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Ecology and silviculture of Osyris lanceolata (African sandalwood) : an aromatic tree of Tanzania

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ECOLOGY AND SILVICULTURE OF *OSYRIS LANCEOLATA* (AFRICAN SANDALWOOD): AN AROMATIC TREE OF TANZANIA

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ABSTRACT

Studies on ecology and silviculture of African Sandalwood (*Osyris lanceolata)* were carried out in Tanzania between January 1999 and February 2001 to provide basic information required to develop strategies and guidelines for management and conservation of the species. The studies had six major objectives: to assemble and collate existing information from literature and other secondary sources on the biology, ecology, silviculture and resource role of *O. lanceolata;* to assess and characterize its current populations in Tanzania in terms of size class distribution, reproductive biology, natural regeneration and associated plants; to analyse the oil quality and composition of its wood, and identify compounds that have market potential; to identify its host plant species and study their relationships; to develop methods of efficient seed storage and pre-treatment techniques and efficient vegetative propagation of the species. The results of the literature search revealed a dearth of information on the species in Africa as a whole and Tanzania in particular. The results of the study on population status of *Osyris lanceolata* in Tanzania showed that the populations were stable despite many years of exploitation. Populations with high density were found in the northern part of the country, which is more arid than the south. Regeneration took place through both seeds and rootstocks with the latter source accounting for 61% of the total regeneration. There was limited reproductive success in the populations studied due to either low level of pollen production or limited pollinators' movement. Assisted pollination increased the reproductive success. The quality and quantity of sandalwood oil produced varied between populations. The best quality and quantity of oil was obtained from the population in the north yielding as much as 8.45 ± 0.54% oil containing 32.2 ± 1.2% of santalol. Other compounds of commercial importance were also found in the oil including lanceol (56.7%), bisabolol (5.1%), nuciferol (3.7%) and bisabolene (3.3%). The results of the study on host plant identification and their interactions revealed that *O. lanceolata* was non-host selective but had preference. The most preferred hosts included *Rhus natalensis, Dodonaea viscosa, Tecomaria capense, Catha edulis*, *Apodytes dimidiata*, *Brachystegia spiciformis*, *Maytenus senegalensis* and *Aphloia theiformis*. Among the preferred host species *B. spiciformis* and *R. natalensis* promoted the early growth of *O. lanceolata* according the nursery experiment carried out in the present study. The same study showed that *Casuarina equiesetifolia* an exotic host species gave comparatively early growth performance of *O. lanceolata* as observed with *B. spiciformis* and *R. natalensis* probably due to its light crown that cast little shade and nitrogen fixing capability. The results of the study on seed storage method and pre-treatment technique revealed that the best storage method for seeds of *O. lanceolata* was 20% moisture content of seeds stored at 3-5°C. The best pre-sowing treatment technique was complete removal of the seed coat before sowing which gave a germination of up to 66.5% followed by soaking seeds in hot water which yield a germination of about 57.5%. The results of the study on vegetative propagation revealed that *O. lanceolata* can easily be propagated through both stem cutting and marcotting. The best rooting success was achieved in June and September. Application of auxins also proved useful in promoting rooting in both stem cuttings and marcotting with IBA at 50 ppm being the best in most respects. Nodal position also influenced rooting success, with cuttings from the basal portion being better than those of terminal origin. Based on the results of these studies it is recommended that further work is required by including more populations within Tanzania and in neighbouring countries to confirm the findings of the present study and identify populations with better quality of oil for improvement of the species through selection and breeding. The avoidance of the current destructive method of harvesting to encourage natural regeneration, promotion of planting using the methods developed to increase the resource and detailed chemical study to identify compounds with market potential are some of the other recommendations.

DEDICATION

To my wife Judith, my children Ester, Amos and Frida, my parents Paschal and Bernadetha who shared the pains of loneliness while away for studies and all the toiling tax payers of Finland and Tanzania who sacrificed their resource and gave this study a priority.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of Variance
C/N	Carbon/Nitrogen ratio
CEC	Cation Exchange Capacity
FAO	Food and Agriculture Organization of the United Nations
FINNIDA	Finnish International Development Agency
HADO	Hifadhi Ardhi Dodoma (Land Conservation in Dodoma)
IBA	Indole-3-Butyric Acid
IBPGR	International Board for Plant Genetic Resource
KIRDEP	Kondoa Integrated Rural Development Programme
MC	Moisture Content
MPTS	Multi Purpose Trees
NTSP	National Tree Seed Programme
P	Probability value
SIDA	Swedish Agency for International Development
TAFORI	Tanzania Forestry Research Institute
TIRDEP	Tanga Integrated Rural Development Programme
USAID	United States Agency for International Development

CHAPTER I

INTRODUCTION

1.1 Background

Realization that some non-timber forest products contribute a lot to the economy of rural communities has made them increasingly important. In fact some non-timber forest product have a value exceeding by far that of timber (Simons, 1996). Tanzania is no exception and many plant species are already known for their potential as source of non-timber forest products. Some of these are *Allanblackia stuhlmanii*, a potential species for soap production (Glendon, 1946; Lovett, 1983), *Uvaria leptocladon,* a species known to contain benzylated dihydrochalcones, and is used traditionally to treat cerebral malaria (Nkunya *et al.*, 1993), and *Osyris lanceolata,* famous in fragrance and perfumery industry (Mbuya *et al.*, 1994). The role of non timber forest products and growing extractivism activity in Tanzania, and Africa in general, underline that the multiple use potential of the forest resource is economically important (Minja, 1994; Simons, 1996; Demetria, 1998).

Osyris lanceolata Hochst & Steud is one of the sandalwood plant species known for producing fragrant scented wood from which sandalwood oil is extracted (Hill, 1937; Walker, 1966; Iyenga, 1968, Srinivasan *et al.*, 1992; Mbuya *et al.*, 1994). Sandalwood oil is one of the most expensive essential oils and its official export price for quality oil by 1996 was US$ 1500 per Kilogram. The price was expected to continue rising following the increase in demand that has been increasing from year to year (Nasi and Ehrhart, 1996). The oil is used in luxury cosmetics, perfume and fragrance industry. The excellent blending properties and its antiseptic properties make sandalwood oil valuable as a fixative for other fragrances (Winter, 1958; Walker, 1966; Saad, 1983; Srinivasan *et al.*, 1992; Coppen, 1995; Radomiljac *et al.*, 1999). The use sandalwood oil extends beyond perfumery to the treatment of various human disorders. It is useful as a popular sedative in oriental medicine and is considered to have narcoleptic effect (Okugawa *et al.*, 1995). It has a chemo-

preventive effect and thus used in treating inflammatory and eruptive skin diseases (Dwivedi and Zhang, 1999), useful in treating bronchitis, dysuria, gonorrhoea, and urinary infection (Okasaki and Oshima, 1953; Winter, 1958).

Osyris lanceolata is indigenous to Tanzania. Aromatic plants such as *O. lanceolata* have been in use in Tanzania and elsewhere as an invaluable source of natural perfume, cosmetics, flavors, fragrances, medicine, dyes and as a dietary supplement for many years. These are derived either from heartwood, roots, bark, leaves, flowers and seeds of aromatic plants (Hill, 1937; Chauhan, 1989; Khan *et al.*, 1993; Hines and Eckman, 1993). *Osyris lanceolata* commonly known as East African sandalwood is now used as a substitute/supplement of Indian sandalwood, *Santalum album* in the production of essential oil used mainly in fragrance industry and general perfumery (Walker, 1966; Iyenga, 1968; Srinivasan *et al.*, 1992). In general, sandalwood, which is a common name given to some species of the family Santalaceae, is among the most popular aromatic plant group in the world that have been in use in the perfumery and fragrance industry for many centuries (Hill, 1937; Metcalfe, 1950; Schery, 1954; Walker, 1966, Heywood, 1978). The species produce a highly valued aromatic heartwood from which the essential oil containing santalol is extracted. Of all the sandalwood species, *Santalum album* (East Indian sandalwood) is known to be the mother of all in the production of this essential oil (Srinivasan *et al.*, 1992).

Sandalwood oil is extracted from heartwood of the stem and root of sandalwood trees. Since roots are preferred due to relatively high oil content compared to an equal amount of wood from stem and other parts processed (Srinivassan *et al.*, 1992), trees are mostly harvested by uprooting (Mbuya *et al.*, 1994). As a result, the widely used Indian sandalwood species, *Santalum album*, has now been reduced to few stands of trees of inferior populations (Errickson *et al.*, 1973; Rai and Salma, 1990). The decline in the resource base in India has mainly been attributed to careless method of harvesting with no consideration of establishing plantation, due to outbreak of spike disease and the recent incidence of heart rot (Hill, 1937; Iyenga, 1968; Rai and Sarma, 1990; Harsh, 2000; Shankaranayana *et al.,* 2000). As a result

of the decline in the resource base of Indian sandalwood, several other species have been identified and used as substitutes or supplement. In Western Australia, *Santalum spicatum* was identified in early 1960s and since then the species has been exported to various market places (Walker, 1966; Errickson *et al.*, 1973; Srinivasan *et al.*, 1992). It is reported that, at the moment, all large trees of *S. spicatum* have been harvested, leaving only small trees, being scattered here and there (Errickson *et al.*, 1973). Other species, which have been identified and used as substitutes of Indian sandalwood, include *Santalum lanceolatum* from Australia; *Santalum yassi* from Fiji; *Fusanus spicatum* from Australia; and *Amyris balsamifera* from west India (Walker, 1966; Srinivasan *et al.*, 1992). In East Africa, *Osyris lanceolata*, commercially known as East African Sandalwood, was identified in early 1900s and has been in use as a substitute or supplement of Indian sandalwood since then (Dale and Greenway, 1961; Eggling and Dale, 1962; Mbuya *et al.*, 1994; Naves and Ardizo, 1954; Walker, 1966).

Osyris lanceolata is a widely distributed species, mainly in the tropics and some parts of the Mediterranean region (Heywood, 1978; Miller, 1989). In the tropics, it is mostly found in East Africa including Somalia and Ethiopia and southward through Zambia, Zimbabwe to some parts of Botswana (Dale and Greenway, 1961; Eggling and Dale, 1962; Miller, 1989; Beentje, 1994). In the Mediterranean region, it occurs in a variety of Mediterranean-type scrublands of Southern and West Spain, Persian and Iberian Peninsulas and Canary Islands (Herrera, 1984a; Bramwel and Bramwel, 1974). The species is also found in North Africa (mainly Egypt and Algeria) and South Africa, particularly in areas with Mediterranean type of climate (Bramwel and Bramwel, 1974; Palmer and Pitman, 1972; Palgrave, 1977; Saad, 1983).

Although *Osyris lanceolata* occurs in many countries, its use as a substitute/supplement of Indian sandalwood is not reported from any of the countries apart from Tanzania. According to Dale and Greenway (1961), Eggling and Dale (1962), Breintenbach (1963), and Walker (1966), populations of the species from Tanzania are currently the only ones used as a substitute or supplement. Even within Tanzania, harvesting is reported to be concentrated only in

few places (S.T Mwihomeke and Mabula, personal communication). The reasons for this location preference by the sandalwood industry are not yet known. Some traders of the species claimed the main criteria for selective harvesting had been high stocking of some populations that makes the overall harvesting economical. However, the possible existence of quality differences among populations is speculated (Fazal, M, personal communication) with the possibility of provenances from Tanzania being superior. However, nothing more could be said more as no studies has been done and get data to confirm this.

Before *Osyris lanceolata* was recognized as an important raw material for production of sandalwood essential oil, the species had commonly been used in Tanzania and elsewhere in Africa as an important source of local medicine. Its bark and root decoction is used as medicine against diarrhea, urinary diseases such as inflammation of bladder and gonorrhea. Fruits form an important dietary supplement. Fiber obtained from roots is used in basketry, while the strong red dye yielded by the bark and root is used in skin tanning (Brenan and Greenway, 1949; Palmer and Pitman, 1972; Beentje 1994; Mbuya *et al.*, 1994). Following its identification as a substitute for East Indian sandalwood, *O. lanceolata* has been heavily exploited in Tanzania. It is reported that harvesting of the species has been so rampant, involving removal of the whole trees as the roots are believed to have more oil compared with an equal amount of wood from other parts of the plant (Srivanisan *et al.*, 1992; Mbuya *et al.*, 1994).

While harvesting had been going on to date, no inventory has ever been carried out to quantify the resource base in Tanzania. As a result, the current resource situation and its future prospects are not known (Mbuya *et al.,* 1994). The threat facing *O. lanceolata* in Tanzania has recently been realized and it was one of the biodiversity issues discussed during the workshop on "Setting Forestry Research Need and Priories" which was held in Moshi, Tanzania in 1997 and at the Eastern Arc Biodiversity Conference that was held in Morogoro, Tanzania in 1997. Due to unregulated and destructive methods of harvesting that have been taking place for almost five decades in Tanzania, it currently doubted that, some of the gene pool of the superior genotypes of the species may have already been lost, thus reducing the

species to inferior populations as happened with other sandalwood species. Having realized the threat facing *Osyris lanceolata*, the Workshop and Conference drew the attention of scientists and policy makers for urgent actions (Mwang'ingo and Mwihomeke, 1997).

Resource assessment or inventory is considered to be a vital and basic tool that provides knowledge on the amount of resource available, its distribution, characteristics of various populations and changes occurring in them. These in turn provide a picture on the future prospects of the resource and how possibly it can be managed in a sustainable way (Leek, 1965; Bawa and Krugman, 1991). It is also an important process to assist in the identification of potential sources for genetic improvement. It is well known that sampling for *ex-situ* conservation of plant species relies on the population characteristics and variation existing in it. Areas, where *in-situ* conservation programs can be employed as a means of safeguarding the genetic pool, too can be identified through assessment of the species population structure (Kemp and Palberg-Lerche, 1994).

Successful management of *O. lanceolata* in the wild also depends on a thorough knowledge on the biology of the species (Janick *et al.*, 1982). Biological aspects such as flowering and fruiting, plant-pollinator interactions and sexual systems of the species are vital for successful management of genetic resources. *O. lanceolata* is a dioecious species. To facilitate its conservation and management, attention needs to be given to the characteristics of the stand structure with respect to age and spatial distribution of genders. Tree breeding programs are highly dependent on such biological aspects of a species (Bawa and Krugman, 1991). Where *ex-situ* conservation is required, they dictate the design and amount of sampling that need be done during seed collection to cover the genetic diversity existing in the population (Bawa and Ng, 1990; Simons, 1996a).

The timing at which seeds and vegetative material can be collected for propagation is also highly influenced by the phenology of the species (Hartmann and Kester, 1997; Simons, 1996a). The periods of flowering and/or resumption of active growth are well known to be correlated with success of vegetative propagation in many

plants (Nand and Anand, 1970; Joshi *et al.,* 1992). The timing of flowering between males and females and their spatial distribution need to be known as these may have a big influence on the overall pollination and seed production of the species (Baker, 1976; Cruden, 1976).

The pattern of interaction between the species and pollinators is another important biological phenomenon that can influence pollination success. Where extreme pollinator specialization, occurs, management of the pollinators may become as important as the management of the species itself (Wiebes, 1979; Bawa and Krugman, 1991). However, little study on most of these biological aspects are reported in *O. lanceolata* in the whole of tropical African range which in turn add to overall complications in the management of the species.

The most desirable means by which the sustainability of *Osyris lanceolata* can be ensured is through domestication. Being a marketable species, its domestication stands a very good chance of being accepted by farmers as the benefits of the species are already known to them (Mbuya *et al.,* 1994). In addition, domestication is a useful tool for *ex-situ* gene conservation for such threatened species. It also indirectly facilitates *in-situ* conservation by enabling the local communities to have the resources on their land. This reduces their dependency on the natural forest where the species can be safely conserved (Simons, 1996a; Baricevic *et al.*, 1997; Dawson, 1997; Lulandala, 1998). Since 1990s when the Government of Tanzania banned further harvesting of the species, the emphasis has been encouraging individuals, private firms and government institution to domesticate the species as another source of income. The move also aimed at safeguarding the species in the natural populations from further deterioration before basic information to guide its conservation and management are drawn. However, the species has never been successful domesticated in Tanzania or elsewhere due to lack of basic information on the silviculture of the species. No propagation methods, nursery techniques and field procedures for its domestication have yet been developed. Seeds of the species are reported to have dormancy problems in addition to being recalcitrant (Mbuya *et al.*, 1994; Msanga, 1998).

Trials to propagate the species through seed have had no success due to difficulties of germinating the seeds brought by dormancy problems. Germination was reported to be poor, hardly reaching 50 % in a very sporadic way over eight weeks (Msanga, 1998). The seed coat is generally thin but impermeable to water, which might be the cause of the germination problem. Msanga (1998) suggested that the application of some pre-sowing treatments might improve the rate of germination although no trials have been carried out to investigate the effectiveness of various pre-sowing treatments. Supplies of sound viable seeds have also been a problem as the rate of predation and pathogenic attack in the species seems to be high with most seeds being damaged by a beetle, *Dismegistus sargumeus* that feed on the fruit juice of the species (Personal observation). Also, the recalcitrant behaviour of seeds makes the storage of the seeds difficult (Mbuya *et al.,* 1994). This means the seeds need specialised handling and storage facilities that are usually expensive and not affordable locally. Hence there is need to develop an appropriate method of seed storage and pre-treatment techniques.

Information on ecological requirements of the species had also been scant, as little has been explored in this species especially in characterizing its habitats (Miller, 1989; Beentje, 1994). This knowledge is important for domestication and management of the species. Some of the most important environmental conditions that need to be taken into account are climate, soil and the association of the species with other species of both flora and fauna (Munyanziza, 1995; Dawson and Were 1997). This is essential information needed for successful domestication of the species as it offer guidance in matching sites for domestication or plantation development with the species requirements (Evans, 1982; Jha, 1994).

Osyris lanceolata is a hemiparasitic plant. Plants with this behavior usually require the presence of a host plant for their normal growth and survival. Plantation silviculture of parasitic species is well known to be complex than traditional monoculture due to the need to provide a range of host plants to support their survival (Srivassan *et al.,* 1992; Radomiljac *et al.*, 1999). *O. lanceolata*, being a hemi-parasitic species, requires the presence of host plant for its growth and

survival. According to Metcalfe (1950), Rao (1942a), Mbuya *et al.* (1994) and Herrera (1984a) the species rarely survives beyond a year if haustorial connection with a host plant is not established. Furthermore, some parasitic plants are known to be host specific (Ananthapadmanabha *et al.*, 1988). Specific hosts are required to assist the parasite in nutrient and water uptake (Metcalfe, 1950; Miller, 1989; Niranjana and Shivamurphy, 1987; Beentje, 1994; Herrera, 1984a). The main nutrients met through this relationship include phosphorus, potassium and magnesium (Rai and Salma, 1990). Field studies in India have revealed some plants that commonly grow in association with *O. lanceolata* and some are considered to be possible hosts. They include *Euphorbia hirta, Jasminum rigidum, Orthosiphon diffusus, Phyllanthus simplex* and *Lophopogon tridentatus* (Rao, 1942a). In South-western Spain, Herrera (1988a) reported *Pistacia lentiscus, Phillyrea angustifolia* and *Juniperus phoenicea* to be the most frequent hosts. However, no study has been carried out in tropical East African region to identify possible host plants and their potential to support the growth of *O. lanceolata*. Besides, most of the above hosts don't occur in Tanzania.

1.2. Objectives

In the light of the foregoing, the general objectives of the present study were to assemble and collate existing information from literature and other secondary sources on the biology, ecology, silviculture and resource role of *O. lanceolata* and then to determine through original fieldwork the current population status, biology, oil chemistry and ecology of *O. lanceolata* in Tanzania. Developing possible propagation methods of the species for its efficient domestication was also an objective. To be able to fulfill these objectives, several research questions (hypothesis) were raised in relations to specific objectives be fulfilled. These research questions are presented under the specific objectives of each study presented in chapter III-VII of this thesis. The conclusions reached for the set research questions are presented in chapter VIII that covers general discussion, conclusions and recommendations.

1.3. Study sites

The field study was carried out in six natural forest of Sao Hill, Image, Nundu, Gubali, Bereko and Mgwashi located in the southern, central and northern parts of Tanzania between March 1999 and February 2001. The locations of these study sites are shown in Figure 1.1 while Plate 1.1 and 1.2 present the typical physical feature of *Osyris lanceolata* supporting stands as observed at Sao Hill and Gubali stand.

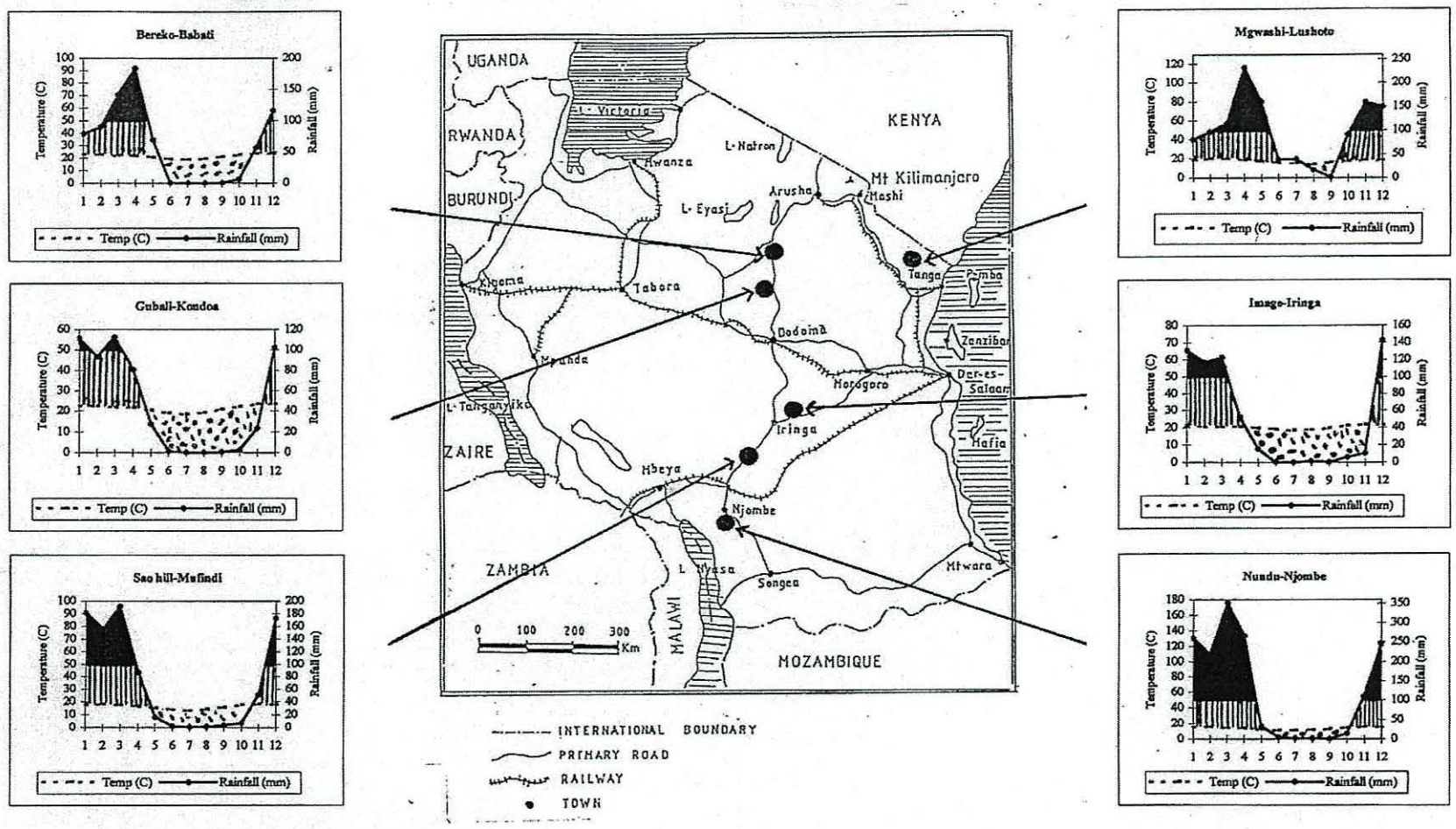
1.3.1 Sao Hill forest

Sao Hill forest is part of the Sao Hill forest plantation project in the Mufindi district in Iringa region located at 8° 18'- 8° 33' S and 35° 6'- 35° 20'E at an altitude of about 1900 m above sea level (Madoffe and Austara, 1993; Ishengoma *et al.*, 1995). It is about 600 km south west of Dar es Salaam (the capital city of Tanzania) and 90 km south of Iringa township. It is the largest state owned plantation with a gazetted area of about 95,000 hectares. Of these, 65000 hectares is suitable for production forestry and the rest is unsuitable being covered by either broad swamps or underlain by hardpans of rocks and stones (Mgeni, 1986).

The climate of Sao Hill is largely influenced by altitude and topography. It is characterized by one rainy season with a mean annual rainfall of 900-1300 mm. Minimum rainfall is about 800 mm while 2000 mm is the maximum. The rainy season is between November and April with showers or drizzle in May. Temperature fluctuates between about 23 °C maximum and 10 °C minimum. Frost is common in the months of June to August (Nykvist, 1976a; Mgeni, 1986; Nshubemuki *et al.*, 1996).

The soils of the area are granitic in origin, being deep and relatively uniform in physical structure, well drained and mostly of sandy clay loam texture. The chemical status of the soil is generally low with nitrogen, organic carbon, available phosphorus and exchangeable bases being below the optimum level for most agricultural crops. The soils are moderately acidic at the top and become more neutral with increasing depth (Nykvist, 1976b).

Figure 1.1 Map of Tanzania, showing study sites and their climatic condition of rainfall and temperature



Source: Adapted and modified from Mgeni (1986)

Much of the land that is not yet subjected to cultivation is covered by grass. The common species include *Loudetia simplex, Themeda trandra, Hyperrhenia* sp. and *Pennisetum maximum*. The areas which were formerly cultivated are colonized by rhizomatous grasses *Imperata cylindricum, Digitaria abyssinica* and *Pennisetum clandestinum*. The natural tree and shrub vegetation is rather scattered occurring predominantly in clumps or individually around rocky knolls or gullies. The common woody species include *Brachystegia* sp., *Albizia* sp., *Croton* sp., *Erythrina abyssinica, Cussonia kirkii, Parinari curatellifolia* and *Protea uhehensis*. The section of the forest, which was sampled for the present study, was dominated mainly by *Osyris lanceolata, Maytenus heterophylla, Tecomaria capensis, Dodonaea viscosa* and *Rhus natalensis.*

1.3.2. Image forest

Image forest reserve is in Iringa district, about 60 km from Iringa Township along Iringa-Dar es Salaam road. It is located at 36° 08'E - 36° 12'E and 7° 28' S - 7° 35' S at an altitude of about 1900 m above sea level. The climate of the area is influenced mainly by topography. The mean annual rainfall of the area is about 600 mm ranging between 450 - 720 mm per year. The rain is unimodal with one rainy season between December and April. Temperature fluctuates between the monthly means of 27 °C maximum to 14 °C minimum. June, July and August are the coldest months with maximum mean monthly temperature of 25 °C during the day and 12 °C during the night (FAO, 1984).

The topography of the area is composed of series of undulating hills and valleys with slopes of more than 40% being the major feature. The vegetation is mainly composed of *Brachystegia* and *Isoberlinia* woodlands. The most dominant species are *B. spiciformis, B. utilis* and *B. glaberrima* and *Isoberlinia. Osyris lanceolata* tress are scattered here and there within the woodland. The secondary forests and areas at lower altitudes are dominated by upland dry sclerophyll forests with *Combretum sp., Albizia sp.* being common. Soils are predominantly laterized low-humic red earths, being fertile initially, but with frequent cultivation, fertility is lost rapidly. However, where serious soil deterioration has not taken place, recovery of the vegetation is usually rapid (Gilchrist, 1952).

Plate 1.1. Physical feature of *Osyris lanceolata* supporting stand as observed at Sao Hill stand, Tanzania. Note the tree cover and bushy characteristics of the site

Plate 1.2. Common physical features of *Osyris lanceolata* supporting stands as observed at Gubali. Note the rocky characteristics and sparse vegetation cover

1.3.3. Nundu forest

Nundu forest reserve is in Njombe district located 200 km south of Iringa Township on the road to Songea. The reserve is part of the continuous forest reserve currently under the management of Njombe district council. The reserve is located at 34° 45', 34° 55' E and 9° 23', 9° 30' S at an altitude of about 1900 m. The site receives a mean annual rainfall of 1508 mm. The maximum rainfall recorded over the past 10 years is about 3800 mm. The maximum mean monthly temperature is about 19.4 °C while the minimum is 7.9 °C (FAO, 1984; NMD, 2000).

