Mean Average

Table 2: Periods of phenological events in *O. lanceolata*

Events	1st Cycle	2nd Cycle	Remarks
Flower buds initiation	Jan/Feb	Sept/Oct	Budding continues till early June for 1st Cycle and early November in 2nd cycle.
Flower buds opening	Jan/Feb	Sept/Oct	Buds opening continues till early June for 1st Cycle and early November in 2nd cycle.
Fruit initiation	Feb/Mar	Oct/Nov	Fruit initiation continues till mid-June for 1st Cycle and late November in 2nd cycle.
Fruit maturation	April/May	Nov /Dec	Fruit initiation continues till Mid July for 1st Cycle and early December in 2nd cycle.

Reproductive Biology in Kenya's *O. Lanceolata* Populations

In Kabarnet, 40% while in Kitui 30% of the flower in control setup developed into fruits. In assisted pollination treatment (*P),* 50% of the flowers developed into fruits in both Kabarnet and Kitui population. Less than 5% of the flowers that had their anthers removed (A), developed into fruits. In Kabarnet population, 58.67%, while in Kitui 63.33% of the fruits matured in control (*C*). In assisted pollination (*P*), 65.38% and 77.43% of the fruits matured in Kabarnet and Kitui respectively. On average, 20% and 33.3% of the fruits had full embryo in Kabarnet and Kitui populations respectively in a control setup while in the assisted pollination, 62.94% and 69.84% fruits had full embryo in Kabarnet and Kitui populations' respectively. In the anther removed setup, about 1.5% of the fruits that developed had empty embryo when crosschecked in both populations. The fruits that were proved to have full embryo in assisted pollination treatment (*P),* were significantly higher (*p=0.034*) at $p<0.05$ in both populations (Table 4).

The ANOVA for the fruiting, fruit ripening and full embryo seeds that formed in the two populations showed no significant differences between reproductive stages in both populations.

Discussion

Phenology of O. lanceolata

The first cycle of *O. lanceolata* flowering cycle in Kenyan populations begun in late January/early February. Fruit maturation started in February/March and ripening was within April/May months. In the second cycle, flowering starts in late September/early October, fruiting stated in October/November and ripening within November/December. There was no distinct delineation of the reproductive stages from one to the successive developmental stages of *O. lanceolata* in Kabarnet and Kitui populations. Observations by the natives revealed that months in which flowering occurs tend to vary yearly depending on the prevailing weather conditions. Previous studies of flowering and fruiting of other species in Santalaceae family for instance *S. album* in Java, China and Indonesia revealed that flowering time occurs from June to October (Ma *et al*., 2005). In India, flowering of *S. album* occurs from July to September with continuous staggered flowering

occurring in other months of the year (Baskorowati, 2011; Ratnaningrum & Indrioko, 2014).

Table 3: Phenology of both male and female *O. lanceolata* trees.

Events	Sex	Range Duration in days	Variance	[1]SD	Mean days	Cumulative days
Flower budding to flower bud opening	[2]F	4 – 6	1	1	5	5
	[2]M	5 – 9	2.5	1.58	7	7
Flower bud opening to flower withering	F	12 – 17	3.5	1.87	14.5	19.5
	M	14 – 20	4.67	2.16	17	24
Fruit initiation to fruit maturation	F	29 - 36	6	2.45	32.5	52
Fruit maturation to fruit ripening initiation	F	13 - 17	2.5	1.58	15	67
Ripening initiation to active fruit ripening	F	16 – 33	28.9	5.3	26	83
Total		**74-109**		**16**	**109**	

[1] SD= Standard Deviation [1] F= Female [1] M= Male

Table 4: Relative success rates in female reproductive cycle in Kitui and Kabarnet.

Treatment	Population	Number of flower buds	% flowers to fruits	% of raw fruits to ripe fruits	% of ripe viable fruits	Average success (%)	% of ripe empty fruits
[5]*C*	Kabarnet	50	40.0	58.67	20.0	26.65	80.0
	Kitui	50	30.0	63.33	33.3		66.67
[6]*P*	Kabarnet	50	50.0	65.38	62.94	39.74	48.0
	Kitui	50	50.0	77.43	69.84		46.0
[7]*A*	Kabarnet	50	5.0	2.0	0	0	2.0
	Kitui	50	3.0	1.0	0	0	1.0

Key: C=control / natural pollination treatment, P=assisted pollination treatment, A=anther emasculated.

The species flowering period took 109±16 days to complete one cycle. The phenology of male and female trees differed significantly in both Kabarnet and Kitui populations. Female bud initiation and buds opening was about 2 to 3 days before the male, which agrees with the study on Tanzanian populations of *O. lanceolata* by Mwang'ingo *et al.* (2007). In the Kenyan populations, male flowers opened late and withered 3 to 4 days later after the female flower had withered. The withering of male flowers marked the end of flowering events in male trees but the beginning of fruit initiation in female trees. The withering of male flowers days after female flowers may have been a reproductive strategy of *O. lanceolata* to ensure that there is successive maximum fertilization of the female flowers as was also suggested by Ayasse & Arroyo (2011) in their study on pollination and reproductive biology of plants. However, *O. lanceolata* species efficiency in utilization of the pollen grains produced may have been stalled by the fact that female trees flowered earlier than male trees. Again, the Kenya population of *O. lanceolata* might be facing a challenge of few pollinators available for pollen grain transfers. This was also observed by Mwang'ingo *et al.* (2007) in the Tanzanian populations. The dearth of pollinators observed in the populations of *O. lanceolata* during the study suggests that there are barriers to pollen transfer between trees and therefore low rate of cross-pollination in these populations. This could be contributing to the observed low regeneration.