The vegetation of the area is composed mainly of thickets of secondary to upland humid evergreen and dry upland sclerophyll forest. In the dry sclerophyll forest, secondary woodland and scrub of *Agauria* and *Myrica* are predominant. Lianas are frequent but epiphytes are rare. Most of the tree species are evergreen and many are sclerophyll, particularly at the lower altitudes. Typical trees present are *Aphloia theiformis*, Myrsine malanophloeos, *Apodytes dimidiata, Albizia gummifera, Ilex mitis* and *Dombeya sp.* The thicket secondary upland humid evergreen forest consists of compact growth of woody shrubs and small trees up to the height of eight meters with occasional emergent trees. Some lianas and scramblers are present but little ground flora. Most trees are evergreen. In addition to *Osyris lanceolata*, the bulk of the thicket is composed of *Apodytes dimidiata, Maesa lanceolata, Ilex mitis, Tecomaria capensis, Myrica salicifolia, Catha edulis, Agauria salicifolia, Albizia sp.* and *Olea sp.* (Gilchrist, 1952).

Soils of Nundu are humic red earths and partially deteriorated forms of the soils exist under upland forests.

1.3.4 Gubali forest

This forest is located at 4°56'S and 35°42 E in Kondoa district, in Dodoma region which is within the semi arid central zone of Tanzania that is considered to be the heart of country's dry lands (Darkoh, 1987).

According to the climatic record of 1931-1980 and the recent data from the Department of Meteorology, the area receives an annual rainfall of about 640 mm occurring within 60 days in a year (FAO, 1984; Mbegu, 1988, NMD, 2000). Years of comparatively less rainfall than 640 mm are regular and expected and the likelihood of rainfall to be less than 500 mm is calculated at 1:6. The actual rain season is between October/November–April/May with short dry spell from January to late February. At 2123 mm per year, the evapotranspiration in the region exceeds average rainfall nearly four times (Christianson *et al.*, 1991). This makes crop and animal failure and even famine to be recurrent dangers in the area (Wenner, 1983; KIRDEP, 1992; Mwatebele, 1999).

The topography is generally flat, with altitude between 1000 and 1500 m above the sea level. A number of ranges dissect the general plain of the region from northwest to southeast. Some ranges are prominent outcrops rising to 1800 m above sea level. About 10% of the total area in the district is designated as Kondoa eroded area, presenting a classical example of soil erosion in the whole of Tanzania. Physically, most of the district is dominated by inselbergs, undulating hills and pediments (Mgeni, 1985; Ostberg, 1986).

The vegetation in the area is categorised as bushland, woodland, wooded grassland and grassland. The usual *Brachystegia/Julbernardia* or miombo woodland occur in most parts. Common species include those of *Osyris lanceolata*, *Brachystegia, Isoberlinia, Acacia, Albizia, Commiphora, Combretum* and *Euphorbia*. Grass cover is dominated by species of *Aristida, Eragrostiella diffusus, Hyparrhenia and Setaria*. Most grass species and herbs are short lived. The vegetation is usually devoid of grass undercover due to overgrazing and uncontrolled annual bushfires that are common throughout the year (Christianson *et al.,* 1991).

In most areas, soils have developed directly from geomorphologically metamorphic rocks. Texture wise they are coarse loam to sandy loam. They are low in organic matter have low bulk density and are low in water retention capacity and base exchange (Tosi *et al.*, 1982). Cultivation of sorghum, maize and millet and grazing are major land use and at least 90% of the total population in the area relies on

agriculture. The main animals kept include cattle, sheep, goat and donkeys (KIRDEP, 1992; Mwatebele, 1999). The traditional bush fallow system that ensured sustainable yield broke a long time ago due to explosion of both the human and bovine populations. This has given way to delicate combination of sedentary agriculture and stock keeping. The livestock units per km^2 are estimated at 40.8, exceeding the allowed carrying capacity of 12 livestock units by 240% (Mbegu, 1988).

1.3.5. Mgwashi forest

Mgwashi forest is within Lushoto district in Tanga region and forms part of the West Usambara Mountains. It is located at 4° 49' S and 38°31'E. Climatic data from Mazumbai forest reserve show that the annual rainfall of the area is about 1150 mm. This rain is received in bimodal pattern with short rains in October-December and long rains in March –June. The short rains account for 25% of the total rainfall and are less reliable. Long rains have their peak in April (Lundgren and Lundgren, 1979). In general areas located to the east and south east of the mountains receive more rainfall as they are the first areas to receive moisture laden south-easterly trade winds from the Indian Ocean. The wind becomes drier as it passes over the western, northern and northeastern parts of the mountains. Consequently, the leeward side where Mgwashi is located gets less rain with some lower slopes receiving even less than 700 mm per year (Mwihomeke, 1987). The mean monthly temperature of the study site is 17.3 °C with the maximum of 20.8 °C and minimum of 13.8 °C (Lundgren, 1978; Mwihomeke, 1987).

The physical environment of the study site is typical of the West Usambara Mountains terrain. There is a pronounced plateau with its greater part lying at an altitude between 1200 to 1300 m above sea level. The terrain is generally steep with slopes of 15–50% being common. This terrain makes the area susceptible to severe soil erosion and landslides especially on deforested, cultivated and overgrazed lands (TIRDEP, 1976). Soils of the West Usambara are classified as dystric nitisols and lithosols with the former being dominant while the later are the common inclusions (FAO-UNESCO, 1977). They are derived from gneissose precambrian metamorphic

rocks with varying quantities of pyroxene, hornblende and biotite (Wiesum *et al.*, 1985). They are considerably weathered with fine to medium texture and they tend to be clay loam or sandy loam in texture. The inherent soil fertility is low to very low and largely depends on the presence of high levels of organic matter associated with forest cover. Surface run off is very high which accelerates both soil erosion and leaching (Moore, 1971a; Mwihomeke, 1987).

The vegetation composition of Mgwashi forest can be described as Somali-Masai scrub according to the description of vegetation of Africa by White (1983) and that of Mwihomeke (1987). Bordering this forest is the deciduous bushland and thicket vegetation of the adjoining semi-arid lowlands. It is characterised by small widely spaced trees over a grass stratum and is mainly composed of species such as *Osyris lanceolata*, *Euphorbia candelabrum, Protea madiensis, Acacia sp., Rhus sp., Dodonaea sp., Combretum sp., Maytenus sp.,* and *Catha sp.* The areas are generally unsuitable for agriculture and are extensively used for animal grazing.

1.3.6 Bereko forest

This study area is in Babati district, about 15 km south of Babati on the Great North road from Dodoma to Arusha. It forms part of Bereko Forest Reserve, a government owned forest under the administration of the Babati district council, which is estimated to cover an area of 9840 ha. It is located at 35°47'E and 3°45'S at an altitude of 1200 m above sea level. The mean annual rainfall is about 750 mm per year, most of which falls within November and May. The dry season is during the months of June to December. The mean monthly temperature is about 23 °C with the minimum monthly is 14.5 and maximun of 25.2 °C (Lindsrom, 1998; NMD, 2000).

The vegetation composition is typical miombo woodland, which is characterised by sparse vegetation cover usually of low stature trees. *Leguminoceae* species, the most important being *Brachystegia utilis, B. spiciformis* with various species of *Acacia* and *Isoberlinia* dominate tree cover. Other important species include *Combretum, Dalbergia, Strychnos* and *Osyris lanceolata* (Moore, 1971b). Soils of Babati are classified as red earths (Berry, 1971).

1.3.7 Iringa Zonal Tree Seed Centre

This centre where most of the nursery studies were carried out is located at $35^{0}41'$ E, $7^{0}46'$ S and 1640 m above sea level within the municipality. This zone is one of the three centres of the Tanzania National Tree Seed Program (NTSP) established in 1989 with the aim of procuring tree seeds for National and International supply (NTSP, 2000).

The mean annual rainfall of the centre is about 580 mm, the minimum being 450 mm and the maximum 720 mm per year. The rain is unimodal with one rainy season that is, between December and April sometimes extending to June. Temperatures fluctuates between the mean monthly of 27 °C maximum to a minimum of 14 °C. June, July and August are the coldest months with maximum mean monthly of 25 °C during daytime and 12 °C during night hours (NMD, 2000).

1.4 Summary

The study reported in this thesis was undertaken:

i. to provide information urgently needed by scientist, foresters, development agents and policy makers on the current population of *O. lanceolata* in order to develop strategies for conservation and management if the species
ii. to develop efficient method of for its propagation in order to assist farmers in the domestication of the species on farmlands to diversify their product and income and at the same time provide a means for *ex-situ* conservation of the species.

This thesis has 8 chapters. Chapter I comprise this introduction, which is made up of the background information to the study, the justification and the objectives. It also includes the description of the study sites. The current state of knowledge on *Osyris laceolata* is presented in Chapter II which consist of four major sections. Section 2.1 reviews the biology of the species including taxonomy and phenological aspects. Section 2.2 covers while section 2.3 introduces the silviculture of the species. The

last section 2.4 covers the resource role of *Osyris lanceolata*. Chapter III consists of 4 sections. Section 3.1 comprises literature review on population studies, assessment and its characterization. Section 3.2 describes the methodology employed in assessing and characterising the populations of *Osyris lanceolata* in Tanzania. The results obtained and the discussions are presented in Section 3.3 and 3.4, respectively. Chapter IV deals with the oil chemistry of *Osyris lanceolata.* The identification of host plant species of *Osyris lanceolata* and their relationships are presented in Chapter V. Chapter VI is concerned with storage and pre-treatment of seeds of *Osyris lanceolata.* Chapter VII deals with vegetative propagation of *Osyris lanceolata* and has 4 sections. Literature on vegetative propagation is presented in Section 4.1. In Section 4.2 methods of propagating *Osyris lanceolata* by stem cutting and air layering are described. The results of the propagation experiments are presented in Section 4.3 and these are discussed in Section 4.4. Finally, all the results of the present study are discussed and consequent conclusions and recommendations are made in Chapter VIII.

CHAPTER II

OSYRIS LANCEOLATA: CURRENT STATE OF KNOWLEDGE

2.1 The biology of *Osyris lanceolata*

2.1.1 Taxonomy and systematics

O. lanceolata Hochst & Steudel. commonly known as East African Sandalwood (Dale and Greenway, 1961; Breintenbach, 1963) belongs to the family Santalaceae. Santalaceae is a family of tropical and temperate herbs, shrubs and trees most of which if not all are hemi-parasites. While they are able to manufacture their own complex food substances through photosynthesis, they require the presence of host plants from which they absorb water and minerals through haustoria connections. They are mostly root parasites but a few are epiphytic branch parasites (Rao, 1942a, b; Metcalfe, 1950, Heywood, 1978).

Several trees of this family are known as sandalwood and they provide wood having a characteristic smell. Through wood distillation, an essential oil used in medicine and perfumery is obtained (Walker, 1966; Curtis, 1967; Errickson *et al.*, 1973).

The family Santalaceae is characterized by plants having bluish green leaves or yellow green leaves with no stipules, their arrangement being often alternate or opposite. They are simple, entire and sometimes reduced into scales. The flowers are usually small greenish or white, regular, hermaphrodite or unisexual and actinomorphic. The calyx of the species is greenish or petaloid often fleshy, adnate to the ovary with 3-6 lobes vulnate or slightly imbricate with no petals. The number of stamens is the same as and opposite to the calyx being attached to the tube below perianth. The ovary is inferior or half inferior being a one celled, simple style with 1-3 ovules. The fruit is nutlike or drupaceous, being indehiscent and dry or fleshy, having one seed with no testa and much endosperm (Breintenbach, 1963; Heywood 1978; Palgrave, 1977; Miller, 1989; Beentje, 1994).

2.1.1.1 Tribes of the family Santalaceae

The family Santalaceae is divided into three tribes namely *Santaleae, Thesiae* and *Anthobleae* currently comprising a total of about 36 genera with some 400 species widespread in temperate and tropical regions of the word (Heywood, 1978; Miller, 1989). The tribe *Santaleae* to which *Osyris* belongs, contains about 27 genera being characterized by an inferior ovary, the receptacle shallowly saucer or cup-shaped and lined with a nectar secreting disc. The *Thesieae* tribe is characterized by the ovary being inferior and the receptacle deeply cup-shaped, without a disc. This tribe comprises about five genera. On the other hand, the *Anthobleae* comprises three genera which are characterized by the ovary bring superior to inferior, the ovules not fully differentiated from the placenta and the pedicel becoming swollen and fleshy as the fruit develops (Heywood, 1978).

2.1.1.2 *Osyris* as a genus

The genus *Osyris* was first described by Linnaeus in 1753. It is a genus with trees or shrubs which are dioecious or subdioecious and possesses leaves which are alternate and entire. The inflorescence is of subumbellate lateral cymes. Flowers have a uniseriate perianth, (3-4) -merous with lobed disc subtended by minute caducous bracts. Female flowers are with inferior ovary, simple style and 3-lobed stigma. Male flowers do not have an ovary and style. Fruits produced in this genus are drupes (Baker and Hill, 1911; Hill, 1915; Miller, 1989).

Three genera of the family Santalaceae i.e. *Osyridocarpus, Thesium* and *Osyris* occur throughout Africa (Baker and Hill 1911; Stauffer, 1961; Gilbert, 1970; Miller, 1989). The key to these genera is (Miller, 1989) as follows.

1. Shrub or trees, sometimes scandent, more than 0.5 m tall; leaves elliptic-oblong, fruit a drupe. 2.
   - Herb or undershrubs to 0.5 m; leaves scale like or linear, fruit a nut, rarely fresh 1. *Thesium*
2. Flowers 3(-4) - merous, plants dioecious 3. *Osyris*
   - Flowers 4 (-5) -merous, bisexual 2. *Osyridocarpus*

The species *Osyris lanceolata*

Many species have been proposed as species name for African Osyris. These are indicated in Table 2.1.

The first description of *Osyris lanceolata* in Tanzania was that of Engler (1895). The species was originally named as *O. tenuifolia* Engl. using the type specimen from Moshi, Tanzania. In 1932, Peter described another three species of *Osyris* from Tanzania that were considered different from *O. tenuifolia,* and were named by him as *O. laeta, O. densifolia* and *O. oblanceolata.* Generally *O. lanceolata* displays a big variation in size and leaf form, and this can be evidenced by a lot of synonyms that have been applied to it to denote different species in different areas shown in Table 2.1.

The increased number of the species of *Osyris*, and the names which were being applied to denote different species prompted Stauffer (1961) to review all the African Santalaceae and see whether the reported species were really new or not. It was concluded that, the various species of *Osyris* reported were actually not different from *O. lanceolata,* as originally described by Hochstetter and Steudel (1832). The confusion arose because the species displays a big variation in morphological attributes especially in size and leaf form, depending on the geographical region where it grows.

The only species similar to *O. lanceolata* and maintained by Stauffer is transferred to a separate genus as *Colpoon compressum,* a species restricted to South Africa. The obvious differences between them are on leaf positioning and flowers. While *C. compressum* has opposite leaves and flower heads produced in the terminals, *Osyris lanceolata* has alternate leaves and flowers which are produced in the axil of the leaves (Brown, 1932; Palmer and Pitman, 1972; Wyk and Wyk, 1997).

Table 2.1. *Osyris lanceolata:* chronology of nomenclature and synonymy

Author and year	Names	Remarks
Hochstetter and Steudel (1832)	*Osyris lanceolata* Hochst. &Steudel	*Osyris lanceolata* named and described for the first time as reported in Exsic. Urio. Itim. Schimper South Africa (type specimen from Spain).
Decaisne (1836)	*O. quadripartita* Decne.	An *Osyris* specimen considered as new and described as *O. quadripartita* (type specimen fom Algeria).
Richard (1850)	*O. abyssinica* A. Rich.	Specimen of *Osyris* from Ethiopia (Tigray) was named by Achille Richard as *O. abyssinica,* because of leaf size and shape differinces compared to the former two species of *Osyris.*
Wight (1852)	*O. wightiana* Wight.	Wight observed a specimen of *Osyris* to differ from with what had already been collected. It was considered a new species and was named after him as *O. wightiana* by Nattaniel Wallich. The origin of the species was presumably India.
Griffith (1854)	*O. nepalensis*	A species of *Osyris* that looked similar to those above was identified by Griffith in 1854. Due to size and texture difference of the leaves, this species was considered as a new and named *O. nepalensis.*
de Candolle (1857)	*O. quadrifida* A.DC. *O. arborea* A.DC.	Specimens of *Osyris* from Ethiopia and Nepal were studied by de Candolle who concluded that both specimens were different from *O. abyssinica* The species from Ethiopia and Nepal, were named as *O. quadrifida* and *O. arborea,* respectively.
Balfour (1884)	*O. pendula* Balf.f.	Balfour obtained a specimen from Socotra and concluded that it was a new species. The species was then by him as *O. pendula*
Engler (1892)	*O. rigidissima* Engl.	A specimen of *Osyris* from Somalia was named by Engler as the new species *O. rigidissima.*

Jackson	(1895)	Six species considered : *O. quadripartita*, *O. quadrifida, O. lanceolata, O. nepalensis O. wightiana* and *O. arborea*	In the first issue of Index Kewensis, *O. quadripartita* and *O. quadrifida* were sunk into *O. lanceolata. O. nepalensis* and *O. wightiana* were sunk into *O. arborea.*
Engler	(1995)	*O. tenuifolia* Engl	An *Osyris* specimen from Kilimanjaro, Tanzania was described as a further new species *O. tenuifolia.*
Pilger	(1906)	*O. divaricata* Pilg.	A specimen from India was named *O. divaricata.*
Baker	(1910)	*O. parvifolia* Baker.	Baker received an *Osyris* specimen collected by Rolf and described it as *O. parvifolia.*
Peter	(1932)	*O. densifolia* Peter *O. laeta* Peter *O. oblanceolata* Peter	In 1932, three specimen of *Osyris* from Tanzania were obtained and considered as new and different species of Osyris : *O. densifolia, O. laeta* and *O. oblanceolata*
Parsa	(1948)	*O. daruma* Parsa	An *Osyris* specimen from Iran was desribed as a new species *Osyris daruma.*
Cufodontis	(1953)	*O. abyssinica, O. rigidissima, O. parvifolia, O. wightiana, O. arborea and O. tenuifolia*	Reduces the species accepted for Eritrea, Ethiopia and Somalia to three. *O. rigidissima* is sunk into *O. abyssinica. O. arborea* and *O. tenuifolia* are sunk into *O. wightiana. O. parvifolia* is retained as the third distinct species.
Stauffer	(1961)	*O. lanceolata* Hochst. & steudel	A review of African Santalaceae, which concluded that the various species described since *O. lanceolata*, are all of that species *O. lanceolata*.
Miller	(1989)	*O. quadripartita*	Miller made another review of *Osyris* occurirng in Ethiopia and other parts of Africa. He agrees with the review of Stauffer (19619 but suggests the name *O. quadripartita* be adopted as it was initially introduced in 1836 while the initial description of *O. lanceolata* was considered to be that by de Candolle in 1857. However, this overlooked the original description by Hochstetter and Steudel in 1832.

2.1.2 Description of *O. lanceolata*

2.1.2.1 Seedlings

Little information has been found in the literature on the mode of seed germination of this species. However, post germination information reveals that once seeds have managed to germinate and establish a root system, seedlings of *O. lanceolata* are able to survive on their own water and mineral supply for a maximum period of one year. After that period, they must have established haustoria connections with other plants to enhance nutrient uptake. If they fail to do so, the survival of the seedling is impaired. Most seedlings are likely to die as a result of this (Herrera, 1984a).

2.1.2.2 Habitat, size and form of mature trees

Osyris lanceolata occurs on the edges of forest and woodland, often along streams in gullies, under cliffs and in dry localities. Also it occupies rocky places on hillside (Breintenbach, 1963; Beentje, 1994, Wyk and Wyk, 1997). The species is an evergreen dioecious shrub or small tree growing to a height of 1-7 m tall depending on soils, climatic conditions and genetics of the tree. In Tanzania, Kenya and Ethiopia, the species grows to a height of up to 7 meters (Breintenbach, 1963; Mbuya *et al.*, 1994; Beentje, 1994). In South Africa, the maximum height growth reported is 6 meters (Palmer and Pitman, 1972) while in the Mediterranean region, the maximum height recorded is 3 meters, with most species being between 1 and 2 meters (Herrera, 1984a; 1988a). Normally it is a highly branched tree with branches sometimes pendant (Miller, 1989). The bark of the mature tree is light gray brown or black (Mbuya *et al.,* 1994). Plate 2.1 shows the multi-stem behavior of *O. lanceolata.*

2.1.2.3 Foliage

The species is evergreen, possessing leaves throughout the year. Mature leaves are bluish green or yellow green and sometimes grey glaucous. They are simple, alternate, entire and shortly mucronate obovate, elliptic or oblong measuring up to 8 cm long but in most cases it is 2.5 cm long or less (Plate 2.2). Veins, more or less immersed, only the midvein raised below and running back down stem in narrow ridge

(Hilliard, 1994; Beentje, 1994; Mbuya et al 1994). The margins are slightly thickened with very short petiole 1-3 mm long (Von Breintenbach, 1963). The species displays an extreme variable in leaf and size leading to its being recognized as new species once it is met in different localities (Miller, 1989).

Plate 2.1. The multi-stem of *Osyris lanceolata*

Plate 2.2. Leaves of *Osyris lanceolata*

Inflorescence, flowers and flower buds

The species produces small flowers in the axils of leaves and they are pale yellow green inconspicuous, in short terminal heads, being either male or female (Plate 2.3). Male flowers are produced singly or in pairs in axial cymes about 1.5 cm long in the axils of leaves. Female flowers are much larger than males, pedicellate, solitary and axiliary, the inflorescence bearing 3 flowers. Flowers of both sexes secrete nectar. Female flowers develop to fleshy single seeded fruits (Dale and Greenway, 1961, 1983; Miller, 1989; Beentje, 1994; Mbuya *et al.*, 1994).

Joshi (1960) made an observation on the floral development of the species and noted that, floral organs develop in acropetal succession and those of the androecium follow the primordia of the periath. As the carpels grow, they enclose a small cavity where the central placentum develops simultaneously. Gradually this acquires a dome shaped appearance and bears three, occasionally four, anatropous ovules. The development is similar in the male flowers, but the growth of the gynoecium is arrested.

2.1.2.5 Fruit and seeds

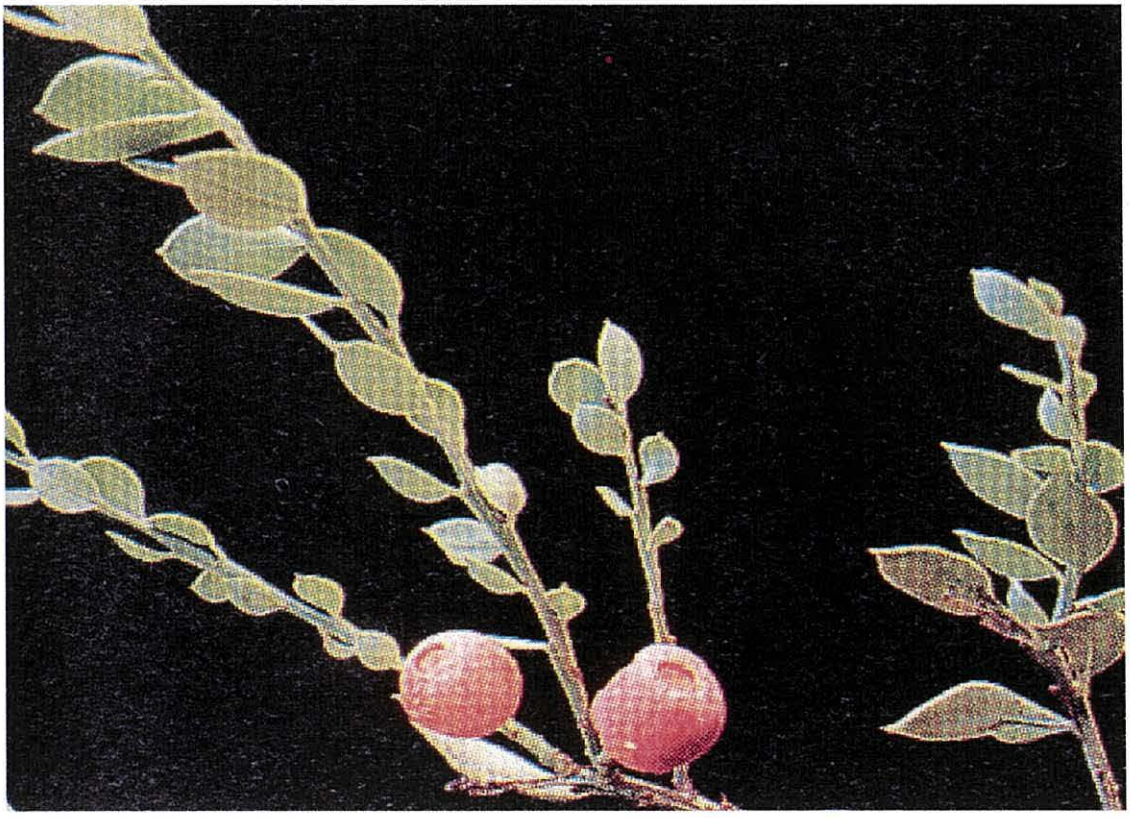
The ripe fruit of the species is a red colored drupe containing one seed (Plate 2.4). The color of the fruit turns to purple black as it matures more (Breintenbach, 1963; Retief and Herman, 1997). There is a great variation in the size and composition of fruit produced between and within individuals as reported by Herrera (1988b). Diameter ranges from 4.8 - 10.1 mm. The flesh mass of individual fruits varies from 95 to 890 mg composing 24-232 mg of seeds and 58-657 mg of pulp. Seeds are known to be recalcitrant, hardly storable for more than two weeks. A kilogram of seeds contains about 11,000 seeds (Mbuya *et al.*, 1994).

2.1.2.6 Chromosome number and dioecity

There is no recent publication describing the number of chromosomes in *O. lanceolata*. However, Darlington and Wylie (1945) reported chromosome number in this species to be 2n = 30.

Plate 2.3 Flowers and flowering in *Osyris lanceolata*

Plate 2.4. Ripe fruits of *Osyris lanceolata*



The species is dioecious i.e. male and female plants are separate. Species displaying this character have been shown to have male and female plants being either randomly distributed (Ngulube, 1996; Oni, 1997; Herrera, 1988a) or individuals of the same sex may be clustered in a population (Freeman *et al.*, 1976; Cox, 1981). The sexes also tend to differ in their vegetative and reproductive features (Hancock and Bringhurts, 1980; Shea *et al.*, 1993; Lloyd and Webb, 1977).

A study carried out in south-western Spain to determine plant size, spacing pattern and overall distribution of the species revealed that, the sex ratio in *Osyris lanceolata* is mostly distributed in 1:1 ratio, though some slight deviation were observed in some populations. Size distributions showed that male tend to be taller than females, although in some cases, few females exceed the height of males (Herrera, 1984a; 1988a).

2.1.2.7 Phenology and Life cycle

O. lanceolata is an evergreen species, retaining its leaves throughout the year. In the Mediterranean region, growth and formation of new leaves have been observed to resume following the autumn rains. Flowering of the species starts by bud initiation in March, being produced exclusively on the new growth of the current year. Buds appear continuously as actively growing shoots elongate. Cessation of growth also arrests flower production. The actual full flowering period is sometimes very long and variable, lasting for about 6 months (Herrera, 1984a; Herrera, 1988b). Table 2.2 summarizes the flowering seasons reported by different authors and from herbarium collections in different places.

In the Mediterranean region, flowering has been observed to take place between March - September for females and almost throughout the year for males. However, the peak flowering period for both sexes is between May and June (Herrera, 1984a; Herrera, 1985). In Tanzania, flowers have been observed between August and February (Brennan and Greenway, 1949). The herbarium collections show a similar

trend although flowering in other months of the year is common. In South Africa, Retief and Herman (1997) reported flowering to take place between October and April, the same time reported in most collections observed at Kew botanical garden herbarium (personal observation).

Table 2.2. Flowering periodicity of *Osyris lanceolata*

Source	Flowering period												Location
	J	F	M	A	M	J	J	A	S	O	N	D	
Herrera, 1885			X	X	X	X	X	X					Mediterranean, 37°26'N, 5°45'W
Evans, J.B. 1957								X					Ethiopia
Barger, W. 1961									X				Ethiopia
Thulin, M and Hunde, A. 1980					X								Ethiopia
Gillet, J.B. 1952											X		Ethiopia
Eggeling, W.J. 1936	X												Uganda
Eggeling, W.J. 1940										X			Uganda
Thomas, A,S. 1940					X								Uganda
Purseglove, 1948		X											Uganda
Hudson, D. 1957							X						Uganda
Clover, E.C. 1963				X									Kenya
Polhill, E. 1966			X										Kenya
Perdue, R.E. and Kibuwa, S.P, 1966												X	Kenya
Wood, D. 1966						X							Kenya
Hepper, F.N and Jaeger, P.L.M. 1978.											X		Kenya
Drumond, R.B. and Feden, A.J. 1974						X							Kenya
Bridson, D. 1984								X					Mufindi, Tanzania
Ruffo, C.K, 1984										X			Lushot, Tanzania
Iversen et al., 1985											X		Lushoto, Tanzania
Greenway, 1963												X	Lake Manyara,Tanzania
Ruffo, C.K and Kisena, C.M. 1987											X		Sumbawanga, Tanzania
Grimshaw, J.M. 1993							X				X		Kilimanjaro, Tanania
Retief and Harman, 1997	X	X	X	X						X	X	X	South Africa
Germishuizen, G. 1978											X		South Africa
Muller, M. 1979		X											South Africa
Burtt, B.L. 1982	X												South Africa
Burtt, B.L. 1984												X	South Africa

The interval between anthesis and fruiting is quite variable due to within-plant variances in developmental rates. Consequently, ripe fruits are produced almost throughout the year. Fruits resulting from the previous year's flower tend to coincide with fruits in the following year, especially those, which are produced from flowers, set earlier in the population (Herrera, 1985). Two fruiting peaks have been observed in the Mediterranean region. The major one appears in winter and a minor one in spring (Herrera, 1988b). Brennan and Greenway (1949) reported fruits to appear in the months February- April in Tanzania. Herbarium collection shows that fruits are likely to be observed at any time of the year. It should be noted that information on phenology is largely based on the Mediterranean region, as little information on this aspect exists in other parts, especially in the tropics. Table 2.3 summarizes the fruiting seasons by various authors and herbaria collections.

2.1.3 Reproductive biology

The information on the sequential events of flowering in this species from other parts of the world seems to be scant apart from those reported by Herrera (1984a; 1985;

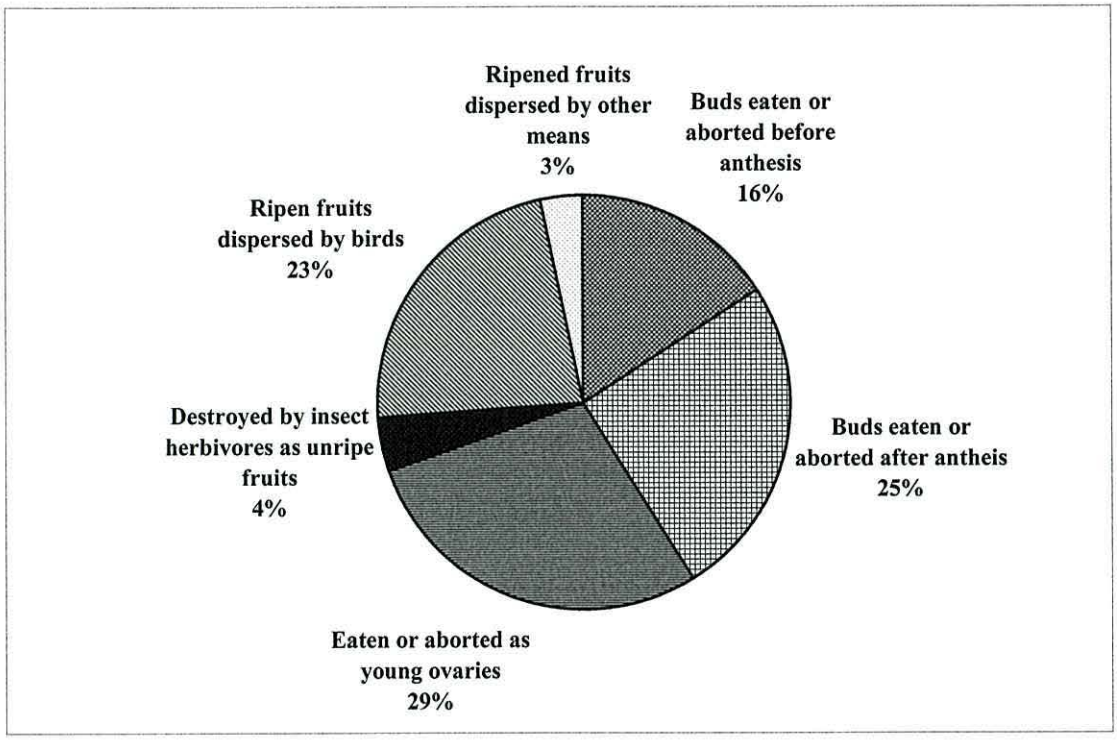
Table 2.3. Fruiting periodicity of O*syris lanceolata*

Source	Fruiting period												Location
	J	F	M	A	M	J	J	A	S	O	N	D	
Herrera, 1985	X	X	X	X	X	X	X	X	X	X	X	X	Mediterranean 37°26'N, 5°45'W
Gillet, J.B. 1952.											X		Ethiopia
Barger, W. 1961									X				Ethiopia
Thulin, M. and Hunde, A. 1980					X								Ethiopia
Bridson, D, 1984								X					Mufindi, Tanzania
Grimshaw, J.M. 1993							X						Kilimanjaro, Tanzania
Greenway, P.J. 1946		X											Hanang, Tanzania
Semsei, S.R. 1956								X		X			Songea, Tanzania
Burtt, B.L. 1982	X												South Africa
Venter, S. 1976												X	South Africa
Muller, M. 1979		X											South Africa
Germishuizen,G. 1978											X		South Africa
Leakey, D.G.B. 1935												X	Kenya
Putseglove, P.J. 1948		X											Uganda

1988b) in the Mediterranean region. Flowering of the species starts by formation of flower buds, which appear on new growing shoots of the species. The interval between flowering and fruit ripening is reported to be extremely variable. Ripe fruits have been observed throughout the year. In the study to investigate the significance of various stages in determining reproductive rates of female *O. lanceolata*, Herrera (1985) observed that the interval between bud formation and peak flowering was about two weeks. After anthesis and flower withering, the ovary is modified to what is called latent ovary. This stage can be retained without evidence of unripe fruit formation for an average of 6 weeks though the range of 1-25 weeks is not uncommon. After unripe fruits have been formed, they may remain dormant for about 15.4 weeks or more without fruit ripening. The average duration between anthesis and fruit ripening in the Mediterranean region is six months with the extreme range of 13 - 54 weeks.

Herrera (1985) also made a follow up on the proportion of buds formed to fruit that reach dispersal stage in the Mediterranean region. It was observed that of flower buds produced 84.3% reaches anthesis stage. Caterpillars feeding on new growth ate most buds that failed. Among the open flowers, only 70% manage to produce a latent ovary, while 51.2% of latent ovary formed unripe fruits. Majority of the latent ovary which failed to form unripe fruits, 98.3% were simply aborted while few (1.7%) were eaten by insect herbivores. 86.4% of unripe fruits manages to reach ripening stage with the remaining portion being destroyed by insect herbivores. Ripe fruits, which were removed by birds from tree, were about 86.8%. This proportion is what was considered successfully dispersed. Thus out of flower buds produced, only 30% reach unripe stage while the percentage of fruits that are dispersed is only 22.4%. Figure 2.1 present the proportion outcome of buds as they move from bud initiation to fruit/seed dispersal. Abortion rate is high for latent ovary resulting in the dry season. It is suggested that, the high rate of abortion is a response toward resource limitation. The species favors development of latent ovary in accordance with resource availability.

Figure 2.1. Reproductive success in *Osyris lanceolata*: proportion outcome of buds as they move from but initiation to fruit/seed dispersal



Source: Summarized from Herrera (1985)

2.1.3.1 Pollination and anthesis

Tropical trees posses a diverse array of pollination mechanisms and usually each species is visited by a large number of visitors, although few of them may be the principal pollinators (Bawa, 1976). In *O. lanceolata*, insects are reported to be principal pollinators. In the Mediterranean region, small-sized flies are reported. However, several ant species have been recorded to feed on species nectar although it is doubted whether they play any significant role in pollination apart from being nectar thieves (Herrera *et al.*, 1984).

2.1.3.2 Seed biology and dispersal

Seeds of the species are recalcitrant in nature and they tend to loose viability within few days. However, there is no data quantifying the period through which seeds can

remain viable. Due to recalcitrance, coincidence of rain season and fruiting is very important for successful natural establishment through seeds (Mbuya *et al.*, 1994). Seeds of the species are mostly dispersed by birds in the Mediterranean region, which eat about 86.3-94% of the fruits (Herrera, 1984b; 1985). The fleshy fruits are normally favored by frugivorous birds, which eat the ripe drupes and defecate the seeds or regurgitate them. The main species of birds responsible for seed dispersal in the Mediterranean region are reported to include *Turdus merula, Sylvia atricapila* and *Sylvia borin* (Herrera, 1984b).

2.2 Ecology

2.2.1 Origin and affinities

The origin of *O. lanceolata* seems to be uncertain, as concise description of its origin has not been found in the literature. According to the tree and shrub selection guide of South Africa by Wyk and Wyk (1998), the species has its bio-geographic origin in Tropical East Africa, particularly Ethiopia. It appears to have migrated into the Mediterranean region via North Africa. The great difference in the size of the species between the Mediterranean region (Herrera, 1984) and tropical East Africa (Breintenbach; 1963, Beentje, 1994; Mbuya *et al.*, 1994) is further evidence of this origin. The dwarf-sized species in the Mediterranean region could be an adaptation by the species to try to undergo some modification to cope with the unfavorable growth conditions of water and minerals stress in the region. On the other hand Herrera (1988b) considers the species to have its origin in Mediterranean region before the current ecological condition of winter-rain and drought conditions become operational when the warm summer-rain tropical climate existed in the area. Even the unusual phenology of *Osyris lanceolata* observed in the Mediterranean region is postulated to be attributed to its tropical relict conditions. The species is considered to be a survivor of an old evergreen tropical flora currently living in the Mediterranean refugia, having changed little since the initiation of Mediterranean climate in the Pliocene (Herrera, 1984b, 1985).

2.2.2 Geographical distribution and range

This species is widely distributed in the tropics and temperate tropical regions, being more concentrated in dry woodlands and shrubs (Heywood, 1978; Miller, 1989). It occurs in the Mediterranean region in a variety of Mediterranean-type scrubland, with the range including the Canary Islands, Northwest Africa and Iberian Peninsula (Herrera, 1984a; Bramwell and Bramwell, 1974). In Africa the species is widely distributed in the continent, mainly in dry woodland forests. It is mostly found in East and Central Africa and southward to Zambia, Namibia, Zimbabwe and South Africa (Lugard, 1933; Miller, 1989; Brennan and Greenway, 1949). India is perhaps the eastern boundary of the species in the tropical region (Rao, 1942a). In most cases, the species is never locally abundant though wide spread (Palgrave, 1977).

The distribution of the species in Tanzania is also wide. It occurs in highland forests and bushes in almost all floristic regions of the country. In northern Tanzania the species is reported to occur in West Usambaras and its adjoining lands (Handeni, Malimbwi-Lushoto, Pare mountains), Motomoto Hills-Kondoa, Mlali Hill-Mpwapwa, Lupembe-Njombe, Mufindi-Iringa, Litenga Hill-Songea, Shishiye-Hanang and Babati (Brenan and Greenway; 1949; Mbuya *et al.*, 1994; Ruffo *et al.*, 1997).

2.2.3 Ecological requirements

The species occurrence ranges from semi humid lowland-woodland, semi-arid lower highlands to semi arid upper highland forests. It prefers areas where the original vegetation has been cleared, forest margins, evergreen bushlands and thickets, degraded woodland and scrub and on mountain slopes (Miller, 1989; Beentje, 1994). The altitude range of the species is about 800 - 2600 m above sea level. In Ethiopia, the species has been reported to occur at 1200 - 2500 m (Breintenbach, 1963) while in Kenya the species is found between 900-2550 m (Beentje, 1994). Troupin and Bridson (1982), reports the species to be occurring at an altitude between 1300-1500

m in Rwanda while in Lesotho it is found at 1500-2000 m (May, 1994). The site of the species occurrence is generally on rocky areas and mountainous slopes, though it is not uncommon in other soil types (May, 1994).

2.2.4 Plant associations

O. lanceolata is an evergreen root hemi-parasite occurring mainly in semi-humid woodlands and upper highland forests on forest margins and bush (Breintenbach, 1963; Beentje, 1994; Mbuya *et al.*, 1994). In semi humid lowlands, the species has been found in areas dominated by *Croton* thickets while in semi arid lower and upper highlands it is mainly associated with *Acacia* forests (Breintenbach, 1963). In the Mediterranean region, the species is reported to occur in a variety of Mediterranean type scrublands (Herrera, 1984a). According to White (1983), *Osyris* is among the common species in the Somalia-Masai region center of endemism particularly in the evergreen and semi-evergreen bush-land and thickets. The species also forms a component of the Mediterranean regional center of endemism in littoral communities along with *Juniperus phoenicea.* It is also present in *Tetraclinis articulata* forest, which is confined in North Africa.

Associate woody species of *Osyris lanceolata* are not much explored. However from herbarium collection, some of the common species found to be associated with *O. lanceolata* include *Apodytes dimidiata, Catha edulis, Syzygium guineense, Trichocladus ellipticus, Mystroxylon aethiopicum, Cassiopourea gummiflua, Aphloia theiformis, Ekebergia capense, Garcinia kingaensis, Dodonaea viscosa, Bersama abyssinica, Cussonia spicata, Rothmannia fischeri, Oxyanthus speciosus* and, *Rutidea fuscescens*. Others are *Warburgia, Podocarpus, Brachystegia* and *Polyscias* from Tanzania. Collections from Kenya, shows *O. lanceolata* to be mostly associated with *Carissa edulis, Flueggea virosa, Tapiphyllum schumannianum, Euphorbia pseudograntii, Vernonia brachycalyx* and *Rhus natalensis*. The other associated community is the *Acacia xanthophloea* woodland in association with *Solanum incunnum, Cordia monoica, Teclea simplicifolia, Justica striata, Pavonia burchellii* and *Sphaeranthus confertifolius*.

In Uganda, the species is found mainly in open woodland in association with *Protea abyssinica, Faurea speciosa, Combretum molle*, *Acacia seyal,* and *Heeria reticulata.* On fire-free slopes *Catha* species are common. In Ethiopia, the species has been found in areas dominated by *Loudetia arundinacea, Combretum molle, Pappea capensis, Dodonaea angustifolia* and *Dichrostachys cinerea* especially on open bush-land and low-woodland steep slopes. At bottom slopes, dominant species are *Terminalia brownii* and *Acacia mellifera*. Other common species include *Erythrina abyssinica, Steganotaenia araliaceae Ozoroa insignis, Zanthoxylum chalybeum*, *Cussonia* and *Grewia sp.*

2.2.5 Parasitism in *Osyris lanceolata*

Like many species in the family *Santalaceae*, *Osyris lanceolata* is a hemi-parasitic plant, and it requires the presence of host plants for better growth and survival performance (Metcalfe, 1950; Miller, 1989; Beentje, 1994). The parasitic nature of the plants is believed to be a strategy for survival in a resource-limited environment where it is mostly found. It assists the species in getting some of the most important requirement of water and minerals (Metcalfe, 1950; Rai and Sarma, 1990). The species is non-host specific as it parasitises roots of many plants through haustoria connection (Rao, 1942a; Niranjana and Shivamurthy, 1987). However, Herrera (1988a) reported some preference in hosts in the overall species with female being slightly more selective compared to males in southwestern Spain of the Mediterranean region.

A follow up on plant development has shown that, during early development, young seedlings may survive up to one year without the presence of host plants. After this period, growth is retarded followed by the their death if they fail to establish haustoria connections (Rao, 1942a; Metcalfe, 1950). The main nutrient requirements met through this connection include phosphorus, potassium and magnesium, which are of crucial importance in plant growth (Rai and Salma, 1990).

Several species have been identified to be the possible host of the species, with the spectrum ranging from grasses to another sandal plant (Niranjana and Shivamurthy,

1987; Rai and Salma, 1990). Some of the reported host plants from India include dicotyledons species such *Euphorbia hirta, Jasminum rigidum, Orthosiphon diffusus, Phyllanthus simplex, Stachytarpheta indica* and *Stylosanthes fruticosa.* Monocotyledon species include *Chryosopogon orientalis, Cyanotis tuberosa, Eragrostis bifaria, Heteropogon contortus, Panicum trypheron* and *Lophopogon tridentatus* and (Rao, 1942a). In South-western Spain, Herrera (1988a), reports a total of 16 species to be the hosts with *Pistacia lentiscus, Phillyrea angustifolia* and *Juniperus phoenicea* being the most frequent hosts. Others include *Myrtus communis, Rosmarinus officinalis, Corema album, Erica scoparia, E. arborea, Daphnegnidium, Quercus cofficera, Pinus pinea, Rhamnus lycioides, Cistus libanotis, Stauracanthus genistoides, Ulex parviflorus* and *Cytisu grandiflorus*

The haustoria connecting the parasite to the host usually arise from the cortex, endodermis and pericycle. To enable its effective penetration, the haustoria of the species can undergo several modifications depending on how easy or difficult it is in penetrating the host root tissues. Where the host tissue offers great mechanical resistance to penetration, a complex anatomical structure largely consisting of vessel members that are wedge shaped may be formed. The sucker will then penetrate the host tissue through mechanical force (Rao, 1942b; Metcalfe, 1950; Niranjana and Shivamurphy, 1987).

2.3 Silviculture and management of the species

Apart from its importance, information on the silvicultural aspects of this species is scarce. It is likely that, its treatment simply as a component of vegetation has led to little investigation in this area.

2.3.1 Natural Regeneration

Natural regeneration of *O. lanceolata* as a means of its spread is currently assumed to take place through seeds and root suckers (May, 1994; Mbuya *et al.*, 1995). It appears that the main means of spread could be through coppicing and root suckering as seeds have problems in germination due to dormancy and recalcitrant

character (Mbuya *et al.*, 1994; Msanga, 1998). The recalcitrant behavior and fruit maturity during the dry season (Personal observation) gives little hope of having much regeneration through seeds.

2.3.2 Artificial regeneration

Little has been done in this area and the only information available is on seed germination. Mbuya *et al.* (1994) and Msanga (1998) reported the possibility of raising the species artificially through seeds. Under normal conditions, 50-60% of seeds sown could be expected to germinate in not less than 8 weeks. Some pre-sowing treatments such as soaking seeds in hot water or scarification of the seeds coats have been suggested to be the possible ways of improving germination. However no trials have been done to see how successful these methods could be. Currently little information exists in the literature on growth of the species beyond seed germination. Mbuya *et al.* (1994) reported the species to be slow growing, requiring the shade of nurse trees at early stage of growth.

2.4 *Osyris lanceolata* as a resource

2.4.1 Sandal oil

Sandalwood oil, which originates from the distillation of roots and heartwood, is considered to be the most important product from this species (Schery, 1954; Walker, 1966; Dale and Greenway, 1961). Sandal oil is largely preferred in various perfume compositions of oriental and occidental types. Highly superior blends are prepared from it using other perfumery material like bergamot, clove and lavender. Sandal oil has earned a prominent place in agarbathi, cosmetics, fragrance and soap industries. It forms a base in making sandal soaps, talcum powder, perfume and incense (Metcalfe, 1950; Curtis, 1967; Heywood, 1978; Naipawer, 1986; Rai and Sarma, 1990; Srinivasan *et al.*, 1992). As the heartwood is the most important in *Osyris lanceolata* and sandals in general as it contain more oil, the sapwood portion is usually scraped out during harvesting aiming to reduce unnecessary load to carry

as shown in Plate 2.5. Also the root system of *Osyris lanceolata* and most sandal species is believed to contain reasonably high amount of oil compared with an equal amount of wood processed from the shoot system (Srinivasan *et al.*, 1992; Fazal, M, personal communication). For this reason, root portion is always included during harvesting of the species.

Apart from its importance as a supremely satisfying source of fragrance, sandalwood oil finds use in medicine. It is used in ayurvedic as well as allopathic systems of medicine (Shineberg, 1967; Rao and Bapat, 1993). It is useful as an antiseptic, antipyretic, antiscabietic,diuretic, expectorant, stimulant and for treatment of bronchitis, dysuria, gonorrhea and urinary infection (Okasaki and Oshima, 1953; Winter, 1958). The constituents of the benzene extract of the bark are reported to exhibit an excellent insect growth inhibiting property and chemosterilant activity (Shankaranarayana *et al.*, 1980).

Plate 2.5. *Osyris lanceolata* wood ready to be sold. Note the removal of sapwood and inclusion of the root system

2.4.2 Wood

The wood of the species is of great value (Hill, 1937; Walker, 1966; Beentje, 1994). It is close grained like ivory and ebony and is used in the manufacture of carvings, furniture, combs, chess pieces, cosmetic boxes and other curios of exquisite beauty (Shineberg, 1967; Heywood, 1978). The wood is insect proof (Saad, 1983).

2.4.3 Traditional uses

Local communities utilize *Osyris lanceolata* as an important source of medicine. Its bark and root decoction is used to treat diarrhea, gonorrhea and other urinary diseases and chronic mucus infections (Saad, 1983; Brennan and Greenway, 1949). Fruits form an important dietary supplement. Fibers from roots are used in basketry, while the strong red dye yielded by bark and root is used in skin tanning (Palmer and Pitman, 1972; Beentje 1994; Fichtl and Adi, 1994; Mbuya *et al.*, 1994). *Osyris lanceolata* being an evegreen tree/shrub with long flowering period encompassing almost the whole year in some locality, forms a good forage plant. Honeybees collect both nectar and pollen from the species (Fichtl and Adi, 1994).

CHAPTER III

THE STATUS AND CHARACTERISTICS OF POPULATIONS OF *OSYRIS LANCEOLATA* IN TANZANIA

3.1. Background information and objectives of the present study

3.1.1. Introduction

Following its identification in early 1900s as a substitute of Indian sandalwood, *O. lanceolata* has been heavily exploited in Tanzania. Where possible roots are also excavated, as these are believed to contain more oil compared with other parts of the plant (Mbuya *et al.*, 1994). However, no inventory has ever been carried out to quantify the resource base. As a result, the current resource situation and its future prospects are not known (Mbuya *et al.,* 1994).

The increased demand and threat facing *O. lanceolata* in Tanzania was first brought to the attention of scientists during the workshop on "Setting Forestry Research Need and Priories" which was held in Moshi, Tanzania in 1997. The matter was brought up again as one of the biodiversity issues that were discussed during the "Eastern Arc Biodiversity Conference" held in Morogoro, Tanzania in 1997. It was realized that unregulated and destructive harvesting have been taking place for almost five decades in Tanzania, and there is a possibility that some of the gene pool of the superior genotypes have been lost, ultimately reducing the species to inferior populations. For this reason, the workshop and conference drew the attention of scientists and policy makers to take some measures for an efficient management and conservation of the species (Mwang'ingo and Mwihomeke, 1997).

The present study was, therefore, undertaken to assess the resource base in Tanzania in order to obtain some of the needed information that would help guide the development of management and conservation strategies for the species. Key issues addressed in the present study include the current distribution and status of the populations of the species and their characteristics.

3.1.2 Population structure and status in plant communities

Population status of a species is determined based on assessment of the population structure in terms of size class distribution of the species. Knowledge on the structure of the population is of considerable importance in the management of any species or forest in general. It is a vital and basic tool that provides knowledge on the amount of resource available, its distribution, characteristics and changes occurring in the population. These in turn will provide a picture on the future prospects of the resource and how possibly it can be managed in a sustainable way (Leek, 1965; Bawa and Krugman, 1991). It is also an important process that could assist in the identification of potential sources for genetic improvement. It is well known that sampling for *ex-situ* conservation relies on the population characteristics and variation existing in it. Areas where *in-situ* conservation programs can be concentrated as a means of safeguarding the genetic pool could as well be identified through assessment of the species population structure (Kemp and Palberg-Lerche, 1994).

A common characteristic of size class distribution of most tropical trees in a population is the pronounced absence of saplings and juveniles. This type of size class distribution results when regeneration of a species is severely limited for several reasons, with most seedlings dying before becoming established (Richard, 1952; Whitmore, 1975). A stable self maintaining population is characterised where there is a smooth decrease in the number of individuals from the smaller to large size classes, with the intermediate class being well represented (Leek, 1965).

Variation in size class distribution can occur between populations. Morphological characteristics of trees may also vary within a population as well as between populations. Several factors both biotic and abiotic have been found to influence size class distribution and morphological characteristics existing within and between populations. The major abiotic factors include water and nutrient availability, soil acidity/alkalinity, soil salinity, presence of heavy metal in the soil and extremes of temperature. The major biotic factors involve the degree of exposure of the population to disturbances such as fire, browsing and other human impacts (Crawley, 1986).

Availability of water in the soils is a prime determinant of species structure. It regulates the growth of height and spacing of plants in an ecosystem (Titley, 1982). Water use efficiency is a strategy that plants adapt in areas where water is in short supply so as to conserve the minimal amount obtained. In this case leaf area may be highly reduced or stomata are set in deep pits in the epidermis or leaf surface may be covered with hair or leaves may possess thick and waxy epidermis (Kozlowisk, 1972, Levitt, 1972). The difference in leaf morphology reported in *Osyris lanceolata* by Herrera (1988a), Mbuya *et al.* (1994) and Breintebach (1963) in Mediterranean region, Tanzania and Ethiopia, respectively could be attributed to the difference in water availability among the sites.

Another important factor influencing population variability is temperature. Extremes of temperatures between sites contribute to plant size and stature differences as the rate of biochemical reactions are temperature dependent (Went, 1953; Crawley, 1986).
One of the edaphic factors that influence the morphology and size class distribution of a species is soil nutrient status (Bell, 1982). Species from low nutrient environments are characterised by small size. Leaves are too characteristic, being small, leathery and long-lived (Chapin, 1980, Vitousek, 1982). Soil acidity exerts its most important ecological effect by influencing nutrient availability and the concentration of potentially toxic ions such as aluminium (Clymo, 1962; Fitter and Hay, 1981). Soluble aluminium interferes with nutrient uptake and can inhibit root growth, particularly that of seedlings (Clymo, 1962). Low soil pH is associated with phosphorous deficiency (Crawley, 1986). Soil salinity also has an influence on plant growth and the principal effect of it is felt via the plant's altered osmotic balance. Most plants growing on salty areas grow only poorly while those in non-saline soils do well (Wainwright, 1984).

Presence of heavy metals in the soil such as lead, cadmium, zinc, copper, nickel, chromium and mercury may exhibit important effect on growth of certain plants leading to their stunted growth (Bremner and Bradshaw, 1976). These metals are known as environmental pollutants. Their effects on plants are felt through direct toxicity, leading to stunting and chlorosis; antagonism with other nutrients, often leading to symptoms of iron deficient; and inhibition of root penetration and growth (Antonovics *et al.*, 1971; Hughes *et al.*, 1980; Crawley, 1986).

The degree of exposure of the site to disturbances such as fire, browsing and other human impacts can result into difference in plant size and architecture. Frequent fires destroy the vegetation and retard growth of many plants depending on its intensity. To avoid being destroyed, plants in fire environment tend to build some resistance and other features including development of thick fire resistant bark, regeneration by sprouting from root suckers or surviving stems and possession of underground specialised organs like lignotubers that can not be easily killed by fire (Crawley, 1986).

Human and browsing animals create a characteristic population with species whose morphology gives them a certain tolerance to bruising, compression and other physical abuse. Human impact has a big influence where the species have a good commercial value. Removal of the species will normally concentrate on big trees of good form, leaving behind small sized ones of poor form to form a population (Errickson *et al.*, 1973). Hence the extent to which two communities are exposed to this pressure may result into sites having trees of different sizes with different distribution pattern.

However, little and perhaps no study to characterise the habitats and their influences on the structure and morphology of *Osyris lanceolata* has so far been conducted in Tanzania and perhaps in the whole of tropical region.

3.1.3. Natural regeneration in plants and its success

So far little study has been carried out to determine the regeneration status of *Osyris lanceolata* in Tanzania or elsewhere. Natural regeneration is an important process in perpetuating plant communities in their natural habitats. It is the process through which new plants replace their predecessors of the same species (Walter, 1971; Misra, 1989). It is the process that shows the dynamic of the forest and determines the fate of any particular species in the future (Viana, 1990). Natural regeneration of tree species takes place either through seeds or vegetative means mainly through root or coppice sprouting. The same way as regeneration by seed, regeneration of plants through vegetative means also depends on the immediate existence of the parent tree in an area or parent might have existed in the area in the past. The main form of vegetative

regeneration is through root suckering and coppicing. These usually occur following disturbances imposed on the natural communities such as logging and land clearing (Mugasha, 1978). Although it has been claimed that *Osyris lanceolata* regenerated by seed and root suckers, no evidence exists in the literature on the occurrence of regeneration by root suckering.