Species in the same genera tends to have similar flowering patterns especially if growing in environments with similar ecological conditions (Kochmer & Hardel, 1986; Grime *et al.*, 2014). A study on constraints and competition on flowering phenology in Japan, North and South Carolina showed that most populations of the same genera growing in similar ecological conditions had similar flowering timing (Kochmer & Handel, 1986; Pei *et al.*, 2015). In this case the ecological conditions in the Tanzanian populations (annual rainfall, temperatures, soil characteristics and species hosts) are similar to the prevailing ecological conditions in the Kenyan sites (Mwang'ingo *et al.*, 2007; Mukonyi *et al.*, 2011). This therefore suggests that the flowering phenology of *O. lanceolata* in Kenya and Tanzania are similar. They therefore experience same reproductive processes. Slight variations in the flowering phenology might be as a result of slight variations in prevailing climatic conditions and presence of pollinators (Sakai, 2001; Dantec *et al.*, 2015). This might affect flower initiation and end dates or length of phenology events.

The Kenyan *O. lanceolata* flower bud took 5 to 7 days to open into active flower since initiation. The results on flower bud opening to active flowers did not deviate far from those observed from the study by Mwang'ingo *et al.* (2007) in Tanzanian populations. Studies of Australian species have reported variations in bud opening; for instance, *S. lanceolatum* flower opening

takes 12 to 24 hours while the S. austrocaledonicum Vieill have flower buds that open and close within 24 to 48 hour (Page & Tate, 2010). These are shorter duration of flower buds opening as compared to the *O. lanceolata* species.

Reproductive biology of *O. lanceolata*

The flowers of *O. lanceolata* were found within axillary panicles consisting of not more than 5 flowers per inflorescence in female and about 5 to11 flowers in male trees. A study by Page and Tate (2010) on the breeding behaviour self-, intra and inter-specific cross-compatibility of *S. lanceolatum*, a species in the same family (Santalaceae) in Australia observed that the number of flowers per inflorescence on *S. austrocaledonicum* and *S. album* trees ranged from 20-40 flowers. The floral morphology of *S. album*, *S. austrocaledonicum and S. lanceolatum* were observed to be similar (Page & Tate, 2010; Butaud, 2015). The flowers of *O. lanceolata* were 4.6 mm long. Page & Tate (2010) observed that *S. lanceolatum and S. austrocaledonicum* flowers were approximately 3.0 mm long while *S. album* flowers were 2.5mm long. The stigma of the sandalwood trees as studied by Page & Tate (2010) had 3 to 4 lobes just like the *O. lanceolata*. *O. lanceolata* also had an inferior ovary that swell to become a single seeded drupe after fertilization just like *S. austrocaledonicum*, *S. album* and *S. lanceolatum* (Page & Tate, 2010).

Assisted pollination significantly increased the quantity of seeds with full embryo formed by 39.7% compared to natural pollination that resulted to only 26.7% seeds with full embryo. This might indicate that the female flowers were receiving low pollen grains in the natural pollination, hence reducing pollination success in the natural (Setsuko *et al*., 2013). The pollen grains produced were either low in quantity, few pollinators were present or the pollinators were limited in the distance they can cover from tree to tree hence less flowers being fertilized. A study by Ma *et al*. (2006) on fragmented populations of *S. album* (family Santalaceae) in India found that 2-3% of open pollinated *S. album* flowers set seed compared to 26.7% in the study. The percentage in *S. album* increased to 14.0% during artificial pollinations. This suggests that the species is mainly outcrossing. *S. lanceolata* in Australia is also outcrossing species (Page & Tate, 2010). This suggests that seed setting in members of Santalaceae family can be improved by using assisted pollination.

The result of reproductive biology of *O. lanceolata* in Kenya suggests that the species is outcrossing. Few fruits formed (1.5 %) from flowers in which anther had been removed. However, the seeds that formed in this case were not viable. This result therefore suggests that this species has the ability of parthenocarpic fruit development but does not form clonal seeds. In *S. album* and *S. lanceolatum* (China and Queensland populations) had no seeds set on flowers isolated from open pollination by bags (Ma *et al.*, 2005). Most of the seeds formed in *O. lanceolata* natural/control pollinations in Kenya had poorly developed embryo or damaged embryo. A reproductive biology study by Sindhu Veerendra & Ananthapadmanabho (1996), on *S. album* showed that self-crosses yielded 1.3 % viable seed on isolated trees whose flowers were restricted by pollination bags. About 3.1% of the self-pollinated flowers formed seeds in 20.0% and 50.0% of *S. lanceolatum* and S. *album* tested genotypes respectively (Tamla *et al*., 2011). As observed in *S. lanceolatum*, most species in the family Santalaceae showed that though the species were mainly outcrossing, there is some degree of selfing (Page & Tate, 2010). This could be attributed to low number of pollinators or extent of the populations' fragmentation in the regions.

Conclusion

This study has shown that *O. lanceolata* in Kenya takes about 109 days for one entire flowering cycle in Kabarnet and Kitui populations. The species in Kenya has two flowering phase starting in late January and September in the first and second cycles and ends in May and December respectively. The study also showed that assisted pollination significantly increased the quantity of viable seeds produced by 40% compared to natural pollination that had about 27 %.

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Conflict of interest statement

To the best of my knowledge, none of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper. There is "No Competing interests are at stake and there is No Conflict of Interest" with other people or organizations that could inappropriately influence or bias the content of the paper.

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