The success of natural regeneration also depends on the potential for regeneration largely determined by the availability of seeds and seedlings, and the potential for re-growth, determined by the availability of conducive environment such as light, moisture and nutrient (Viana, 1990).

Regeneration from seeds begins with the dispersal of seeds from the mother trees to suitable sites for germination. For successful germination, the dispersed seeds must be viable and must escape from predators. They must also encounter suitable germination sites in terms of light, moisture and temperature. All these factors together with nutrient relations and herbivory control the growth and reproduction from seeds (Harper *et al.*, 1965; Hutching, 1986; Bazzar, 1991). Dispersed viable seeds can immediately germinate if they meet favourable environmental conditions or can be stored in the soil bank as dormant seeds waiting for the condition to come. The time of stay in the soil seed bank varies depending on the species. Some tree species produce dormant seeds that may stay in the soil for a long time without loosing their viability while others produce seeds that can hardly stay for a week. It is due to the presence of seed bank in the soils that have made some species to germinate even in places where the mother trees are not evident (Harper, 1977; Hurka and Neuffer, 1991).

The amount of viable seeds produced also influences the regeneration that can be obtained. The higher the quantity of seeds produced, the higher is the chance of regeneration (Janzen 1970; Howe, 1980; Hubel, 1979, 1980; Peterson and Pickett, 1995). Viable seeds also have to escape from predators if they have to germinate. It is known that on tropical mainland especially in the dry forests, more than 90 % of all tree species have more than 50% of their seeds killed by predators or fruit fungi between fruit set and seed germination. This places regeneration of many plants through seeds to be problematic (Janzen and Yanes, 1991).

The spatial distribution of regenerating seeds and hence seedlings of a particular plant are determined among other factors by the pattern of seed rain (dispersal) about each parent. This is influenced by wind and water movement or by abundance and activity of animal dispersal agents (Bawa, 1976; Hutching, 1986). Generally seed dispersal is viewed as an adaptation to increase the chance of survival of the offspring of a particular species. It enhances survival probability by removing the offspring from the mortality factors acting on density dependent or distance dependent manner or both (Janzen, 1970; Connell, 1971). Pathogens, seed predators, herbivores, allelopathy, and competition between off springs and parent are among the factors that can affect seedling growth near the mother trees but they are escaped through seed dispersal. Seed dispersal also increases the probability of survival of the offspring by sending it to new or vacant sites or to more suitable habitats for regeneration (Howe, 1980; Augspurger, 1983; Ernst, 1991).

3.1.4. Reproductive biology in plants and its success

Success management of *Osyris lanceolata* partly depends on a thorough knowledge of the reproductive biology of the species (Janick *et al.*, 1982). Biological issues such as flowering and fruiting, plant-pollinator interactions and overall sexual systems of the species are vital. With *Osyris lanceolata* being a dioecious species, for facilitation of its conservation and management special attention is needed to characterization of stand structure with respect to age and spatial distribution of genders. Successful breeding programs for species rely on these and other biological aspects (Bawa and Krugman, 1991). Where *ex-situ* conservation is required, they dictate the design and amount of sampling that need to be done during seed collection to cover the genetic diversity existing in the population (Bawa and Ng, 1990; Simons, 1997). Knowing exactly at what time some phenological events occur is important as timing of seeds and vegetative material collection for propagation are highly influenced by phenology of the species (Hartmann and Kester, 1997; Simons, 1997; Nand and Anand, 1970; Joshi *et al.,* 1992). The timing of flowering between males and females and their spatial distribution need to be known as these may have an important influence on the overall pollination and seed production of the species (Baker, 1976; Cruden, 1976; Bawa, 1980b).

Among the important phenomena that form a basis of the reproductive and breeding system is the phenology of the species, including the flowering and fruiting pattern and pollination ecology. It provides a sound basis for conservation and improvement programme for any species (Bawa, 1976). The timing and quantity of flowering can influence the extent to which mating is random. Where the species flowers at different times, chances of mating either between populations or within individuals of the same population may be reduced. This may reduce the effective breeding population size and crossing may be limited (Bawa, 1976). Relatedness within the population may therefore be higher than expected and this in turn will affect the degree of diversity that is sampled when seed is collected. The number of flowers and seeds borne by the tree also affect their contribution toward the pollen and seed genetic pools, which has an implication for sampling variation and also for the prospect of genetic improvement (Simons, 1996a). also the amount of flowers produced in a plant is positively correlated with the number of fruits and seeds produced. In years of poor flowering, less seeds is expected (Bullock and Bawa, 1981; Oni 1997).

While studies on flowering and fruiting periodicity have mainly been derived from community wide studies (Frankie *et al.* 1974; Appanah, 1985), individual tree species in the tropics display much variation in the timing, duration and frequency of flowering. Even within the species there is sometimes considerable difference with regard to the time when the species flower (Frankie *et al.,* 1974). According to Hubel and Foster (1983), more than 50% of tree species in the tropics have been observed to have only 2-3 mature individuals per hectare. Yet these species display complex flowering patterns with pollination being exclusively by animals ranging from tiny wasps to large bats (Appanah, 1981; Bawa *et al.*, 1985).

In dioeceous plants (separate male and female plants), studies on reproductive biology are even more complex. Complexity increases especially where the product of economic interest is seed or fruit. The optimal yield requires an appropriate number of mixture of male and female plants (Bawa and Krugman, 1991). The distribution of male and females has an influence on pollination as pollinators are mostly attracted to males than females. The male flowers usually offer more floral rewards to pollinators in terms of pollen and nectar compared to female. Thus, sometimes as a strategy of being

pollinated, some female flowers have a tendency of mimicking male flowers (Baker, 1976; Bawa, 1980a, 1980b). Generally fruit set which is the percentage of flowers that produce fruits, reflect the number of flowers visited by pollinator. On the other hand, seed set which is a percentage of ovules in pollinated flowers that develops into seeds reflect the number of times a flower is visited by the pollinator. More visits result into transfer of sufficient pollen to the stigma to assure maximum seed set. Thus from management perspective, pollination biology is important in the overall fruit and seed development (Cruden, 1976). As a consequence of this, where domestication of the species is to be considered, then the ratio of male and female species and how they are distributed need to be taken into account to facilitate pollination.

Another interesting aspect in species experiencing dioecity character such as *O. lanceolata,* the mode of distribution between male and female plants in the community/population is an important subject. Often spatial segregation between male and female sexes along the gradient of habitat quality has been revealed (Hutching, 1986; Shea *et al.*, 1993). This tendency can have a big influence on the pollination of the species especially when the distance between males and females clusters is so big (Simons, 1996a). Some reasons have been put forward to explain why clustering of male and female individuals into separate patches may occur. These include differences in resource requirement between sexes. Fruit producing females need more energy for sexual reproduction than males (Wallace and Rundel, 1979, Popp and Rinartz, 1988). Thus when sex clustering occurs, females tend to be in resource rich areas (Freeman *et al.*, 1976; Lloyd *et al.*, 1980).

Cox (1981) put forward another likely reason with regard to sex related niche differences. It is explained that, the difference may be the result of selection against deleterious inter-sexual competition, or consequence for selection against for increased inter-specific competition ability. This proportional difference was described by Onyekwelu and Harper (1979) as the Jack sprat effect or inter-sexual niche partitioning as also described by (Cox, 1981). However, according to Bawa and Opler (1977), a random distribution of males and females is optimal for distribution of pollen and dispersal of seeds. Random distribution facilitates pollen reaching females flowers and reduces mass predation of concentrated seeds.

Evidences have shown that fruit and consequently seed formation may take place in some plants without sex fusion (apogamospermy). In this case the progeny have exactly the same genetic constitution as their parents. Compared with other asexual reproduction, this reproduction means has an added advantage that the seed produced is capable of being dispersed and the potential for extended dormancy (Asker, 1980; Donald, 1986). Species, which have been shown to display this behaviour, include *Antennaria parlinii* (Bayer and Stebbins, 1983) and *Uapaca kirkiana* (Ngulube, 1996).

While these reproductive aspects are of importance in the overall management of any species, little study has so far been conducted on this species apart from that of Herrera (1984a 1985) conducted in the Mediterranean on annual cycles of the species and its pre-dispersal reproductive biology. No other studies are reported on these aspects in the whole of tropical region where it occurs abundantly or somewhere else to provide an overview of the aspects in various regions.

3.1.5. Objectives

The present study was, therefore, carried out with the aim of exploring some basic information that will guide the development of conservation and sustainable management of the species. The specific objectives were:

1. to characterize the current status of *O. lanceolata* in Tanzania with a view of:
   i. assessing the current status of the populations of *O. lanceolata* in Tanzania in terms of size class and gender distribution of the species,
   ii. characterizing the ecological aspects of these populations in terms of associated species, climate and soil characteristics, and
   iii. assessing the status of natural regeneration in these populations
2. to assess the reproductive biology of the species by examining:
   i. the phenological events that occur between flowering and fruiting
   ii. the reproductive success of the species through pollination experiments

To enable fulfilment of these objectives, the following research questions were posed in regard to study on population structure:

i. Where and how much *O. lanceolata* resource is available in the country?
ii. What are the ecological attributes of the sites that support growth of *O. lanceeolata* in terms of soil and climate?
iii. What species occur along with *O. lanceolata* and are its potential hosts
iv. How do morphological attributes of the trees vary within and among populations of *O. lanceolata*?
v. Is this variation reflecting environmental or genetic factors and what is the relative importance of each?
vi. What are the major means through which *O. lanceolata* regenerates and to what extent does each means contribute to the overall regeneration and recruitment of the species?
vii. What is the distribution pattern of male and female *O. lanceolata* within population?
viii. What is anticipated duration of the various phases of the reproductive processes?
ix. What is the reproductive success of the species? Does the distribution of males and females in a stand influence the reproductive success of the species?

3.2 Material and methods

3.2.1 Study sites and criteria for selection

The studies were carried out in six forest sites (described herein as populations) in Tanzania. These sites are shown in a map of Tanzania presented in fig. 1.1.They included Bereko in Babati district, Gubali in Kondoa district, Mgwashi in Lushoto district, Image in Iringa district, Nundu in Njombe district and Sao Hill in Mufindi district. The later three sites are located in the southern part of Tanzania herein referred as the southern ecozone while the former three are located in the central and northern part of the country, referred herein as belonging to the Northern ecozone. The detailed description of the sites is presented in Chapter 1, Section 1.3.

These study sites were chosen because of the occurrence of O*syris lanceolata* in them based on library study and reconnaissance survey that was carried out prior to the

actual field studies. They also represent the different physiographic/floristic regions of Tanzania. Following the reconnaissance survey, the final selection of the six sites was based on a number of criteria including the occurrence of reasonable tree density of *Osyris lanceolata*, accessibility and homogeneity of the population in each site as recommended by Oni (1997). According to Oni (1997), a site for population study should preferably meet two basic criteria among others:

i. Homogeneity: The experimental site to be selected should contain at least 100 mature trees of interest over an area that is uniform in vegetation type as much as possible. Since *Osyris lanceolata* is a small tree/shrub (Palgrave, 1977; Mbuya *et al.,* 1994), mature trees were defined arbitrary as those individuals with dimension of at least 3 cm in diameter at a height of 50 cm above ground.
ii. Accessibility: The sites should be easily accessible to ensure easy reach and mapping of all trees of the species of interest in a sample plot.

3.2.2 Assessment of climate and soil characteristics of the study sites

The following climatic and soil characteristics of each site were assessed. Data on rainfall, temperature and evapo-transpiration for each site were obtained from the climatic data of FAO (1984), which covered a period of 30 years, supplemented by recent data from the nearby meteorology stations. Data from other stations that were not included in the FAO (1984) source were obtained from the Tanzania National Meteorological Department.

To assess the soil characteristics of the sites, soil samples were collected from each of the study sites to get an overall impression of soil fertility status. Samples were collected in each site from three different locations, selected purposely close to areas where trees of *Osyris lanceolata* were highly concentrated. At each location a trench was dug to expose the soil horizon. Leaving the organic layer, two samples at a depth 0-25 and 25-50 cm were collected, making a total of 6 samples from each site. Upon collection, the samples were bagged in black polyethylene bags and transported to Mlingano Agricultural Institute soil laboratory where the soil analysis was carried out. The properties analyzed included soil pH measured potentiometrically in 1:5 soil to solution suspension in water (Van Lagen, 1996); total organic carbon through wet

oxidation-redox titration method (Tiessen and Moir, 1993), total nitrogen determined by measuring the amount of ammonium evolved (Kjedahl method) as described by McGill and Figueiredo (1993), available phosphorus determined calorimetrically through Olsen method using ammonium molybdate as a coloring reagent (Halloran, 1993). Other soil properties analyzed include exchangeable bases (calcium, magnesium, potassium and sodium) and cation exchange capacity were determined through $BaCl_2$ method by removing exchangeable bases with an excess of $BaCl_2$. The removed bases were then measured through atomic absorption and atomic emission spectroscopy. To determine C.E.C, Ba was removed from the solution with an excess of Mg_2SO_4. The Mg loss for the exchange of Ba was then measured to determine C.E.C (Van Largen, 1996).

3.2.3 Assessment of populations of *O. lanceolata*

3.2.3.1 Population structure

In order to assess the population structure of *O. lanceolata*, a sample plot was laid out at each site. The plot was laid out by adapting the nearest neighbor distance approach as described by Krebs (1998) and Bullok (1996) with some modification in order to keep the whole plot as compact as possible. In this method, a starting point, which was one of the trees of *Osyris lanceolata* was chosen. From this point, the nearest *Osyris lanceolata* tree was recorded. The exercise continued until more than 100 trees were included in a plot. For the purpose of mapping, both bearings and distances from one tree to another were recorded. Sometimes, a divergence to this procedure had to be adopted to make sure that the population is kept as intact as possible as the nearest neighbor approach was becoming a unidirectional. Trees were numbered serially using aluminum tags as they were identified and measured.

For every tree that was identified, the following data were recorded: the sex of the tree determined by critical observation of the type of flowers produced aided by presence or absence of fruits; tree total height measured using calibrated ruler stick; tree diameter measured at height of 50 cm above ground using caliper; crown diameter computed as a mean of two crown measurements made along two directions

at 90 degrees to each other intersecting at the base of the tree. Other data collected included: branching height measured vertically from the ground to the base of the first branch using a calibrated ruler stick, number of primary branches arising from the base of the tree crown and the number of stems per tree.

The recorded distances and bearings from one tree to another were used to prepare maps by marking on grids and marking the position of each individual tree and its sex. The maps were then used to determine con-specific nearest neighbors that were grouped into four categories: male-male, male-female, female-male and female-female. The first term in each category showed the sex of reference individual while the second showed the sex of its neighbor. The frequencies of these categories were scored in each population and were used to determine the distribution of males and females within a stand as described by Ngulube (1996). The neighbor distances between trees were used to calculate the mean distance between trees and the mean area occupied by an individual *Osyris lanceolata* in a population. According to Muller-Dombois and Ellenberg (1974), the area occupied by an individual tree in a stand is given as:

Area occupied by a tree = (mean neighbor distance) ^{2}

From the mean area occupied by an individual tree, stocking or density of *Osyris lanceolata* per hectare was estimated as well as the total area of the stand assessed.

Stocking/density per hectare = (1 ha =10,000 $m^{2)}$)/area per tree in m^2

The trees were later summarized according to size classes using the tree morphological data collected in each population. Trees were grouped into:

- five height classes of ≤ 1.5, 1.6-3.0, 3.1-4.5, 4.6-6.0 and > 6 m;
- six diameter classes of ≤ 3.5, 3.6 -5.5, 5.6-7.5, 7.6-9.5, 9.6-11.5 and > 11.5 cm;
- five branching height classes i.e. ≤ 0.5, 0.5-1.4, 1.5-2.4, 2.4-3.4 and >3.4 m;
- six crown diameter classes of ≤1.5, 1.5-2.4, 2.5-3.4, 3.5-4.4, 4.5-5.4 and >5.4 m;
- three classes of primary branches of 1, 2 ad 3 branches; and
- five classes of number of 1, 2-3, 4-5, 6-7, >7 stems.

Trees were also summarized according to their sexes and their morphological characteristics in order to see the differences between sexes in each study site. Allometric relationships between morphological attributes were also explored. Correlation analysis was also employed to explore the relationships between morphological attributes and rainfall amounts and soil characteristics of each site.

3.2.3.2 Status of natural regeneration

Sampling plots each measuring 25 x 40 m (0.1 ha) were laid out at 50 m intervals along an established transect in each study site selected for the population study. The total number of plots laid out in each study site varied depending on the size of the study site. Thus, the number of plots laid out at Bereko, Gubali, Image, Mgwashi, Nundu and Sao Hill were 36, 39, 39, 28, 29 and 43, respectively.

In each plot regeneration was assessed in two positions in relation to the mother trees. These included regeneration occurring under the crowns of *Osyris lanceolata* and outside the crown. Regeneration was also categorized into two types i.e. seedlings (plants whose height is less than 50 cm) and saplings (those exceeding 50 cm in height but less than 2 cm in diameter). The source of regeneration was ascertained and two sources were recognized: seed origin i.e. individuals with no evidence of expanded base or sign of root stock origin and coppice/rootstock origin i.e. individuals arising from a stump or with evidence of root stock origin with expanded base. These regeneration assessment procedures have already been used in several studies such as those of Ngulube (1996) in Malawi assessing regeneration *Uapaca Kirkiana* and Oni (1997) in Nigeria when assessing regeneration of *Parkia biglobosa.*

The abundance of regeneration between sites, sizes, origins and distances from mother trees were compared through analysis of variance. Separation of means where significant differences were observed was done through Tukey's pair wise comparison.

3.2.3.3 Assessment of associated plant species

The same sampling plots laid out for regeneration assessment (Section 3.2.3.2) were used for the study of associated plant species. In each plot, all woody plants whose diameter was at least 3 cm at the height of 50 cm above ground were identified. Identification of the species was done *in situ* by a botanist and where not possible, plant material (samples) was taken to Lushoto herbarium where further identification was done. A list of plant species identified as common species associated with *Osyris lanceolata* was later produced for each site. The data was used to make comparison of associate species occurring between sites using Sorenson index of similarity developed in 1948 as described by Pielou (1977) and Krebs (1998). Given plant composition at site A and B, the Sorenson index of similarity is computed as:

$S = 2a/(2a+b+c)$.

Whereby a = Number of species common to both site A and B.

b = Number of species occurring in site A but not in B

c = Number of species occurring in site B and not in A

Sites were considered similar in vegetation composition when the similarity index was more than 0.5 while dissimilarity was declared if the computed index was below 0.5.

Other data taken along with species identification was the diameter of each individual associated plant, its height and frequency of occurrence in each plot. Frequency data was used to compute the mean stocking of each individual species per plot, which was then converted into per hectare basis. Diameter measurements were used to calculate basal area of individual plants that was thereafter multiplied by stocking per hectare to compute the total basal area per hectare contributed by each species. The total basal area of each species was then used to describe its percentage contribution to the total basal area of each site.

3.2.4 Reproductive biology assessment

3.2.4.1 Reproductive processes

Owing to lack of field technicians to collect data according to prescription given and large cost associated with it, only one site was used for this experiment i.e. Sao Hill to provide a general view of what happens in this species.

Ten randomly selected trees comprising five from each sex were assessed for this study. During flower initiation, which commenced in mid February, five reproductive shoots from each tree were randomly selected. On each shoot, all flower buds were counted and marked. Thereafter, the stages of reproductive development and the length of period required for completion of each stage were observed and recorded at two days intervals. The reproductive stages monitored and recorded followed a modification of Dafni's (1992) five scale points. In female trees the stages of development monitored included bud initiation to active flowering, flowering to stigma withering, fruit initiation, fruit initiation to formation of mature unripe fruit and unripe fruits to formation of ripe fruits. On male trees only two basic stages were monitored i.e. bud initiation to active flowering and active flowering to anthers withering. As the development from one stage to another did not take place simultaneously, the observation and recording of time taken for each stage of development was made on the basis of 25%, 50% and more than 75% of the marked buds.

3.2.4.2 Reproductive success

This experiment was done based on the observation that a number of seeds were found empty during the fruit development study described above (Section 3.2.4.1.) and lack of pollen was postulated to be the cause. Three treatments involving natural pollination, assisted pollination and restricted pollination were laid out for the study of reproductive success. Five large female trees were objectively selected to ensure that the size of each tree was large enough to accommodate all the three treatments. In each tree all three treatments were applied, each to five shoots selected randomly within the tree. In the assisted pollination treatment, pollens were carefully collected

from male flowers using a fine brush and applied to the receptive stigmas of female flowers for consecutive three days as described by Ngulube (1996) and Simons (1996a). The number of opened flowers that received assisted pollination in a shoot was counted and recorded while unopened buds were removed whenever they were formed. In natural pollination, open flower buds were counted while unopened buds and buds that followed later were removed. In the restricted pollination, shoots bearing female flowers were bagged before anthesis using a closely woven nylon cloth (Plate 3.1). Before bagging, unopened buds were counted to make sure that reasonable number of buds was present. Successive buds formed later after bagging were not removed, as opening would have created a chance of some pollens to get in. Removal of bags was done following stigma withering as a sign of deceptiveness (Ngulube, 1996).

The data recorded in this experiment included reproductive success, i.e. number of buds, flowers or fruits that moved from one stage to another by counting the number of flower or fruits retained in the shoot out of the total marked at weekly intervals.

Plate 3.1. Restricted pollination treatment for evidence of agamospemy behavior

Fruit and seed set were assessed as soon as fruit ripening took place. The seed embryo status was examined by visual inspection after cutting the seeds transversally. Seeds were then scored as filled (embryo present) or empty (no embryo).

Data were analyzed using simple descriptive statistics. A T-test was also applied to compare reproductive success between natural and assisted pollination.

3.3 Results

3.3.1. Soil and climatic characteristics of the six populations studied

3.3.1.1. Soil characteristics

There was a distinct pattern of soil chemical property difference between the populations of the Northern and Southern ecozones. Table 3.1 summarises the soil chemical properties of the six populations of *Osyris lanceolata* in Tanzania. Soil pH in the southern ecozone i.e. Nundu, Image and Sao Hill populations was slightly acidic while that of the northern ecozone (Bereko, Gubali and Mgwashi) was slightly alkaline. Similarly, soils of the southern ecozone had high carbon and nitrogen content compared with the Northern ecozone. The highest carbon and nitrogen containing soils were those of Nundu with mean values of 4.67% and 0.41%, respectively while Bereko had the least contents with only 0.62% carbon and 0.07% nitrogen. Soils of the Northern ecozone were relatively rich in available phosphorous and exchangeable bases with Bereko being the richest in available phosphorus (2.26 mg/kg). Nundu had the lowest phosphorous content (0.34 mg/kg). Mgwachi was the richest (7.73 me/100g) in exchangeable bases while the lowest exchangeable bases were found in the soil of Sao Hill that had 3.36 me/100g.

Although there was no similar pattern of difference in cation exchange capacity (CEC) between Northern and Southern ecozones, difference existed between the six populations. Soils of Mgwashi had the highest CEC (7.2 me/100g) while those of Gubali had the least (3.46 me/100g). Nundu and Bereko soil had almost the same CEC

as those of Mgwashi. Base saturation also differed between the Southern and Northern populations with all Northern populations having 100% base saturation. Soils with the lowest base saturation were those of Sao Hill with only 74.2%. However, Image soils in the south had similar base saturation (91%) compared with those of the Northern zone. Exchangeable cation was high in soils of Bereko (0.06 ms/cm), followed by those of Gubali (0.04 ms/cm). Nundu, Sao Hill, Image and Mgwashi soils had similar exchangeable cations (0.02 ms/cm).

Table 3.1 Soil chemical properties of the six populations studied

Property	Soil depth (cm)	Study site/population					
		Nundu	Bereko	Gubali	Image	Sao Hill	Mgwashi
Soil pH (1:2:5-H_2O)	0-20	6.23	7.70	7.37	6.63	6.20	7.17
	20-40	6.27	7.70	7.20	6.67	6.17	7.30
	Mean	6.25	7.70	7.28	6.65	6.18	7.23
Organic Carbon (%)	0-20	5.46	0.88	1.08	1.83	3.07	1.64
	20-40	3.88	0.37	0.74	2.38	2.89	1.29
	Mean	4.67	0.62	0.91	2.10	2.98	1.47
Total Nitrogen (%)	0-20	0.44	0.07	0.09	0.21	0.28	0.14
	20-40	0.38	0.06	0.24	0.20	0.21	0.11
	Mean	0.41	0.07	0.16	0.21	0.25	0.13
Available P	0-20	0.47	2.30	2.44	0.56	0.49	0.62
(mg/kg)	20-40	0.21	2.22	1.97	0.73	0.39	2.61
	Mean	0.34	2.26	2.21	0.65	0.44	1.62
Exchangeables bases (Ca,	0-20	5.46	6.81	4.66	4.48	3.98	7.70
Mg, K, and Na - me/100g)	20-40	3.76	6.22	2.83	5.34	3.38	7.76
	Mean	4.61	6.52	3.74	4.91	3.68	7.73
Cation exchange Capacity	0-20	7.28	5.31	4.30	5.11	5.35	7.32
(me/100g)	20-40	4.89	4.83	2.62	4.26	4.40	7.07
	Mean	6.09	5.07	3.46	4.69	4.88	7.20
Base saturation (%)	0-20	74.0	100	100	89.3	74.7	100
	20-40	75.3	100	100	92.7	73.7	100
	Mean	74.7	100	100	91.0	74.2	100
Exchangeable cations	0-20	0.03	0.08	0.04	0.03	0.02	0.02
(ms/cm)	20-40	0.01	0.05	0.03	0.02	0.01	0.02
	Mean	0.02	0.06	0.04	0.02	0.02	0.02

3.3.1.2 Climatic conditions

The climatic conditions of the six populations showed large variation in terms of mean annual rainfall, temperature and evapo-transpiration. Table 3.2 summaries these parameters for each of the six populations. The detailed monthly variation in these climatic parameters is presented in Chapter I Section 1.3 and Appendix 2. Nundu, and Sao Hill receive relatively high rainfall compared with all the other four populations.

The other four populations more or less receive equal amounts of low rainfall and thus are classified as populations of semiarid areas. The annual evapo-transpiration rate of all the populations with the exception of Nundu exceeds the annual rainfall with Gubali being the driest with the evapo-transpiration rate more than four times the annual precipitation. Monthly temperatures also vary among populations with Nundu and Sao Hill being the coldest. Frost is reported to be common in these populations (Nykvist, 1976a). Gubali experiences the highest temperature compared to the other populations.

Table 3.2 Climatic conditions of the six populations studied

Population/stand	Mean annual rainfall (mm)	Mean monthly temperature (°C)		Mean annual evapo-transpiration (mm)
		Minimum	Maximum	
Nundu	1500	7.9	19.7	999
Sao Hill	950	10.8	21.7	1280
Image	630	14.0	26.5	1400
Bereko	750	14.5	25.2	1550
Gubali	640	15.3	27.3	2123
Mgwashi	680	13.8	20.8	1150

Source: FA0 (1986); NMD (2001)

3.3.2. Neighbor distance, density and sex distribution in populations of *O. lanceolata*

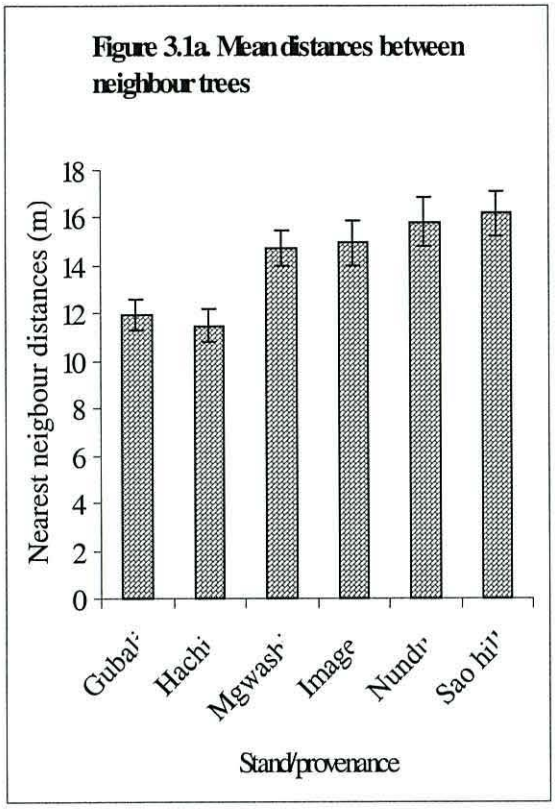
The mean distance between neighbouring sandalwood trees varied significantly among populations ($P<0.01$). The distance ranged from as close as 11.48 ± 0.61 m in Bereko population to as far as 16.18 ± 0.94 m in Sao Hill population (Figure 3.1a). Variation in distance between neighbouring trees was also observed between the populations of the Northern and Southern ecozones ($P < 0.01$). Trees in the populations of the Northern ecozone were closer to each other (12.76 ± 0.40 m) compared with the populations of the Southern ecozone, where spacing between neighbour trees was 15.56 ± 0.56 m (Figure 3.1c). This implies that tree density was higher in the Northern ecozone than in the Southern ecozone.

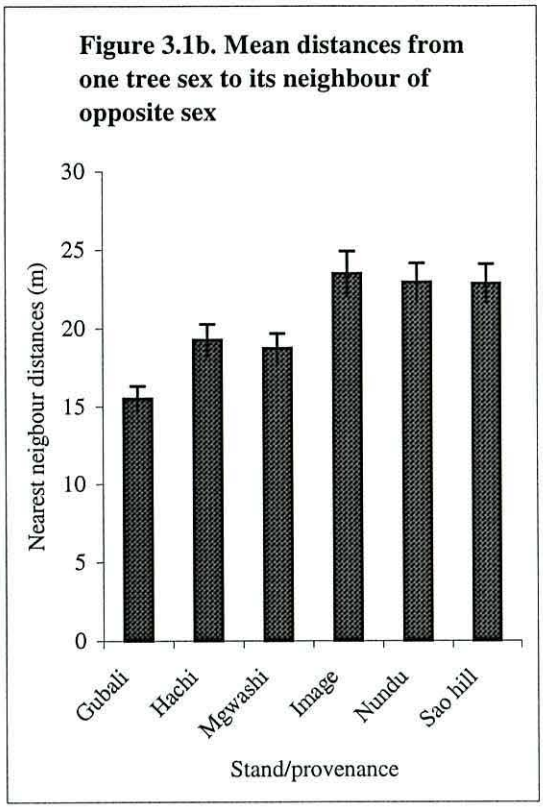
However, the mean basal area per hectare did not differ significantly between the two ecozones ($P = 0.48$) which means trees in the Southern ecozone are relatively bigger in size than those in the Northern ecozone thus offsetting the effect of low tree density. Tree density ranged from as little as 38 individuals per hectare in Sao Hill population to as many as to 76 individuals per hectare in the Bereko population (Table 3.3).

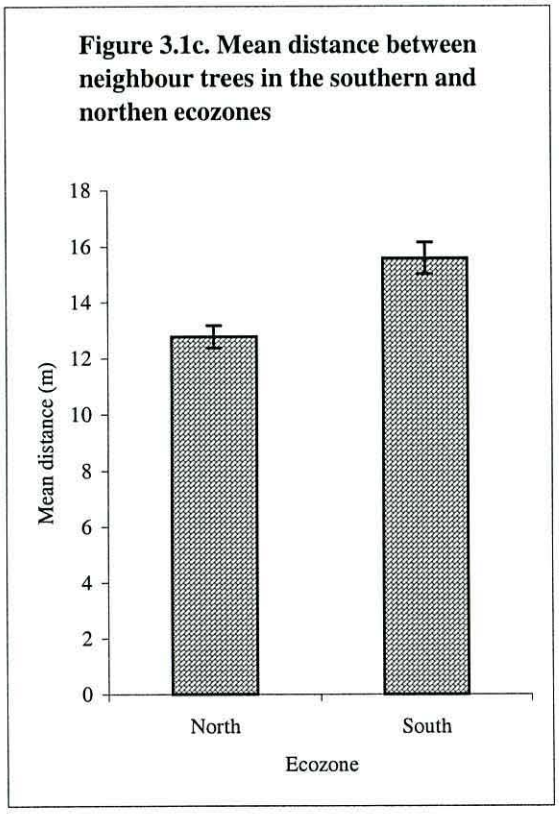
The mean distances from tree of one sex to the neighbour tree of opposite sex also differed significantly among populations ($P < 0.01$). Trees of the opposite sex were closer in the Gubali population (15.51 ± 0.81 m) compared with those of Image, Nundu and Sao Hill. The longest distance between trees of opposite sexes was observed in Image where the mean distance was 23.48 ± 1.41 m (Figure 3.1b). Significant differences also existed between the Southern and Northern ecozones in the distance between opposite sexes ($P < 0.01$) with trees of opposite sex being closer in the Northern ecozone (17.79 ± 0.54 m) compared to the Southern that were spaced at 23.12 ± 0.77 m (Figure 3.1d).

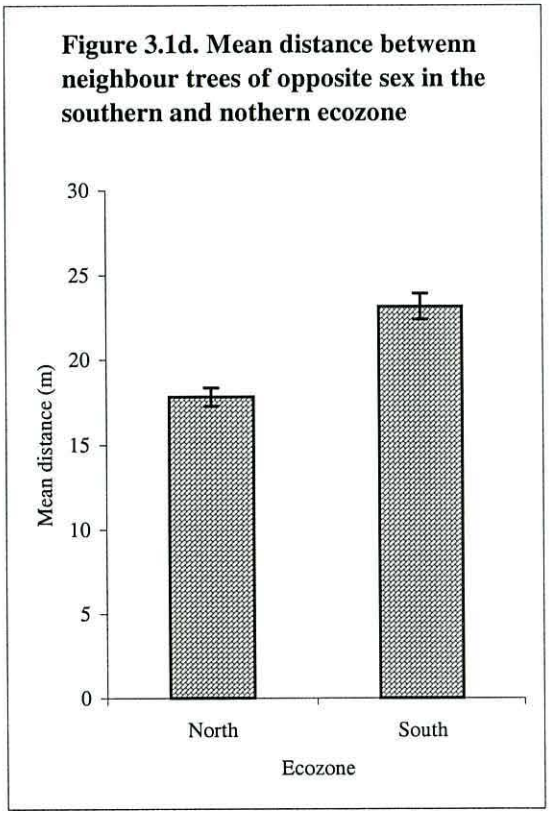
The frequency of occurrence of association between sexes i.e. male to male, male to female, Female to male and female to female as a measure of tree sex distribution in a population, revealed no evidence of sex clustering in any population except Gubali. The sexes were observed to be distributed more randomly rather than following any specific pattern as revealed by the chi-square values computed for the frequency of sex occurrence in the six populations (Table 3.4).

Figure 3.1a-b. *Osyris lanceolata*: mean distance between neighbour trees and distances between tree of one sex and its neighbour of the opposite sex in the six populations.









Out of 851 individuals of *Osyris lanceolata* assessed, 446 individuals were female while 405 were male. Hence no significant difference in the number of individuals was observed between the two sexes when the six populations were considered together (Paired T-test, T = 1.29, P = 0.254). Table 3.5 summarises the number of plants belonging to each sex in each population.

Table 3.3. Tree density and mean basal area per hectare as estimated from the mean neighbour distances and mean tree diameter in each population.

Population	Mean tree diameter (cm)	Mean tree height (m)	Mean area per tree (m^2)	Estimated density/ha	Estimated basal area (cm^3/ha)
Gubali	4.83	3.05	142.8	70.03	1283
Bereko	5.11	3.55	131.8	75.88	1555
Mgwashi	3.59	2.06	216.7	46.15	466
Image	4.86	3.52	223.5	44.74	829
Nundu	8.40	6.49	250.6	39.91	2209
Sao Hill	7.01	4.48	261.8	38.20	1474

Table 3.4. Analysis of sex distribution in populations of O*syris lanceolata*

Population	Chi-square	P-value with DF = 1
Gubali	5.24	0.022
Hachi	0.056	0.813
Mgwashi	0.055	0.814
Image	0.665	0.415
Nundu	0.159	0.690
Sao Hill	0.186	0.666
Overall	0.680	0.410

Table 3.5. Number of individuals of *Osyris lanceolata* belonging to each sex out of 851 individuals assessed in the six populations

Population/district	Females	Males
Gubali – Kondoa	83	68
Bereko- Babati	60	73
Mgwashi – Lushoto	87	64
Image – Iringa	85	78
Nundu – Njombe	67	55
Sao Hill – Mufindi	64	67

3.3.3. Morphological characteristics of *Osyris lanceolata*

3.3.3.1 Mean total tree height

Mean total tree height of *Osyris lanceolata* in the six populations is presented in Figure 3.2a-b while distribution of trees according to tree height classes is presented in Figure 3.2c-d. Significance difference existed in mean total tree height between populations ($P< 0.01$). The mean total tree height ranged between 2.06 ± 0.05 m in Mgwashi population in the Northern ecozone and 6.49 ± 0.17 m in Nundu forest reserve in the Southern ecozone Figure 3.3a. Significant difference also existed between the Northern and Southern ecozone in mean total tree height ($P < 0.01$) with the Southern zone having taller trees (4.69 ± 0.09 m) than the Northern ecozone, which had mean total tree height of 2.86 ± 0.05 m (Figure 3.2b).

No significance difference was observed between males and females in mean total tree height neither between nor within populations as well as between ecozones. The pattern of tree distribution according height classes varied among populations as shown in Figure 3.2c. While most trees in Nundu forest reserve exceeded 6 m in height, in Mgwashi and Gubali most of trees were concentrated within 1.6 - 3 m height class. At Sao Hill forest, most of trees fell within 4.6 – 6.0 height class while in Bereko and Image most trees were in 3.1-4.5 m height class. Ecozones also showed a distinct pattern in height distribution. Trees in the southern ecozone had a normal distribution pattern with most trees being concentrated in mid height class. 3.1-4.5 m height class had the highest frequency in the zone. In the northern ecozone, most trees were concentrated in two diameter class the 1.6-3 m, 3.1-4.5 m with the former height containing more tress (Figure 3.2d).

3.3.3.2. Mean tree diameter

A significant difference existed among populations in tree diameter ($P<0.01$). Bigger trees were found in Nundu Forest reserve, where the mean tree diameter was 8.6 ± 0.32 cm while trees with the smallest diameter were those of Mgwashi with mean diameter of 3.61 ± 0.05 cm (Figure 3.3a). Bereko, Gubali and Image trees did not differ significantly in diameter.

Figure 3.2a-d. Mean total tree height and height class distribution of *Osyris lanceolata*

Figure 3.2a. Mean total tree height in different stands/populations

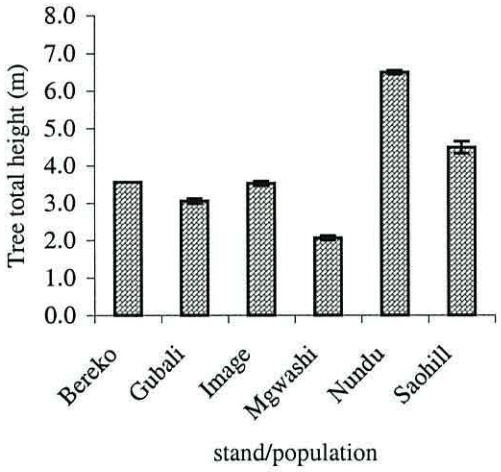


Figure 3.2b. Mean total tree height of according to ecozones

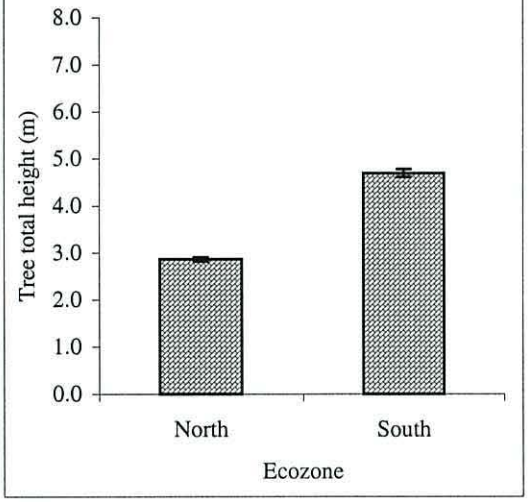


Figure 3.2c. Height class distribution the sampled stands/populations

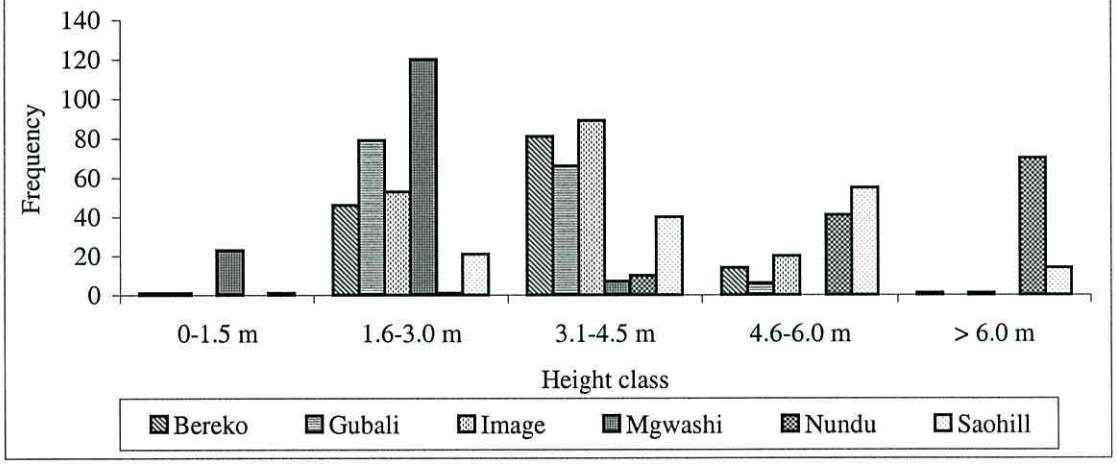


Figure 3.2d. Height class distribution in the northen and southern ecozones populations

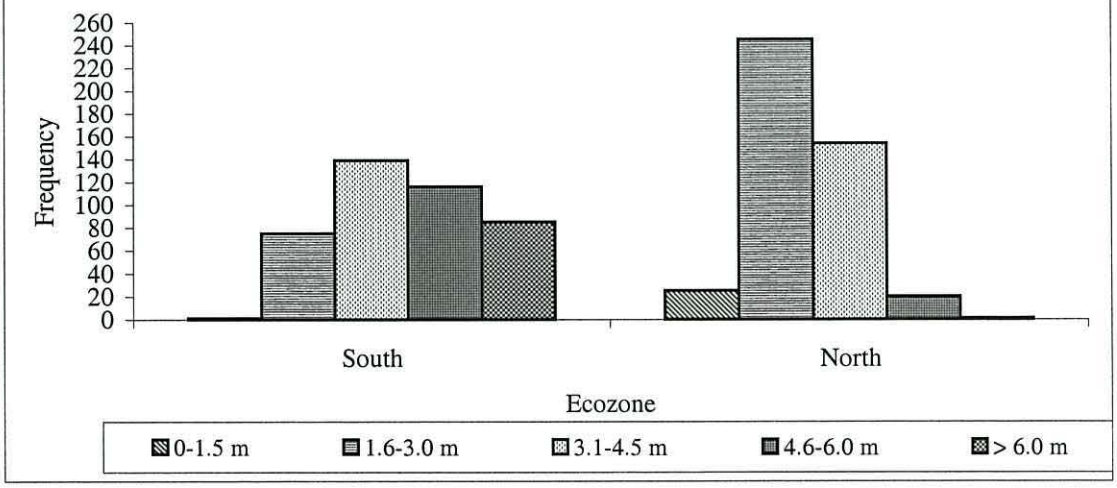
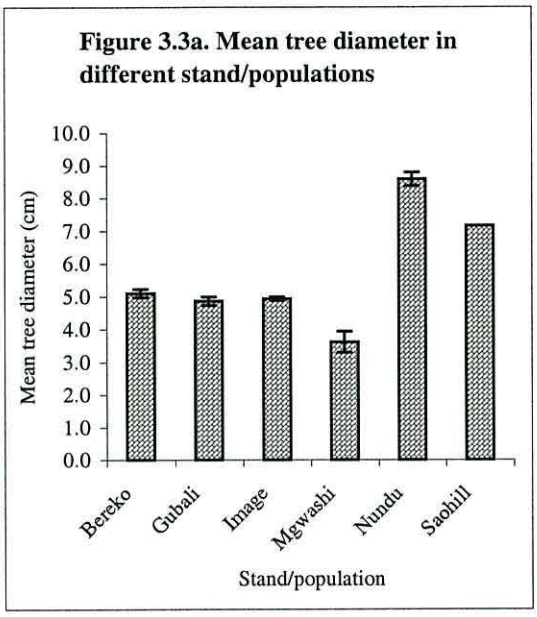
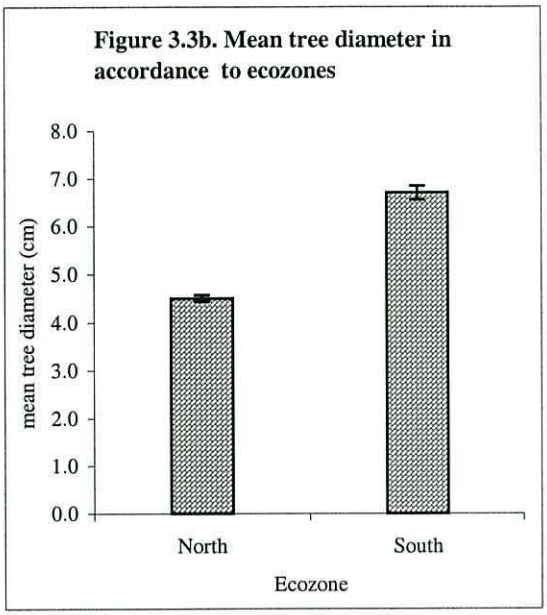
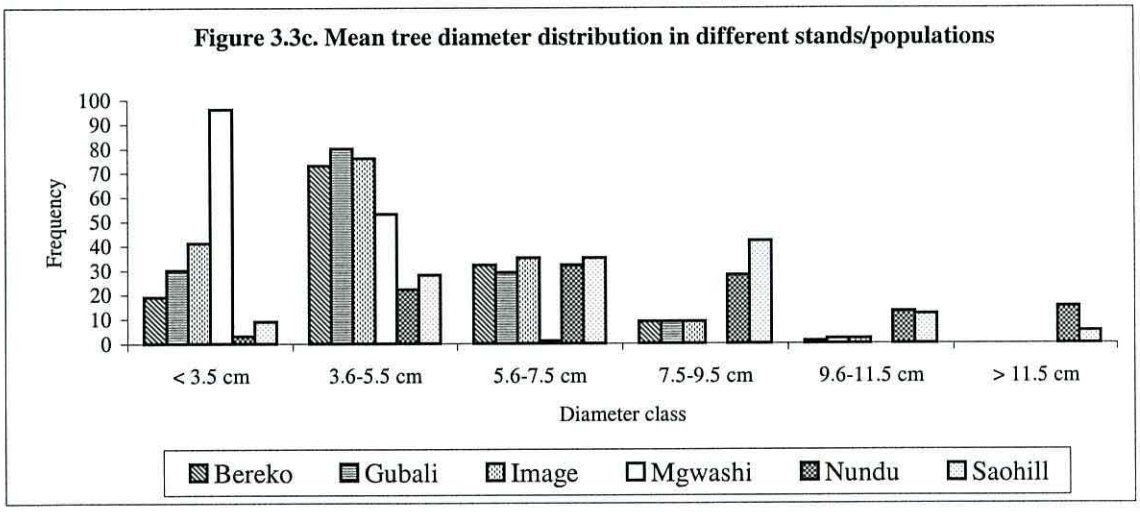
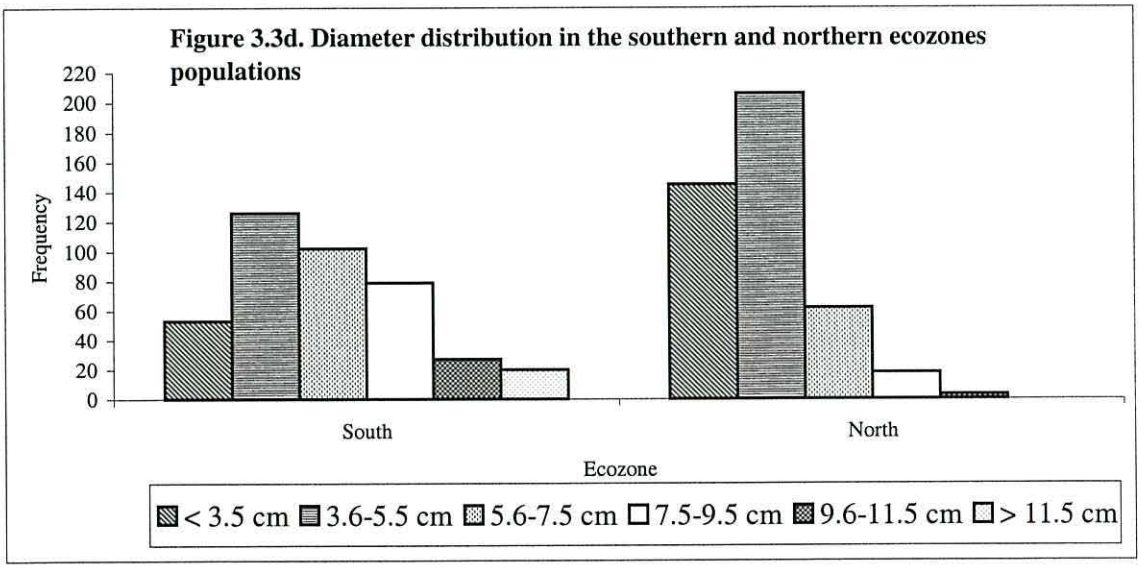


Figure 3.3a-c. Mean tree diameter and diameter class distribution of *Osyris lanceolata*









The two ecozones also differed in mean tree diameter ($P < 0.01$) with the Southern ecozone having bigger trees, with a mean diameter of 6.7 ± 0.15 cm than the Northern ecozone, with a mean diameter of 4.5 ± 0.07 cm (figure 3.3b). No significant difference was observed between male and female trees in mean tree diameter neither within nor between populations as well as between ecozones.

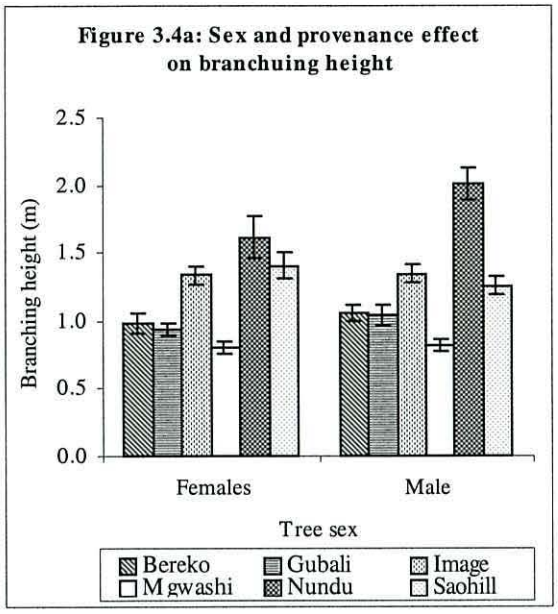
Tree distribution according to diameter classes in each population presented in Figure 3.3c shows that, Gubali, Bereko and Image populations, had most of the trees concentrated in 3.6-5.5 cm diameter class. Nundu and Sao Hill populations, had trees with diameter class distribution which shows a normal distribution pattern with most trees falling within 5.6-7.5 and 7.6 – 9.5 cm diameter classes, respectively. Mgwashi had trees with the smallest dimension with most of them falling within <3.5 cm diameter class (Figure 3c).

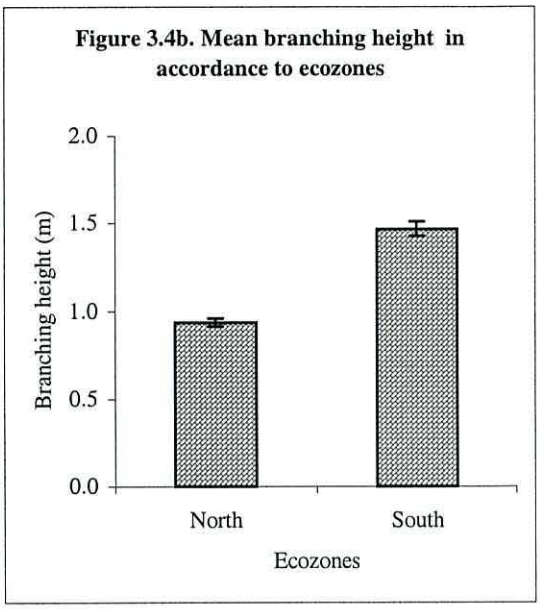
Ecozones had similar distribution pattern as observed in height distribution. Most trees in the southern ecozone were spread in 3.6 –9.5 cm diameter class while the northern ecozone concentrated its trees in < 5.6 cm diameter classes. In both ecozones,3.6-5.5 diameter class that the highest frequency (Figure 3.3d).

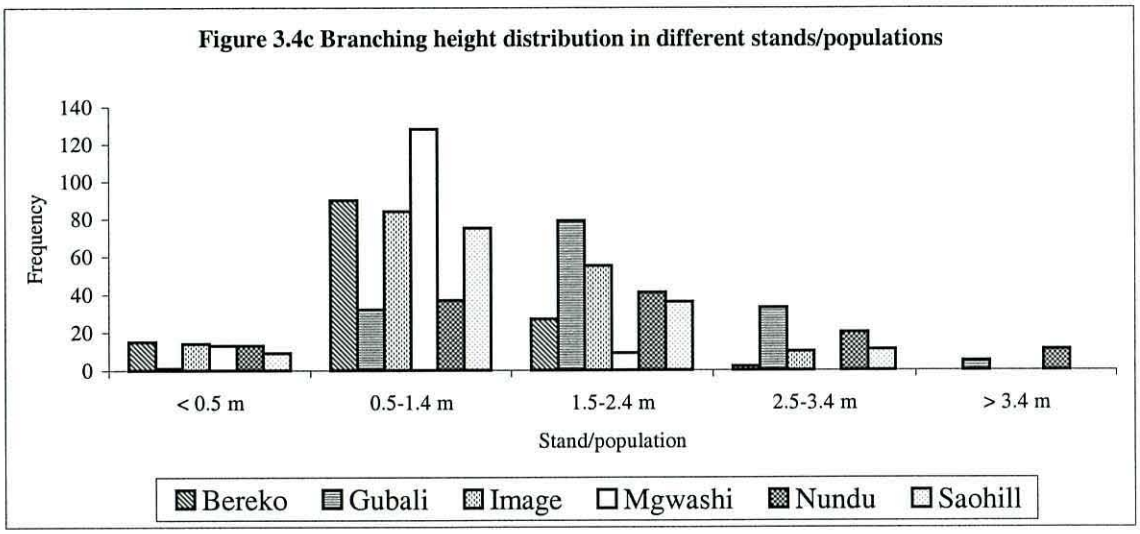
3.3.3.3 Tree branching height

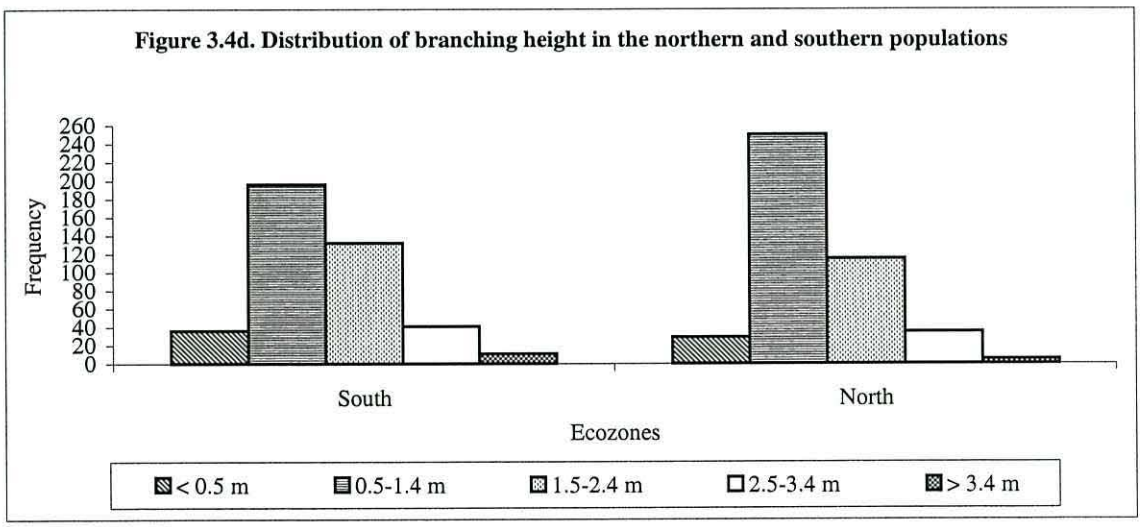
The height at which branches are formed in *Osyris lanceolata* varied between populations ($P < 0.01$) with trees at Nundu branching at relatively higher height above ground than trees from all other populations. While the branching height between the two sexes did not differ among populations, a significant difference existed in the interaction between populations and tree sexes ($P = 0.036$). Male trees at Nundu branched at a significantly higher distance above ground (2.0 ± 0.16 m) (Figure 3.4a) compared with all other trees in other populations including females of the same population. Female trees at Mgwashi had the least branching height (0.80 ± 0.04 m). Ecozone also differed significantly in height at which tree branched. The southern ecozone trees branched at higher point (1.47 ± 0.04 m) compared to those of the northern ecozone that branched at 0.94 ± 0.02 m (Figure 3.4b).

Figure 3.4a-c. Branching height of *O. lanceolata*









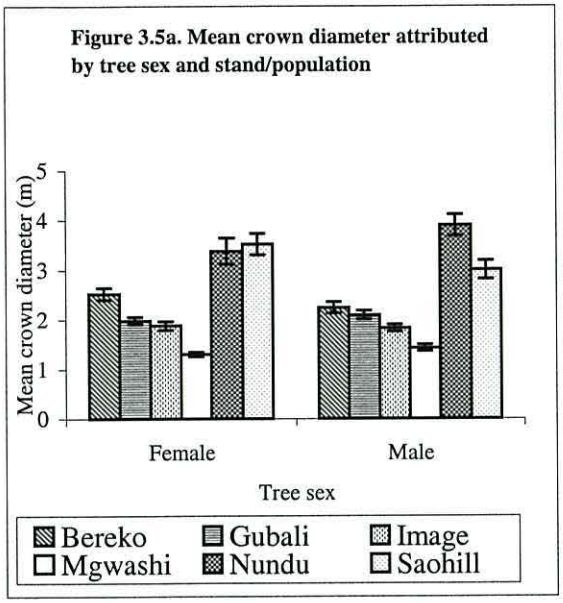
The distribution of branching height in the populations presented in Figure 3.4c shows that most trees at Bereko, Image, Mgwashi and Sao Hill branched at 0.5-1.4 m diameter, while majority at Gubali and Nundu and branched at a height within 1.5-2.4 m. The branching height distribution pattern between ecozones (Figure 3.4d) was similar to that of total tree diameter. In both ecozones, most trees branched within 0.5-1.4 m, 1.5-2.4 m classes with majority being in 0.5-1.4 branching height classes.

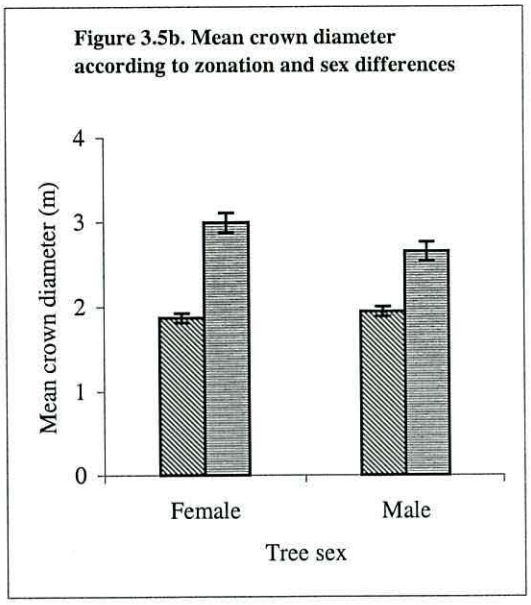
3.3.3.4 Crown diameter

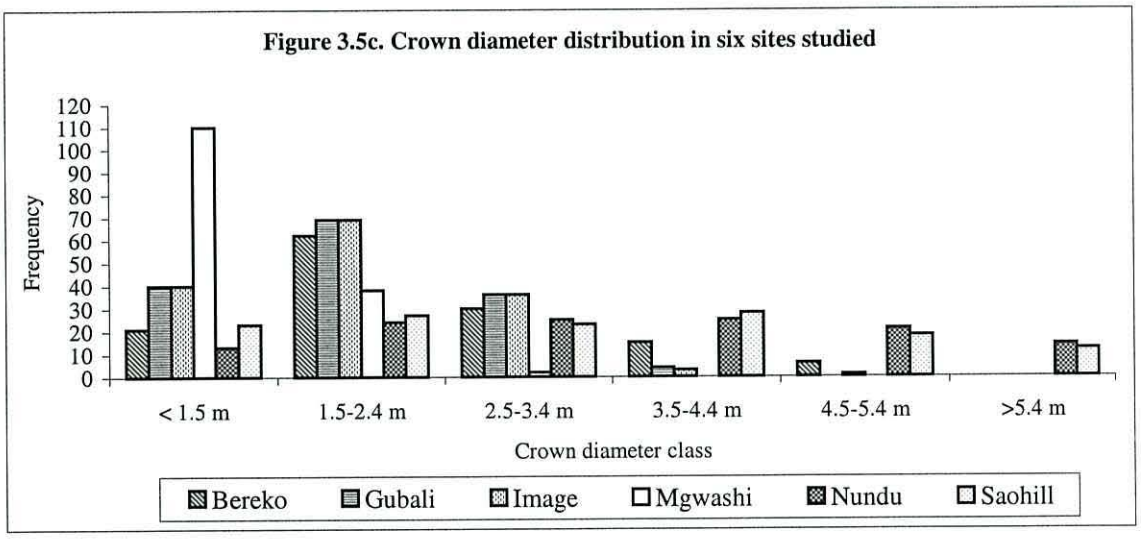
Crown diameter and size distribution of *Osyris lanceolata* is presented in figure 3.5a-d. Crown diameter differed significantly among the populations ($P < 0.01$) with tree of Nundu population having the widest crown diameter (3.66 ± 0.17 m). The smallest crown diameter was found in trees of Mgwashi population that had a mean diameter of 1.36 ± 0.04 m. Tree sexes also differed significantly in the size of the crowns ($P = 0.02$) with female trees having wider crowns (2.41 ± 0.07 m) than males (2.29 ± 0.06 m). There was significant interaction between populations and tree sexes ($P = 0.04$), with male trees at Nundu having the widest crown (3.9 ± 0.21m) with the exception of females of Nundu and Sao Hill. The least crown width was in female trees of Mgwashi that had a mean crown diameter of 1.31 ± 0.05 m (Figure 3.5a). There was also significant difference in crown diameter between Southern and Northern ecozones, with the Southern ecozone populations having trees with wider crowns (2.83 ± 0.08 m) than the Northern (1.90 ± 0.04 m). Tree sexes had a significant interactive effect with ecozones ($P = 0.019$), with female in the southern ecozone having the widest crown (2.99 ± 0.12 m). The smallest crown diameter was found in females of the Northern ecozone (1.87 ± 0.05 m).

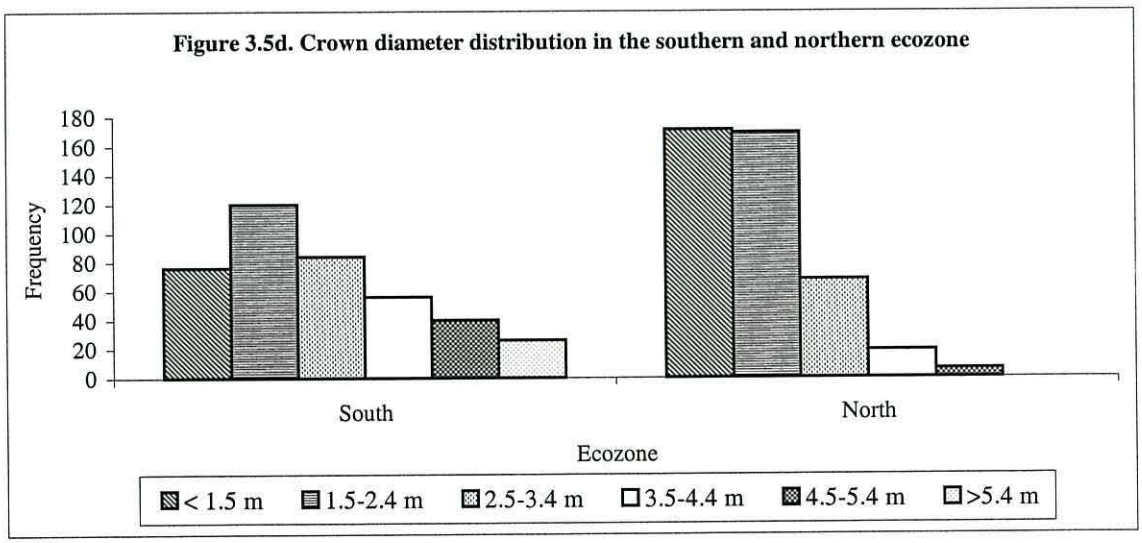
The crown diameter class distribution within each population shown in Figure 3.5c indicates that most trees in Gubali, Bereko and Sao Hill populations have crown diameter in 1.5-2.4 m diameter class while most trees in Nundu population had crown within 2.5–3.4 m diameter class. Mgwashi forest had most of its trees concentrated within < 1.5 m crown diameter class. Ecozone wise, most trees in the southern ecozone were frequent in the 1.5-2.4 m crown diameter class, although crown diameter classes < 1.5 m, 2.5-3.4 and 3.5-4.5 also had reasonable frequency. In the northern ecozone, a large proportion of the trees had crown diameter below 3.4 m (Fig. 3.5d).

Figure 3.5a-c. Mean crown diameter of *Osyris lanceolata*









3.3.3.5 Number of stems per tree

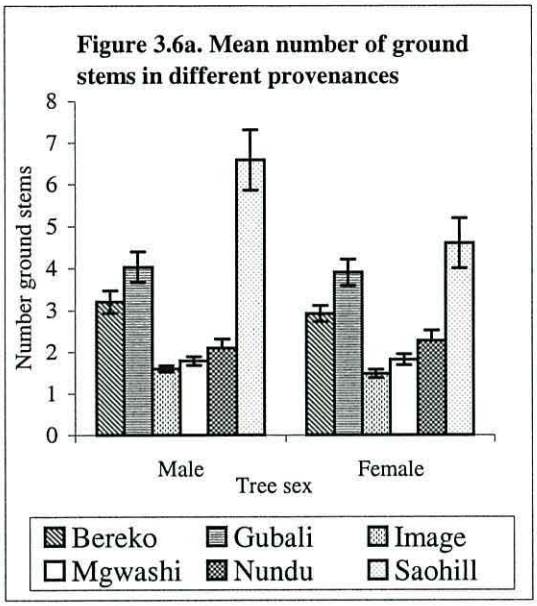
Osyris lanceolata is a multi-stemmed tree or shrub as shown in Figure 2.1. The number of stems per tree varied significantly among populations ($P < 0.01$) and ranged from as little as 1.5 ± 0.06 in Image to as many as 5.6 ± 0.49 in Sao Hill population. No significant difference was observed between males and females when tree sexes were considered alone ($P = 0.062$). However when tree sexes were considered with population there was significant interaction ($P = 0.023$). Males of Sao Hill had the largest number of stems per tree (6.6 ± 0.72). The least number of stems per tree was observed in males of Image (1.47 ± 0.1) (Figure 3.6a).

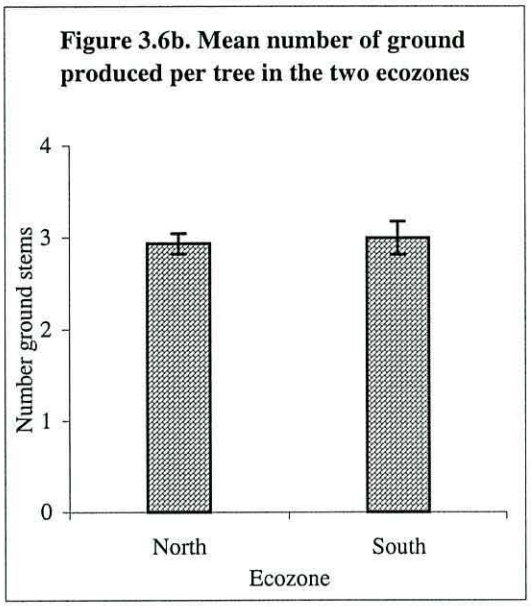
There was no significant difference between ecozones in the number of stems per tree (Figure 3.6b). Tree distribution according to number of stems per tree classes in each population is given in figure 3.6c. Although variation between populations existed, the average number of stems produced per tree was 3, with most trees producing in the range of 2-3 stems per tree. In Image, Mgwashi and Nundu, the majority of the trees were single stemmed while Hachwi and Gubali, had most trees with 2-3 stems. Trees in Sao Hill were the most multi-stemmed, most having more than 7 stems. Zonation wise, most trees had 1-2 stems at both zones (Figure 3.6d).

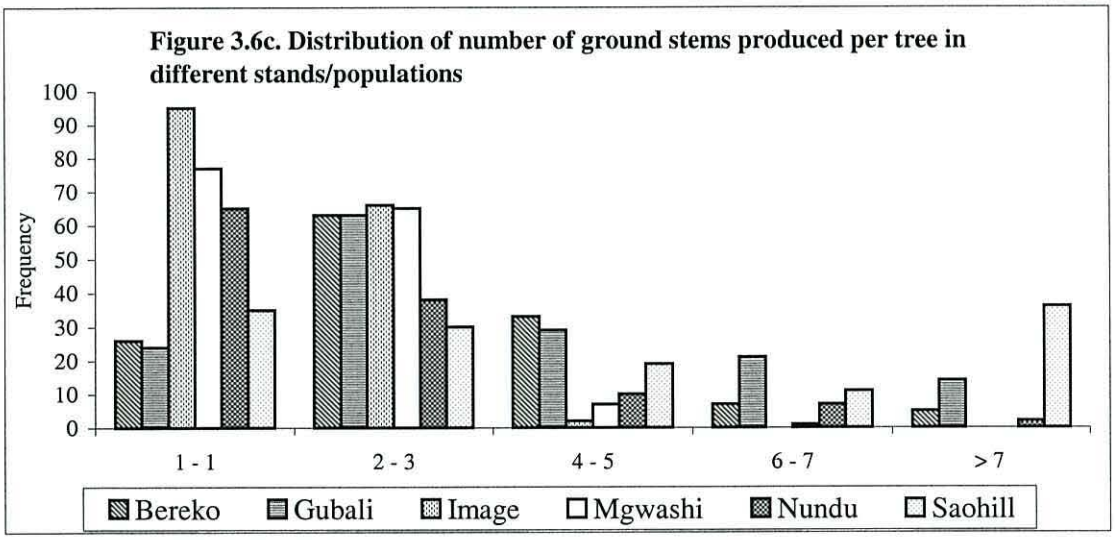
3.3.3.6. Number of branches per tree

The number of branches produced per tree differed significantly among populations ($P < 0.01$). Trees of Gubali had more branches per tree (2.29 ± 0.04) compared with all the other populations. The least number of branches was observed in Nundu population that had 2.05 ± 0.02 branches per tree (Figure 3.7a). Ecozones also differed significantly ($P < 0.01$). Northern ecozone trees produced more branches per tree (2.16 ± 0.02) than southern ecozone which produced 2.08 ± 0.01 (Figure 3.7b). No significant difference was observed between tree sexes. The tree distribution according to number of branches per tree presented in Figure 3.7c shows that most trees produced two branches. However, trees with two or more branches were common. The southern and northern ecozones showed similar distribution patterns in the number of branches produced per tree, with majority having 2 branches (Figure 3.7c).

Figure 3.6a-d. Mean number of stems per tree of *Osyris lanceolata*







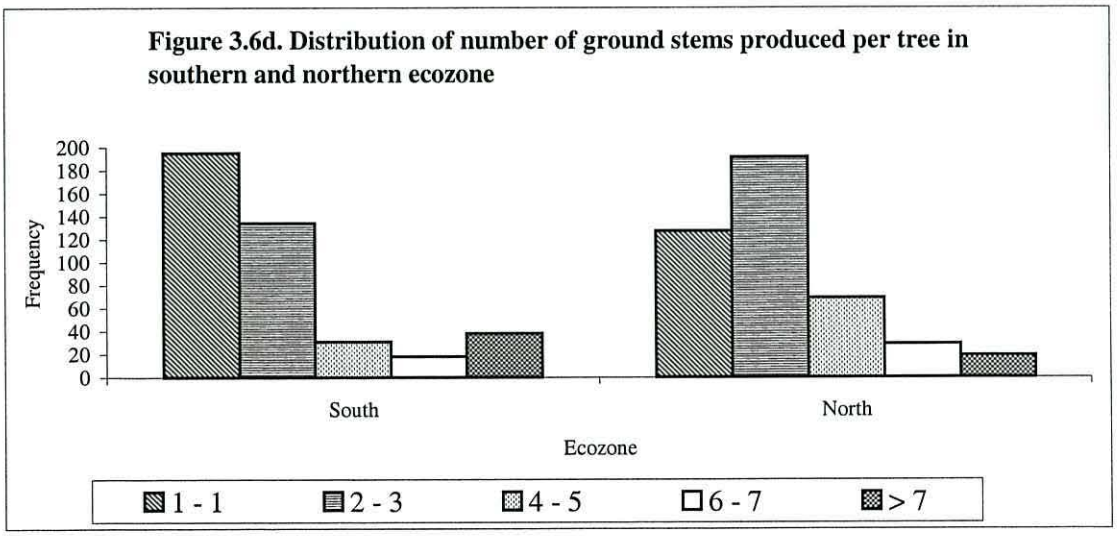
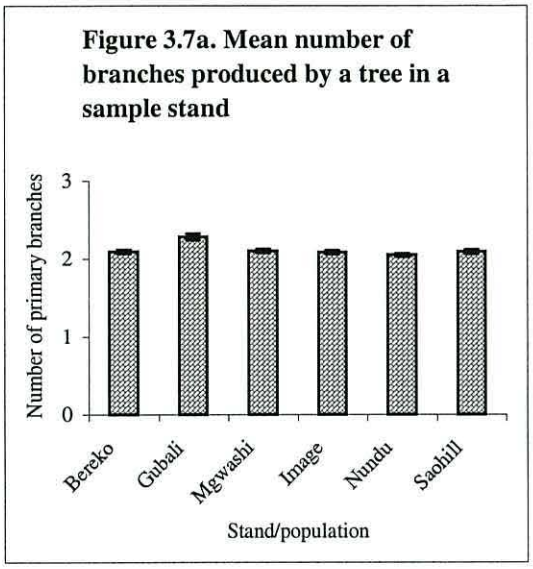
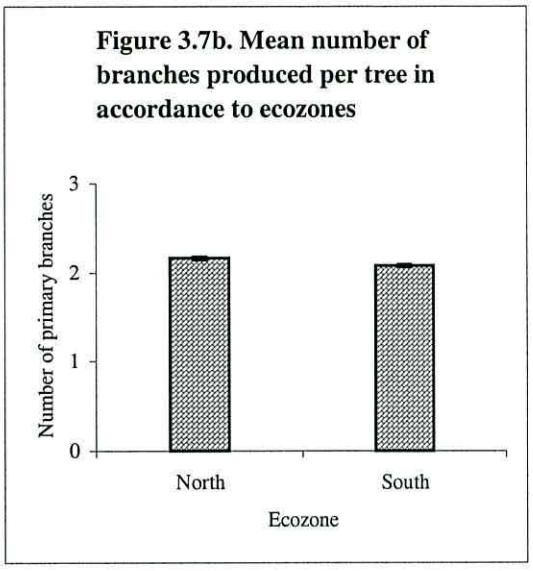
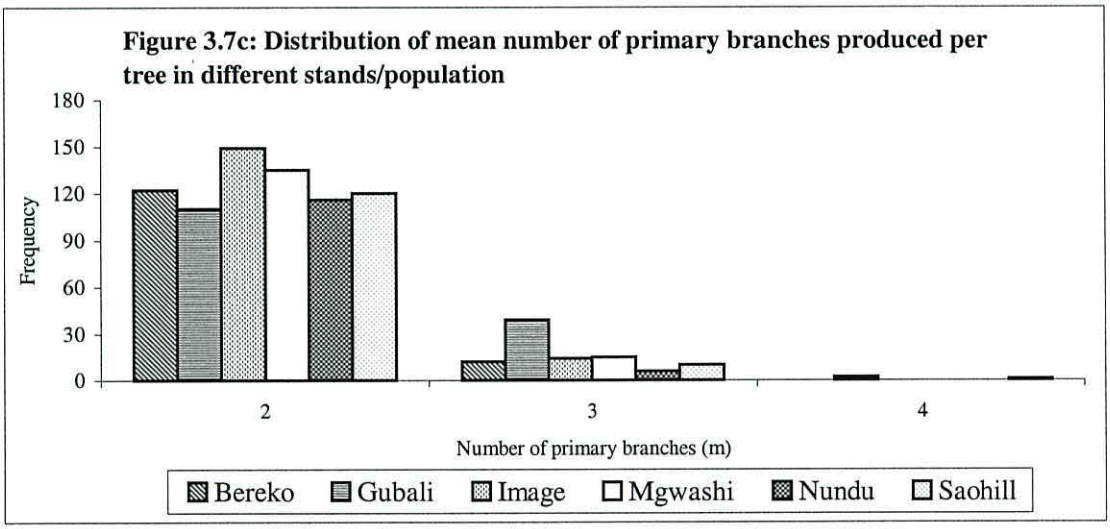
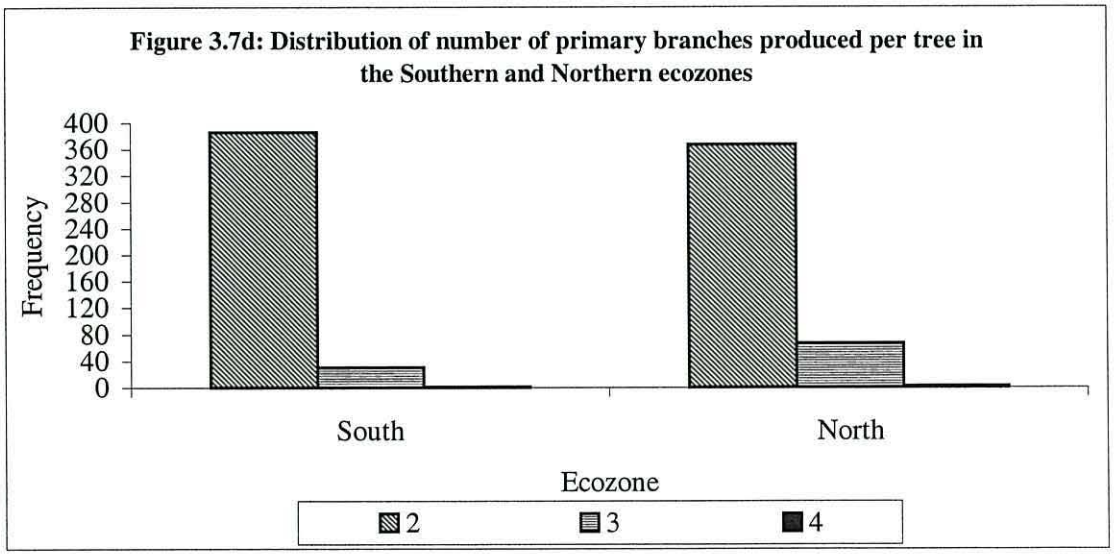


Figure 3.7a-c. Mean number of branches produced per tree in *Osyris lanceolata*









3.3.3.7. Allometric relationship between morphological attributes

The allometric relationships between morphological parameters were explored through linear regression. The results of these analysis are presented in Figure 3.8a-h. Although all the eight regression relationships were significant ($P < 0.01$), strong relationships were only found between tree height and tree diameter ($R^2 = 59.6\%$) as depicted in Figure 3.8a and between crown diameter and tree diameter ($R^2 = 56.6\%$) as shown in Figure 3.8e.

3.3.3.8. Correlation between morphological attributes and site characteristics of mean annual rainfall and soil fertility

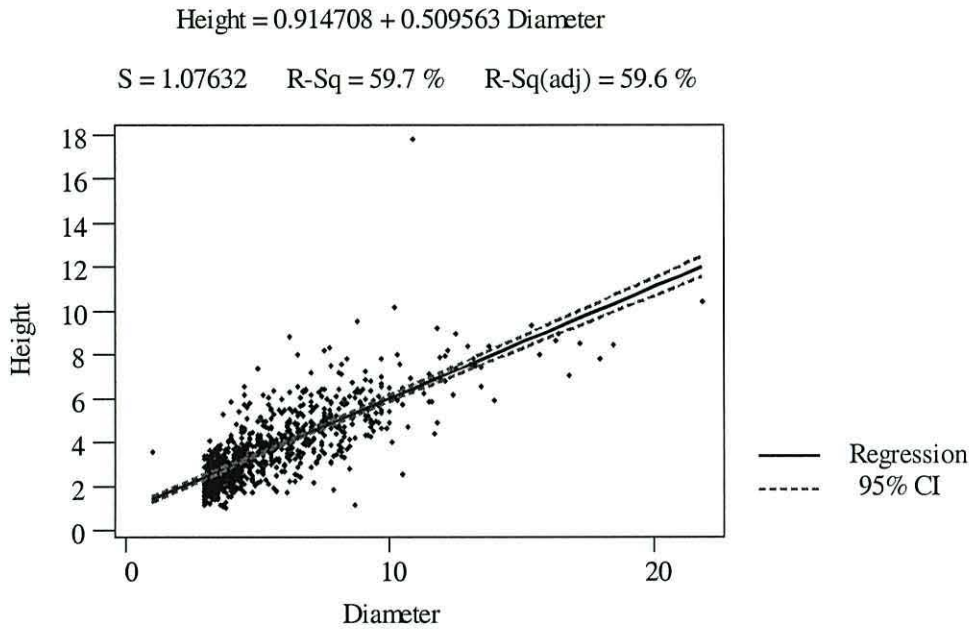
The correlation analysis between various morphological attributes and stand characteristics of mean annual rainfall and soil fertility status is presented in Table 3.6. Mean annual rainfall was strongly and positively correlated with tree height and diameter, crown diameter and basal area per hectare. There was significant negative correlation between soil acidity and tree diameter and positive correlation between soil acidity and crown diameter. Total soil nitrogen had a significant positive correlation with mean total tree height, tree diameter and crown diameter. There was negative significant correlation between available phosphorus and mean tree diameter.

Table 3.6 Correlation between tree morphological attributes and stand characteristics of rainfall and soil fertility status

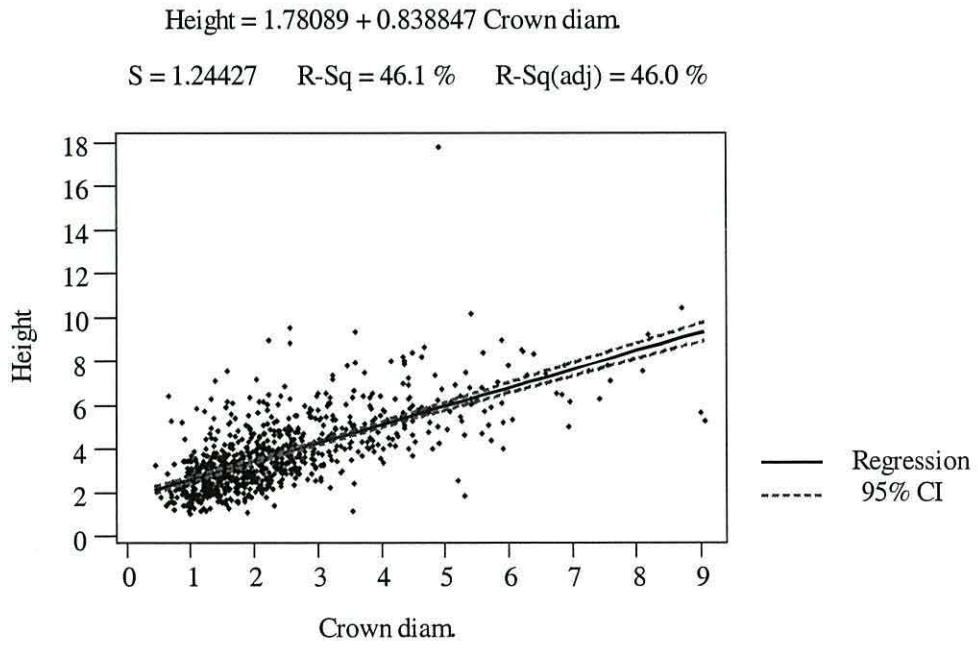
Attribute	Reg & Prob	Tree height	Tree diameter	Crown diameter	Number of stems/tree	Density/ha	Basal area/ha
Rainfall	R^2	**0.905**	**0.899**	**0.839**	**0.037**	**-0.465**	**0.816**
	P	0.013	0.015	0.037	0.944	0.353	0.048
Soil acidity (pH)	R^2	**-0.777**	**-0.853**	**0.845**	**-0.307**	**-0.770**	**-0.517**
	P	0.069	0.031	0.034	0.554	0.073	0.294
Total soil nitrogen	R^2	**0.931**	**0.911**	**0.862**	**-0.034**	**0.653**	**0.674**
	P	0.007	0.012	0.027	0.948	0.159	0.142
Available phosphorus	R^2	**-0.734**	**-0.825**	**-0.805**	**-0.312**	**0.734**	**-0.546**
	P	0.096	0.043	0.053	0.547	0.096	0.263
Base saturation	R^2	**-0.552**	**-0.530**	**0.569**	**-0.230**	**0.211**	**-0.355**
	P	0.256	0.279	0.238	0.661	0.688	0.490

Figure 3.8a-h. Allometric relationship between morphological attributes: Note that probability values for all regression (P) < 0.001

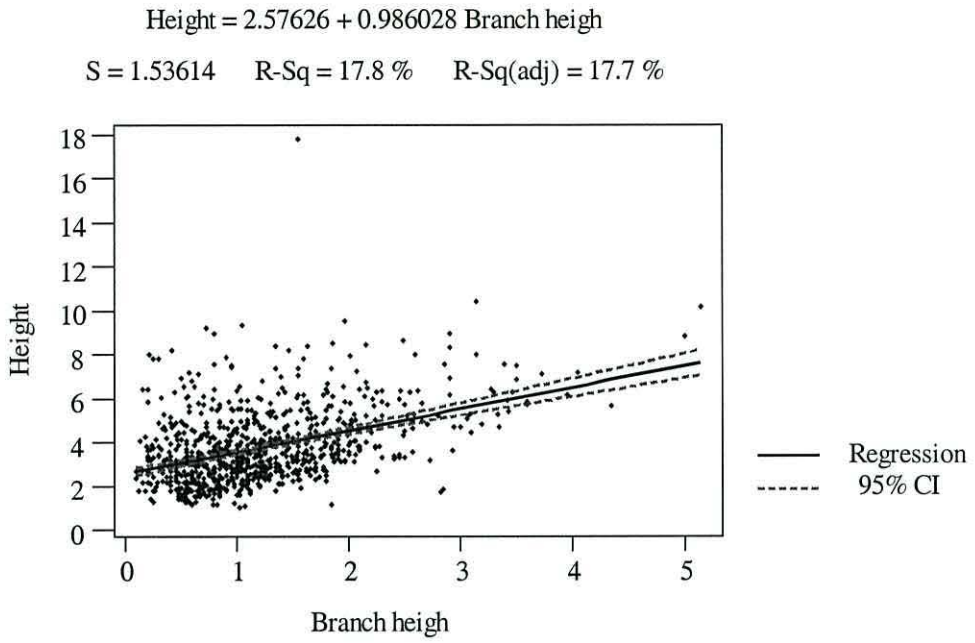
a) Total tree height (m) and mean tree diameter (cm)



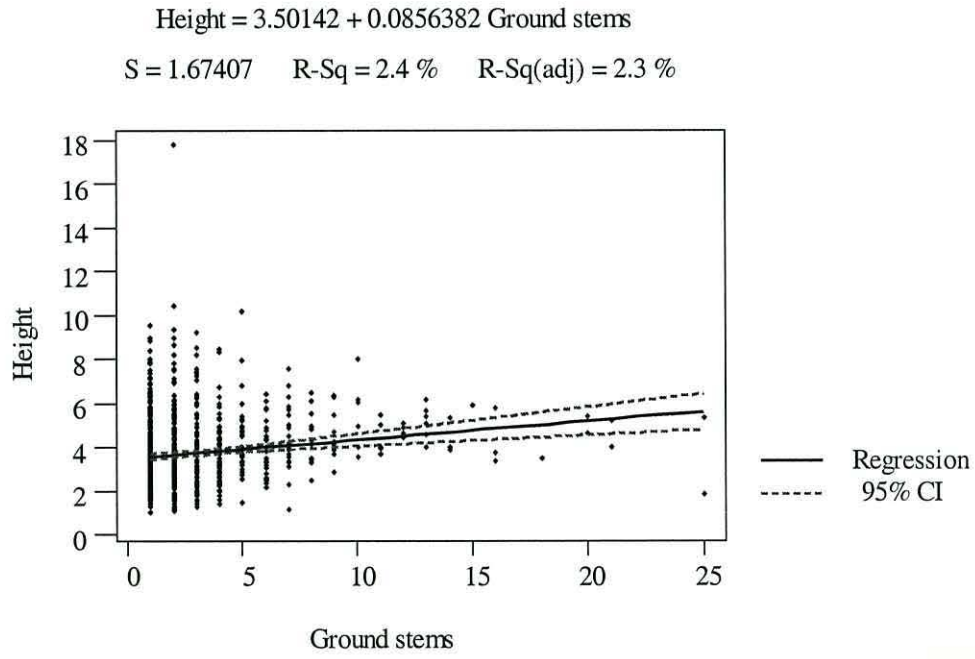
b) Total tree height (m) and mean crown diameter (m)



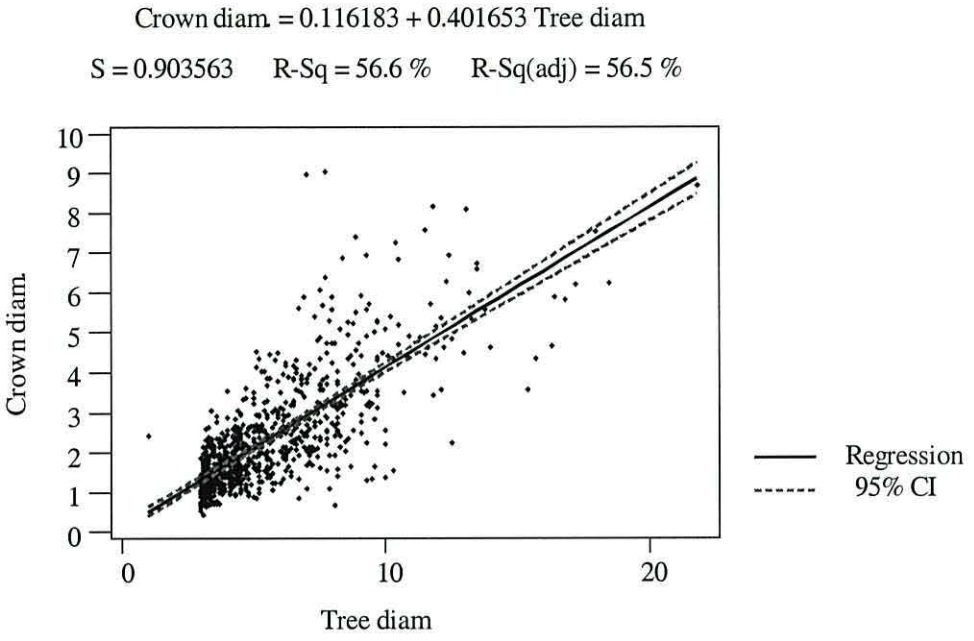
c) Mean total tree height (m) and mean branching height (m)



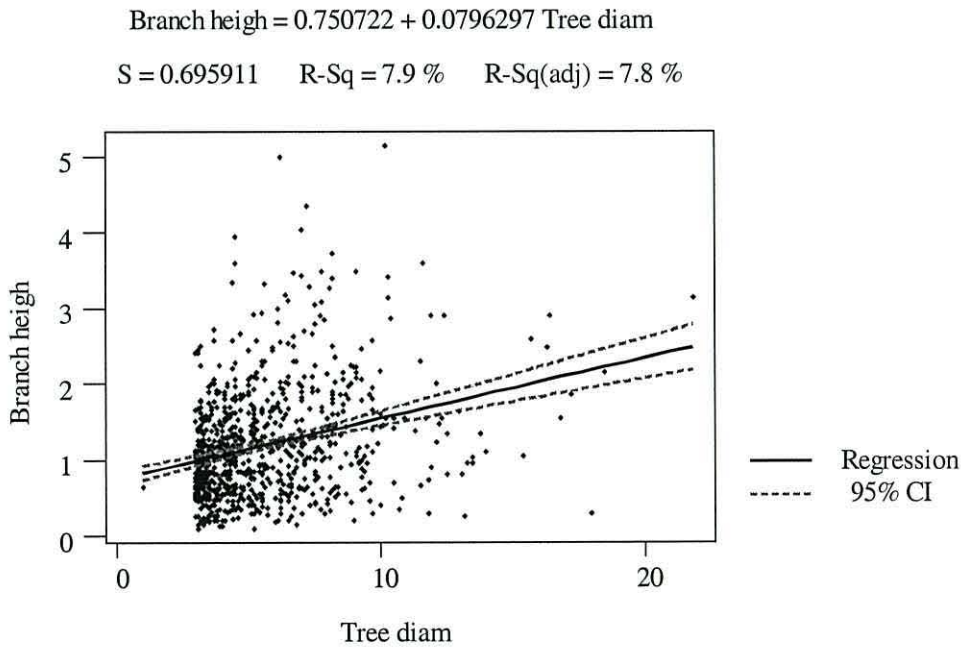
d) Mean total tree height (m) and number of ground stem



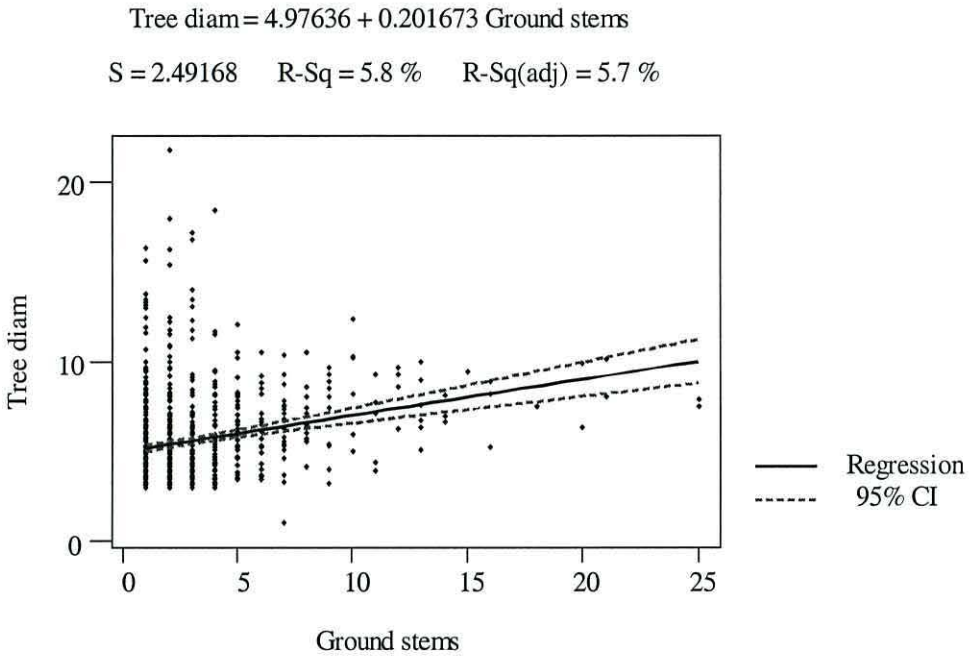
e) Mean crown diameter (m) and mean tree diameter (cm)



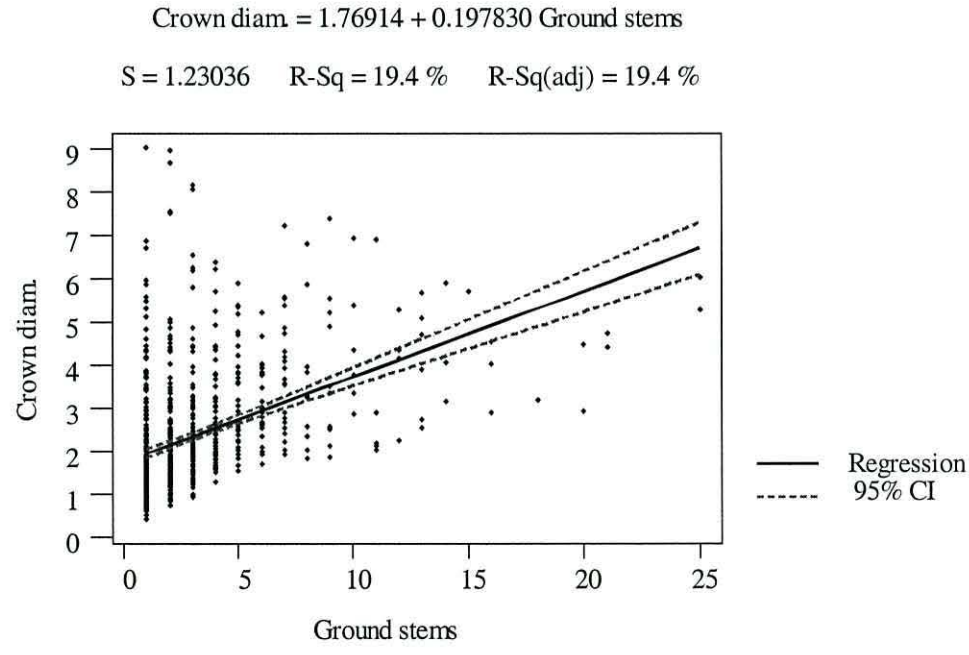
f) Branching height (m) and mean tree diameter (cm)



g) Mean tree diameter (cm) and number of ground stems



h) Mean crown diameter (m) and number of ground stems



3.3.4. Natural regeneration of *Osyris lanceolata*

A study on the natural regeneration in the six populations of *Osyris lanceolata* revealed that recruitment of the species took place through both seed and stump/rootstock. Table 3.7 summarizes the overall regeneration in the six populations. Regeneration abundance differed among sites ($P < 0.001$) with Sao Hill population in the Southern ecozone having the most abundant regeneration (103 ± 8.5 plants ha^{-1}) compared with what was observed in Image and Nundu populations. The least regeneration was in Nundu (48 ± 7.3 individuals/ha). Bereko, Gubali and Mgwashi had similar regeneration abundance.

3.3.4.1 Size of regeneration

Two size classes of regeneration were identified (seedlings and saplings) and they differed significantly ($P < 0.01$). Seedlings were more abundant (51.96 ± 2.51 individuals per hectare) and formed 62% of the total regeneration compared with saplings that were 32.43 ± 1.8 contributing only 38% to the total regeneration (Figure 3.9). Table 3.8 shows the abundance of regeneration in relation to size class

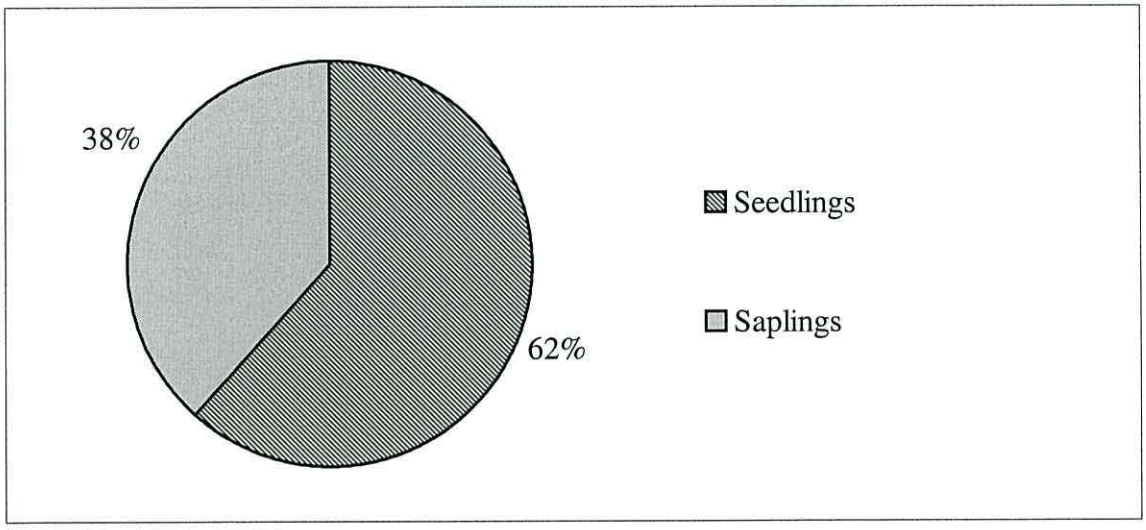
Table 3.7 Overall regeneration (number of individuals ha^{-1}) in *O. lanceolata*

Stand/ Population	Total/ha	Regeneration under mother trees				Regeneration beyond mother trees			
		seed source		coppice/rootstock source		seed source		coppice/rootstock source	
		seedlings	saplings	seedlings	saplings	seedlings	saplings	seedlings	saplings
Gubali	97.0	5.6	3.3	0.6	0.1	24.2	14.2	26.9	22.2
Bereko	93.8	17.4	5.1	2.1	0.3	2.8	1.0	41.3	23.8
Mgwashi	94.3	5.4	3.2	0.7	0.4	16.8	7.9	37.5	22.5
Image	62.6	4.1	1.8	1.0	0.0	14.9	10.3	16.2	14.4
Nundu	47.9	4.8	2.8	1.0	0.7	9.3	5.5	13.1	10.7
Sao Hill	102.8	11.2	5.3	1.4	1.4	18.4	12.6	30.9	21.6

Table 3.8 Regeneration of *Osyris lanceolata* according to size

Stand/population	Seedlings ha^{-1}	Saplings ha^{-1}
Gubali	57.2 ± 4.4	40.3 ± 4.6
Bereko	63.6 ± 7.1	30.3 ± 4.5
Image	36.1 ± 4.3	26.4 ± 3.9
Mgwashi	60.4 ± 6.2	33.9 ± 4.5
Nundu	28.3 ± 6.1	19.7 ± 2.8
Sao Hill	61.9 ± 5.9	40.9 ± 4.4

Figure 3.9 Contribution of seedlings and saplings to total regeneration in *Osyris lanceolata*



3.3.4.2 Regeneration in relation to source

Regeneration of *Osyris lanceolata* was observed to take place through both seeds and coppice/rootstock sources in all the six populations. Table 3.9 presents regeneration of the species at the six populations from the two sources while Figure 3.10 presents relative contribution of the two sources to the total regeneration observed in the six sites. The abundance of regeneration differed significantly between the two sources ($P< 0.01$) with coppice/rootstock source producing more individuals (48.4 ± 2.5/ha) compared with seeds source (36.0 ± 2.2/ha).

Significant difference also existed in the interaction between source of regeneration and population ($P < 0.01$). Regeneration from stumps/rootstock source was the most abundant in Bereko population (67.4 ± 7.6 individuals/ha). The least regeneration was from the seed source in Nundu population that had a mean density of 22.4 ± 4 individuals ha^{-1}. When sapling stage of regeneration was considered alone, coppice/root stock source accounted for 61% of the total individuals. Table 3.10 presents regeneration from the two sources that reached sapling stage in the six populations.

Table 3.9 Regeneration of *Osyris lanceolata* (individuals per hectare) in relation to source of regeneration

Stand/population	Seed source	Coppice/rootstock source
Gubali	47.2 ± 5.1	50.3 ± 4.1
Bereko	26.4 ± 4.2	67.4 ± 7.6
Image	31.0 ± 5.3	31.5 ± 3.8
Mgwashi	33.2 ± 5.6	61.1 ± 5.1
Nundu	22.4 ± 4.0	25.5 ± 5.2
Sao Hill	50.9 ± 5.5	51.9± 6.5

Figure 3.10. Contribution of seed and coppice/rootstock source to total regeneration in *O. lanceolata*

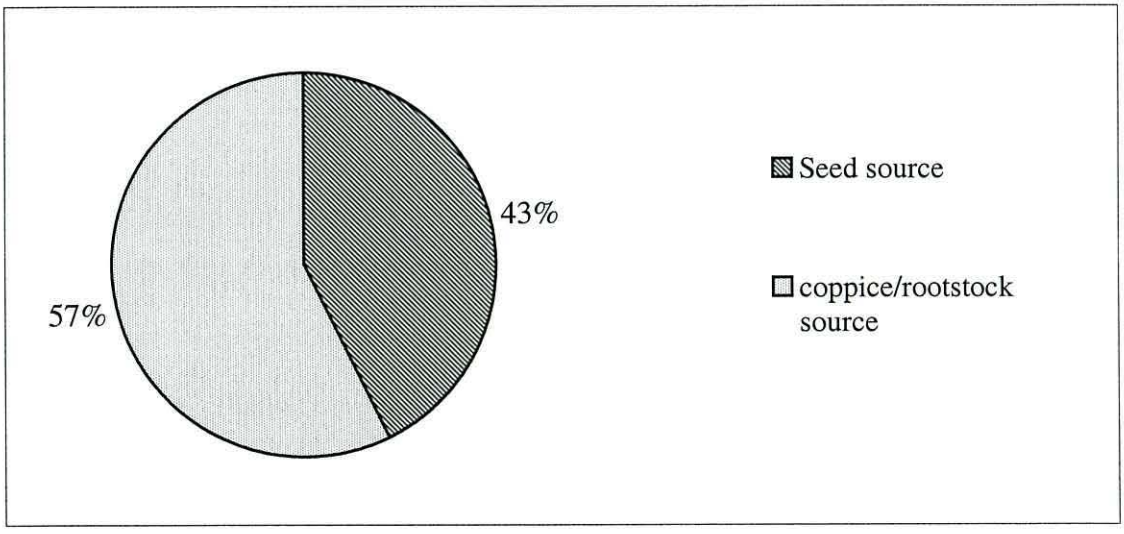


Table 3.10. Natural regeneration of *Osyris lanceolata* that reached sapling stage from the two sources (Individuals/ha)

Stand/population	Seed source	Coppice/rootstock source
Gubali	17.5	22.2
Bereko	6.2	23.8
Image	12.1	14.4
Mgwashi	11.4	22.9
Nundu	8.3	11.4
Sao Hill	17.9	21.6

3.3.4.3 Regeneration in relation to distance from mother trees

Significant difference existed in the abundance of regeneration in relation to distance from the mother trees in all the populations ($P < 0.01$). Of the total regeneration encountered in all populations (1806), 88% regeneration occurred outside the crowns of *Osyris lanceolata* while only 12% was confined beneath tree crowns (Figure 3.11).

The abundance of regeneration in relation to distance from *Osyris lanceolata* trees in each population is summarized in Table 3.11.

Figure 3.11 Proportional of regenerating plants in *Osyris lanceolata* in relation to distance from mother trees

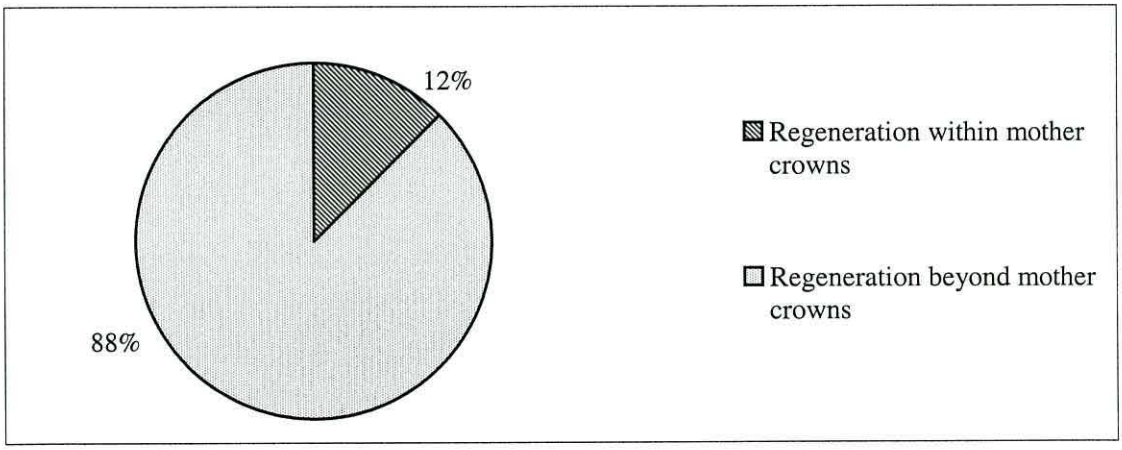


Table 3.11. Total regeneration of *O. lanceolata* in relation to distance from parents that have reached sapling stage

Stand/population	Total regeneration under mother tree crowns	Total regeneration beyond mother tree crowns	Beyond: beneath ratio
Gubali	36	315	8.8
Bereko	24	342	14.3
Image	27	217	8.0
Mgwashi	27	237	8.8
Nundu	27	112	4.1
Sao Hill	83	359	4.3

The ratio of regenerating plants between regeneration occurring beyond and under tree crowns of the mother trees showed a significant difference among sites ($P < 0.01$). The ratio was higher in Bereko population, where regeneration beyond mother crowns was 14 times higher than regeneration under tree crowns. The lowest ratio was obtained (4.1) in Nundu population.

3.3.5. Associated plant species of O*syris lanceolata*

A total of 179 associated species were identified in all the six populations. The species encountered, their density and basal area per hectare and percentage contribution of each species to the total basal area are given in Appendix 1.

In terms of number of associated species, Image forest was the most diverse with a total of 89 species with a mean tree density of 933 individuals per hectare and a total basal area per hectare of about 53370 cm ^{2}. Of all the associated species, *Brachystegia* (*B. glaberrima, B. utilis and B. spiciformis*) were the most dominant making a mean density of 367 (37% of all associated species) individuals per hectare and a total basal area of 34300 cm^2 (63%) of all associated species).

In terms of tree density the highest was found at Nundu which was composed of 42 associated species with a total stocking of 1433 individuals per hectare making a mean basal area per hectare of 57500 cm^2. Of the total species *Aphloia theiformis* and *Myrsine melanophloeos* dominated the site with respective mean stocking per hectare of 466 (32%) and 167 (11%) individuals and a basal area of 15700 cm^2 (26%) and 8500 cm^2 (14%) per hectare.

Mgwashi population was the least diverse with only 35 associated species with an average stocking of 329 individuals/ha and a mean basal area of about 6987 cm^2/ha. The vegetation is dominated mainly by *Euphorbia candelabrum* with a stocking of 77 individual/ha (19.9%) and basal area of 4260 cm^2/ha (56.3% contribution).

According to Sorenson index of similarity the highest similarity index (0.58) was observed between Bereko and Gubali. And the vegetation compositions of these sites were considered similar. The rest of the populations were differed in vegetation composition (Table 3.12). Of the 179 associated species encountered, only *Dodonaea viscosa* and *Rhus natalensis* occurred in all the six populations while *Vangueria infausta,Lannea schimperi* and *Euclea natalensis* occurred in all except Nundu. Out of 79 associated species occurring at Bereko and Gubali, 31 species were common to the two sites. Populations that were extremely dissimilar in vegetation composition were Gubali and Nundu (Index of similarity = 0.04)

Table 3.12 Comparison of vegetation composition of the study sites through pair-wise comparison using Sorenson index of similarity

Stand/population	**Sorenson index of Similarity (< 0.50 – dissimilarity, > 0.50 similarity of stands)**				
	Mgwashi	Bereko	Image	Sao Hill	Nundu
Gubali	0.32	0.58	0.49	0.14	0.04
Mgwashi	-	0.34	0.30	0.27	0.15
Bereko	-	-	0.46	0.21	0.09
Image			-	0.27	0.14
Sao Hill				-	0.46

3.3.6 Reproductive biology of *Osyris lanceolata*

3.3.6.1 Reproductive processes

The mean duration of each phenological event from bud initiation to fruit maturity for both male and female trees of O*syris lanceolata* at Sao Hill are presented in Table 3.13. Flower buds began to be initiated in early February and continued to be formed up to around June. It took 15 - 18 days for half of the flower buds to form active flowers in female plants. Male and female flowers were distinguished by their physical structure. Male flowers had shallow receptacles while female flowers possessed long receptacle that were relatively larger in size.

Of the active female flower formed, half of them withered within 29-31 days, which marked the beginning of fruit initiation and this took place 11 to 13 days after flower formation. In male plants, half of the buds flowered within 12 to 17 day and half of them withered within 31 to 38 days i.e. about 14-20 days since active flowering. While anther withering marked the end of flowering activity in males, stigma withering was the beginning of fruit formation in female plants. After stigma withering, it took only 4.9 ± 1.6 days for 50% of the withered flowers to form young fruits (34.0 ± 2.7) days since buds were initiated).

Table 3.13. Phenological events and the duration they take from initiation to completion in *Osyris lanceolata* at Sao Hill population.

Sex in consideration	Phenological event	Time to reach (Days)			Duration from last event (Days)		
		25%	50%	> 75%	25%	50%	> 75%
Female plants	Bud initiation	-	-	-	-	-	-
	Flowering	12.5±1.9	16.6 ± 1.8	21.1 ± 2.1	12.5 ± 1.9	16.6 ± 1.8	21.1 ± 2.1
	Stigma withering	23.9 ± 2.3	29.1 ± 2.1	33.3 ± 2.4	11.4 ± 2.1	12.4 ± 1.2	12.2 ± 1.5
	Fruit initiation	28.8 ± 2.1	34.0 ±2.7	38.2 ± 1.9	4.9 ± 1.6	4.9 ± 1.2	4.9 ± 1.5
	Unripe-mature fruit	77.0±10.6	105.8±13.1	130.2±12.9	48.2±10.6	71.8±12.7	92.0±15.5
	Fruit maturity	104 ± 9.0	135.8±16.2	162.6±17.0	27.7± 8.8	30.0 ± 1.9	32.4 ± 2.6
Male	Bud initiation	-	-	-	-	-	-
	Flowering	14.5 ± 2.2	18.0 ± 2.5	21.9 ± 2.7	14.9 ± 2.6	18.5 ± 3.1	23.4 ± 3.0
	Anther withering	29.5 ± 3.4	34.8 ± 3.5	41.2 ± 3.9	15.2 ± 2.8	17.1 ± 2.9	8.6 ± 2.7

From fruit initiation until when 50 % of the initiated fruits reached mature unripe fruits, it took 71.8 ± 12.7 days (105.8±13.1 days since bud initiation). At this stage, fruits had changed their color from pale green to deep green. Twenty five percent of the mature unripe fruits became ripe within 27.7± 8.8 days (104 ± 9.0 days since bud initiation) and more than 75% were ripe in 162.6 ± 17.0 days. Ripe fruits were deep orange in color, measuring about 7.5 mm in diameter.

3.3.6.2. Reproductive success

The result of the reproductive success study is summarised in Table 3.14. None of the flower buds that were marked and bagged fruited suggesting absence of agamospermy behaviour in *Osyris lanceolata*.

The results of the natural pollination and assisted pollination experiments show that the two treatments differed in the amount of fruit formed out of the flowers that were marked and treated (P = 0.001). Assisted pollination had a greater proportion of fruits formed with a mean of 79.3 ± 1.5% while natural pollination had 72.9 ± 1.2%.

Table 3.14 Reproductive success in *Osyris lanceolata* in different pollination treatments

Treatment	Reproductive success from one stage to another (%)					
	A to B	B to C	C to D	E	D to F	G
Assisted pollination	78.4 ± 1.5	79.6 ± 1.6	88.4± 1	57.1 ± 1.7	89.6± 1.1	51.4 ± 1.8
Natural pollination	72.9 ± 1.2	82.9 ± 1.4	86.3 ± 1	52.2 ± 1.4	86.8± 1.8	45.4 ± 1.4
Restricted pollination	0	0	0	0	0	0

Legend/key to letters

A = Number of open flowers/ buds marked

B = Number of fruit formed

C = Number of mature unripe fruits produced

D = Number of ripe fruits produced

F = Number of fruits with filled embryos

G = Number of fruits with filled embryos as percentage of total flowers marked

E = Ripe fruits as percentage of total flower marked

Of the fruit formed 79.6 ± 1.6% in the assisted pollination reached unripe mature fruit stage, while in natural pollination, the reproductive success at this stage was 82.9 ± 1.4% but this was not significantly different (P = 0.133) from assisted pollination.

Considering the reproductive success as a whole, from bud initiation to formation of ripe fruits, assisted pollination produced significantly higher (P<0.026) number of ripe fruits (57.1 ± 1.7% of the total flowers marked reached ripe fruit stage). The proportion of seeds with filled embryos also differed significantly between the two treatments (P = 0.012) with assisted pollination having 51.4 ± 1.8% ripe fruits with filled embryo seeds out of the total flowers that were marked.

3.4 Discussions

3.4.1 *Osyris lanceolata* populations and their characteristics

The results of the present study showed that the six populations of *Osyris lanceolata* varied in tree density and morphological parameters. In general, trees of sorthern ecozone, which lies on the lowland areas compared to the southern ecozone, were denser than the southern ecozone whereas trees of the southern ecozone were larger in size than the northern ecozone. The higher density of trees in the northern ecozone indicates that areas with arid and semi-arid climate may be a more favorable habitat for *O. lanceolata* than the semi-humid conditions prevalent in the southern ecozone. In addition to the low rainfall and high temperature conditions of the northern ecozone, the locations of the three populations of *O. lanceolata* were stony and rocky which are characteristic features of sites reported in the literature as suitable for the species (Dale, 1936; Von Breintenbach, 1963; Herrera, 1988a; Beentje, 1994, Wyk and wyk, 1997). This suggests that the northern ecozone may be the natural habitat for *O. lanceolata* while the Southern ecozone is a transition zone between the natural habitat of *O. lanceolata* in the North and the most humid forest zone in the South. Yet, trees were much bigger in size in the Southern ecozone than

in the northern ecozone. This may be due to the difference in rainfall and soil nutrient status. The southern ecozone, which tended to have larger trees receives higher rainfall compared to southern ecozone. This was also further supported by the correlation analysis, which showed a strong positive correlation between rainfall and mean tree total height, tree diameter and basal area per hectare. Similarly within the Southern ecozone Nundu and Sao Hill that receive rainfall amounting to 1500 and 930 mm respectively had bigger trees than Image that receives only 730 mm per year. In the northern ecozone, however, the Mgwashi population that receives relatively higher rainfall than Gubali had lower tree density, basal area and size of trees. It is possible that big trees in Mgwashi were more heavily harvested than in Gubali as this was one of the areas where sandalwood production began in Tanzania.

The variation in stocking and morphological attributes could also be attributed to variation in soil fertility status among populations. Significant negative correlations were found between tree diameter and soil pH and between tree diameter and available phosphorus. This shows that *O. lanceolata* favors soils with low pH. The correlation with P was, however, due to the strong link between pH and P, which tends to get fixed at low pH. On the other hand, total soil nitrogen was positively correlated with almost all morphological parameters. The southern ecozone had soils with a higher nitrogen content compared with the northern ecozone as a result of which they were able to support significantly larger trees.

The findings of the present research are in agreement with reports in the literature. For example, Crawley (1986) outlined some of the major abiotic factors responsible for influencing the distribution and morphological variations of species within and among populations. These included water and nutrient availability, soil acidity and extremes of temperature. The small sized plants and leaf dimensions observed in the northern ecozone (Bereko, Gubali and Mgwashi) could be due to these factors. Where water is scarce, plants tend to undergo modification including small size, development of small waxy leaves, and deep and extensive root systems (Slatyer, 1967; Titley 1982). Temperature has also been reported to play a major role in controlling plant size and stature (Went, 1953 Crawley, 1986). High temperature can result into failure of the

plant cooling system and stomata may close as a response to water shortages. This could cut the overall photosynthesis and result into reduced plant growth (Levitt, 1972). Thus, the small stature of trees in Gubali could be attributed to high temperature. The effect of soil fertility on tree growth is also well documented and of several edaphic/soil factors, nutrient availability is reported to be the most important in influencing species morphological stature (Bell, 1982). Low soil nutrient is generally associated with small sized trees as in the Northern ecozone.

The study on the dioecious characteristics of *O. lanceolata* revealed that no significant difference existed between male and female *Osyris lanceolata* trees in most morphological parameters. Also with the exception of Gubali population no tendency of clustering was observed in both sexes. According to Bawa and Opler (1977), this kind of sex distribution in a population is considered to be ideal for effective pollination in plants. The segregation tendency observed in Gubali where males and females tend to cluster together rather than being randomly distributed may be a strategy by the species to avoid deleterious inter-sexual competition effect on limited resources environment. Clustering far from each other reduces competition that might be detrimental to either of the sexes (Onyekelu and Harper, 1979; Cox, 1981). This may be true in Gubali as rainfall in this area was the least and evapotranspiration was the highest among all the six populations.

3.4.2 Regeneration of *Osyris lanceolata*

From the results of the present study it was shown that *Osyris lanceolata* regenerated from both seed and coppice/rootstock origin. Considering the regeneration that had reached sapling stage that is given a chance to proceed to the adult phase, 61% was of either coppice or rootstock origin. This suggests that regeneration of the species from seeds is still a problem even under natural conditions. Msanga (1998) reported that seeds of *Osyris lanceolata* possess some sort of mechanical dormancy, which hinders their spontaneous germination following their dispersal from the tree.

The season at which seeds are shed could be another reason for little regeneration from seeds observed in the present study. A study on phenological events at Sao Hill revealed that the peak fruiting period in *Osyris lanceolata* is within May-June, which coincides with the beginning of the dry season when water availability could seriously limit seed germination. Water shortage causes failure of seeds to germinate or the germinating seedling might die within a few days (Landsberg, 1984; Hutching, 1986; Bazzar, 1991). In addition, the recalcitrant character of seeds of *Osyris lanceolata* (Mbuya *et al.*, 1994) associated with rapid loss of viability may also contribute to reduced level of regeneration. By the time rains start, which is six moths from the time when ripe fruits were formed, most seeds will already be dead. Coincidence of the rain season and seed dispersal is considered to be most important in recalcitrant seeds where advance regeneration is of more importance than seed bank (Bazzar, 1991).

The small amount of regeneration from seed could also be linked to herbivore and pathogenic attack. These might remove reasonable quantities of seeds and seedlings. According to Janzen and Yanes (1991), seed predation and pathogenic attack are serious problems in many tropical mainland species especially in the dry forest zone where more than 90% of all tree species have more than 50% of their seeds killed by predators and fruit fungi between fruit set and seed germination. Pathogenic attack is usually a serious problem within a few months after regeneration, the major cause being fungi attack (Augspurger, 1990). Seedling death due to herbivores could also be another factor as grazing was observed to be common in most of the populations studied. Herbivore is also reported to indirectly increase seedling mortality as they provide entry opportunity to pathogens, which in turn infect and kill the plant (Watkinson, 1986; Bazzar, 1991).

The low level of regeneration beneath crowns of mother trees observed in the present study is not uncommon (Kimmins, 1987). According to Jansen (1970) and Connel (1971), survival of regenerating seedling is generally lower close to mother trees due to high mortality rate caused by host specific seed predators, herbivore, fungi and pathogens. This hypothesis, which is sometimes referred to as escape hypothesis, makes net regeneration to be at some distances away from the mother

trees. It is a widely accepted hypothesis to explain the cause of low regeneration under mother trees (Gilles, 1992; Lask and Ogden, 1992; Harmer, 1994). According to the observation of Brokawa (1989), Wighma and Cano (1991) in *Myriathes fragrans* seedling density tends to be initially high beneath the parents. However, at later stage, the density is reduced due to high mortality caused mainly by falling branches, shading, drought stress or a combination of these favouring those that are far away. Madsen (1995) relates avoidance of clustering regeneration close to the mother trees as a strategy to avoid within species competition for growth resources.

The variation in abundance of regeneration according to size (between seedling and sapling) observed in *Osyris lanceolata* is also not an exception as it is a common characteristics of the size class distribution of regeneration in many tropical trees. In most cases, there is a pronounced absence of sapling and juvenile trees while seedlings are plenty. This generally results when regeneration of the species is severely limited for some reasons with most seedlings dying before becoming established (Richard, 1952; Whitmore, 1975, Viana, 1990).

3.4.3. Reproductive biology of *Osyris lanceolata*

The reproductive process study revealed that flower formation times differed in male and female plants. Females had their flowers opened almost two days before males. Also anther withering in males was extended by almost five days after the stigma withering ceased. The observations somehow contradicted with what was reported by Herrera (1984a) in the same species in Mediterranean region where female plants tended to begin flowering later than males. The time difference observed between effective flowering and stigma/anther withering could be a strategy by the species to ensure successive fertilization. Similarly, the extension of anther withering a day or two after stigma withering ensures that as many pistils as possible are fertilized before anthers dried off which is also advantageous to the species (Bawa, 1980b; Bullock and Bawa 1981). On the other hand, early flowering of females could be disadvantageous to the species as it decreases the overall fitness by utilizing pollen produced by few plants before a variety of pollens are made available for fertilization from a variety of males (Bullock and Bawa 1981). How *O. lanceolata* adjusts itself to minimize this effect is not known.

The long duration of the transition period between fruit initiation and formation of unripe mature fruits in *Osyris lanceolata* suggests that there is a tendency for the fruits initiated to undergo some sort of dormancy between the two stages. A study carried out by Herrera (1985) in the Mediterranean region on the same species revealed similar situation. The mean duration observed for this transition period was 42 days ranging between 7 days and 175 days.

Results of the reproductive success study suggest that fruit set without fertilization (apogamospermy) does not take place in *Osyris lanceolata* as speculated. The emptiness observed in *O. lanceolata* seeds may indeed be the result of poorly developed ovaries. Consumption of developing embryos by insects at larva stage also accounted for emptiness. It was noted in the study area that there was a tendency for insect eggs to be laid on the flowers of the species. These eggs were enclosed within the fruits during the early stages of fruit development. Upon hatching, the larva stage may have eaten the embryo before getting out through tiny holes that could easily be noted on the fruit cover. Of the total seeds that were declared empty, the larva damage accounted for more than 50 % while the rest was perhaps a result of poorly developed embryo. The likely insect responsible for this damage was a beetle *Dismegistus sargumeus* De Geer that feed mainly by sucking the juice of the fruits. The insect was found to be present in all the study sites samples and those visited during reconnaissance survey. Apart from embryo consumption by larva, the internal content of some seeds were found decayed which suggests that the feeding mechanism of the insect might have introduced some pathogens that killed the embryo or some toxic compounds might have been released by the beetles in the processes of sucking that in turn killed the embryo within the seed.

The reproductive success in the assisted and natural pollination experiments suggests that pollen availability is limiting in *O. lanceolata* as more flowers were aborted in the natural pollination treatment compared to assisted pollination. Poor pollination under natural pollination is a consequence of insufficient pollen production or failure of the pollen to move across trees as observed in fig trees (Wiebes, 1979) and oil palm plantations (Handerson, 1986). One of the factors that

are known to be responsible for pollen failure to reach the target is the distance between male and female plant. The further apart the trees are, the greater the chances of pollen failure to move across (Simons, 1996a). Female and male trees at the study site were observed to be spaced at longer distances from each other (23 m). However, the importance and effect of this distance cannot be overemphasized as the maximum distance between male and female for effective pollination has not yet been established.

The fact that female flowers of *O lanceolata* offer little reward in terms of nectar and are inconspicuous to pollinators and hence rarely visited (Herrera *et al.*, 1984), could have added to problem of pollen availability. This is a common phenomenon in most dioecious species where males tend to have frequent visits compared to females as they offer more floral reward in terms of pollen and nectar. In some plants female flowers are forced to mimic the male character to facilitate their visit (Baker, 1976).

The flowering time difference between male and females observed in *Osyris lanceolata* could have also contributed to pollen limitation in natural pollination leading to fewer fruits that were formed. Time difference in flowering is known to be limiting especially when the interval in flowering between the sexes is too long. By the time one sex is in flower the other might have already been completed or be in its late stage, affecting the whole pollination process (Bawa, 1976).

CHAPTER IV

QUANTITY AND QUALITY VARIATION OF ESSENTIAL OIL FROM *OSYRIS LANCEOLATA* (AFRICAN SANDALWOOD) IN TANZANIA

4.1. Background information and objectives of the present study

4.1.1. Introduction

Osyris lanceolata (African Sandalwood) is one of the sandalwood plant species, which are known to produce fragrant scented wood from which sandalwood oil is extracted (Walker, 1966; Mbuya *et al.*, 1994). The utilization of *Osyris lanceolata* in the production of oil to supplement or blend with that of S*antalum album,* which was becoming scarce and expensive (Srinivasan, 1992; Rai and Salma, 1990), started in early 1900's (Hill, 1937; Dale and Greenway, 1961; Eggling and Dale, 1962; Walker 1966; Iyenga, 1968).

Harvesting of *Osyris lanceolata* for perfumery and overall production of essential oil in tropical Africa has mainly been concentrated in Tanzania although the species is reported to occur in many tropical and Mediterranean countries (Dale and Greenway, 1961; Eggling and Dale, 1962; Walker 1966). Within Tanzania, harvesting of the species is also localised with some parts particularly the northern ecozone being heavily exploited while others including the whole of southern ecozone have remained almost untouched (C.K. Ruffo, personal communication). The criteria used for this selection have been obscured for years and no attempts have ever been made to find out the reasons. Some traders involved in the exploitation of the species claim that the main criteria for the selection of the northern ecozone population were for the high stocking density, which made harvesting economical. The possible existence of quality differences among populations is also speculated as one of the factors (M. Fazal, personal communication). The speculation is considered to be the most influential, although it cannot be overemphasised without solid evidence as no work has so far been carried out to identify the chemical composition and oil content of *Osyris lanceolata* in Tanzania and what extend the compound characterising sandalwood oil quality varies among populations.

4.1.2. Sandalwood oil and its major uses

Sandalwood oil is one of the most expensive essential oils. Official export price for quality oil in 1996 was US$ 1500 per kilogram and the price was expected to continue rising in the following years (Nasi and Ehrhart, 1996). The oil is used in the luxury cosmetics, perfume and fragrance industry. The main buyers are France, USA, UK and the Middle East (Coppen, 1995). The excellent blending properties and presence of a large proportion of high boiling point constituents (particularly santalol) and the antiseptic properties makes it valuable as a fixative for other fragrances (Srinivasan *et al.*, 1992; Coppen, 1995).

The use of sandalwood oil extends beyond the perfumery use. It is useful as a popular sedative in oriental medicine and is considered to have narcoleptic effect (Okugawa *et al.*, 1995). It has a chemo-preventive effect, thus used in treating inflammatory and eruptive skin diseases (Dwivedi and Zhang, 1999), useful in treating bronchitis, dysuria, gonorrhoea, and urinary infections (Okasaki and Oshima, 1953; Winter, 1958). However, the use of oil as a base of fragrance is outweigh by far the medicinal use (Iyenga, 1968; Srinivasan *et al.*, 1992.

4.1.3. Variation in sandalwood oil quality among species

The quality of Sandalwood is in general assessed on the basis of the amount of oil that can be extracted from wood and the quality associated with it (Iyenga, 1968; Verghese *et al.*, 1990). Sandalwood species vary a lot in quality on the basis of which they are graded as primary, secondary and tertiary source. Of all sandals, the East Indian Sandalwood (*Santalum album*) is considered to be the best and the primary source of oil (Walker, 1966; Srinivasan *et al.*, 1992). The secondary source is *Santalum spicatum* from Australia. This species is claimed to have most properties similar to *S. album* and has been subjected to intensive harvesting, the whole excise being exaggerated by the high price that had been offered. By the 1970s only small trees could be traced with little effort to raise the species artificially (Errickson *et al.*, 1973).

Santalum lanceolatum, *Santalum yassi* and *Osyris lanceolata* are regarded as tertiary in their importance. The utilization of this source became important following the fall in supply and rising prices of the primary and secondary sources. The decline in production of *Santalum album* and *Santalum spicatum* is reported to have been attributed to careless and intensive harvesting. The outbreak of spike disease that has been killing *Santalum album* at any age and size in a few months, if not weeks added to the production fall (Iyenga, 1968; Srivanasan *et al.*, 1992). Till now, cultivation of *Santalum album* had limited success and natural populations continue to be the major source (Coppen, 1995).

The choice of *Osyris lanceolata* was mainly based on its scented wood, which appeared to be similar to that of *Santalum album* and some amount of sesquiterpenic alcohol contained in it (Iyenga, 1968).

Of the sandalwood oil quality determinants, the percentage of odoriferous sesquiterpenic alcohol compound known as santalol is considered to be the prime (Iyenga, 1968; Verghese *et al.*, 1990; Srinivasan *et al.*, 1992). The amount of this compound dictates the fixative property and tenacious aroma of sandal oil together with the warm woody character of it. In *Santalum album* oil, this compound forms more than 90% of the total oil while in *Santalum spicatum*, the content of this compound ranges from 70-80%. In *Santalum yassi* and *Osyris lanceolata*, santalol content forms 49% and below 35% of the total oil respectively. *Santalum album* is considered to be the mother of all sandals due to the high proportion of Santalol compound in the oil (Iyenga, 1968; Smith and Moris, 1979; Shankaranarayana *et al.*, 2000)

Other compounds adding to the overall note, odour and quality of sandalwood oil are the amount of α and β santalenes and santalyl acetate. In *Santalum album*, the oil contains 4-11% of the two compounds. Also a number of minor compounds add to the spicy character and overall quality of the oil. These include constituents such as curcumene, farnesene, borneol, dihydrobeta-santalol, teresantalol, isovaleraldehyde, santenone, nortricycloela-santalol, teresantalic acid, santene, nortricycloekasantalic acid, tricycloekasantal, and many others (Srinivasan *et al.*, 1992; Shankaranarayana *et al.*, 2000).

Apart from the general compounds characterising sandalwood oil quality, *Osyris lanceolata* is also known to contain a large amount of essential oil known as lanceol similar to that of *Santalum lanceolatum* (Narves and Ardizo, 1954).

4.1.4 Variation within species: effect of site conditions

As little information is available regarding the quality of oil in *Osyris lanceolata,* much of the information included here is derived from observations made in its related species producing similar oil, particularly *Santalum album* and *S. spicatum* in which much have been explored.

The quantity and quality of oil produced depend on many factors including soil and climate (Shankaranarayana *et al.*, 2000). Wood containing the high proportion of heartwood, which is the most important source of the oil, is believed to come from drier regions, particularly on stony ground. The yield of oil has also been observed to be much higher in sandalwood from poor soils compared to those growing on fertile land. Fertile soils with sufficient rains favour luxuriant growth of the species, but formation of the scented heartwood is quite slow. On steep slopes, where soils are rather poor and rocky and xerophytic conditions prevail, even smaller trees have been observed to have reasonable amount of heartwood and produce more oil (Gunther, 1952; Bhatnagar, 1965). Iyenga (1968) also reported the relationship between environmental conditions and heartwood formation/oil content. It is reported that heartwood formation with much oil is favoured in rocky soils with limited hosts compared to those growing in good soils with several hosts involved. However, little is reported on how far the soil should be poor and xerophytic conditions prevail as excessive nutrient deficiency and aridity are likely to result in stunted growth of any species.

The reason why oil content seems to be higher in wood from drier parts with poor soils than in wetter fertile sites is also uncertain. Srinivasan *et al.* (1992) Related the difference to the presence of sesquiterpene compound in the oil, which is the principal fragrant. This compound is a secondary metabolite and its formation is

favoured under conditions of stress. It is argued that, a significant interrelationship between factors that promote growth and conditions of stress are important for formation of heartwood and oil in sandalwoods.

4.1.5 Variation within a species and within a tree: age/maturity effect

Quantity and quality of sandalwood oil are also influenced by the age at which the tree is harvested and the position within the individual tree. In *Santalum album*, mature trees (30 –50 years) contain 2.8-5.6% and young trees at the age of around 10 years contain 0.2-2% oil. While santalol content in mature trees is high (more than 90% of the oil content) young trees at the age of 10 years is estimated to contain 80-85% santalol (Coppen, 1995). However, the amount of santaly acetate and santalenes, which are also important compounds in determining the quality of oil, are higher in young compared to mature trees. These two compounds decrease with age as they are converted into santalol as the tree ages (Shankaranarayana and Parthasaranthi, 1984).

Within a single tree the content of sandalwood oil is reported to decrease markedly as you move from root to the tip and from the core to the peripherals of the tree. In *Santalum album*, roots are estimated to contain 3.5-6% while stems contain 3-5% oil. The lowest content is in the branches in which the oil content ranges from 1-3%. Likewise the amount of oil extracted from the core (heartwood) is significantly higher than what is obtained from the peripheral parts of the wood, which is composed mainly of sapwood. The difference between the two positions could amount to 20% in *Santalum album* (Shankaranarayana and Parthasaranthi, 1987).

4.1.6 Objectives

The present research was carried out to assess the chemical composition and quantity of sandalwood oil from difference populations of *Osyris lanceolata* in Tanzania. The research also aimed at assessing the variation in quantity and quality of oil among populations and between the root and the shoot within individual trees.

In order to meet these objectives, the following research questions were raised.

i. How much oil and santalol content can be extracted from *Osyris lanceolata*?
ii. Do populations differ significantly in the amount of oil and santalol that can be extracted from *Osyris lanceolata* wood? If populations differ,
iii. What factors are responsible in controlling the amount and quality of oil?
iv. What other chemical compounds, and in what proportions are contained in oil from *Osyris lanceolata* wood?

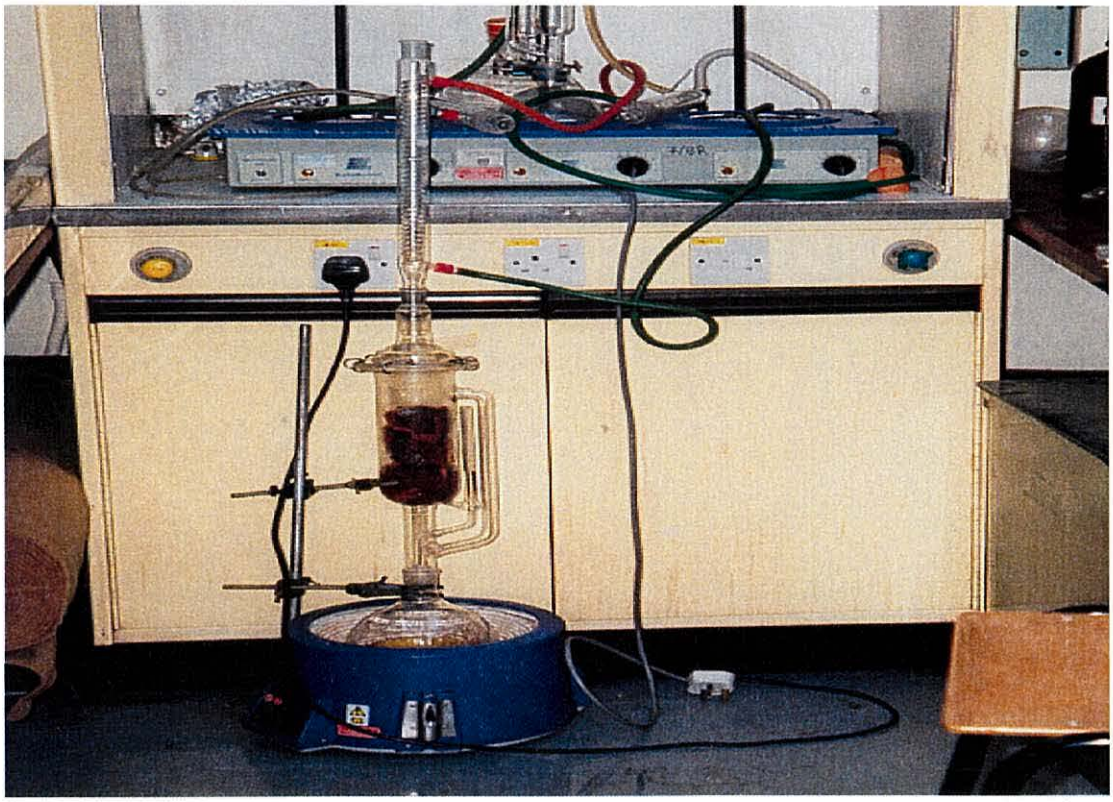
4.2. Materials and methods

4.2.1 Extraction of sandalwood oil and quantitative assessment

Wood samples from six populations were collected and analyzed for oil content to assess the variation among the six populations and the difference in oil content between the root and shoot of the same tree. From each of the six populations i.e. Nundu (Njombe), Sao Hill (Mufindi), Image (Iringa), Gubali (Kondoa), Bereko (Kondoa) and Mgwashi (Lushoto), three mature trees were randomly selected. From each tree four samples were drawn to include two samples from the root and two from the shoot. The root samples were taken from two depths in the soil: root sample 2 (R2) from 5-8 cm and root sample 1 (R1) from 25-28 cm depth. The shoot samples were also taken at two heights above ground: shoot sample 2 (S2) at the ground level 5-8 cm and shoot sample 1 (S1) at 25-28 cm height above ground. In total, 12 samples were taken from each population, making a total of 72 samples for the six populations. The wood samples were dried and taken to the laboratory at the University of Wales Bangor, where oil extraction and further analysis was done.

The dried wood was later chopped and ground into powder before being subjected to stem distillation. A small sample of ground wood (15-25g) was weighed using an electronic weighing scale. This weighed amount was placed in small thimbles, which were then placed in soxhlet apparatus for extraction using dichloromethane (CH_2Cl_2) as an extract. Plate 4.1 shows the set up of the equipment used in the extraction.

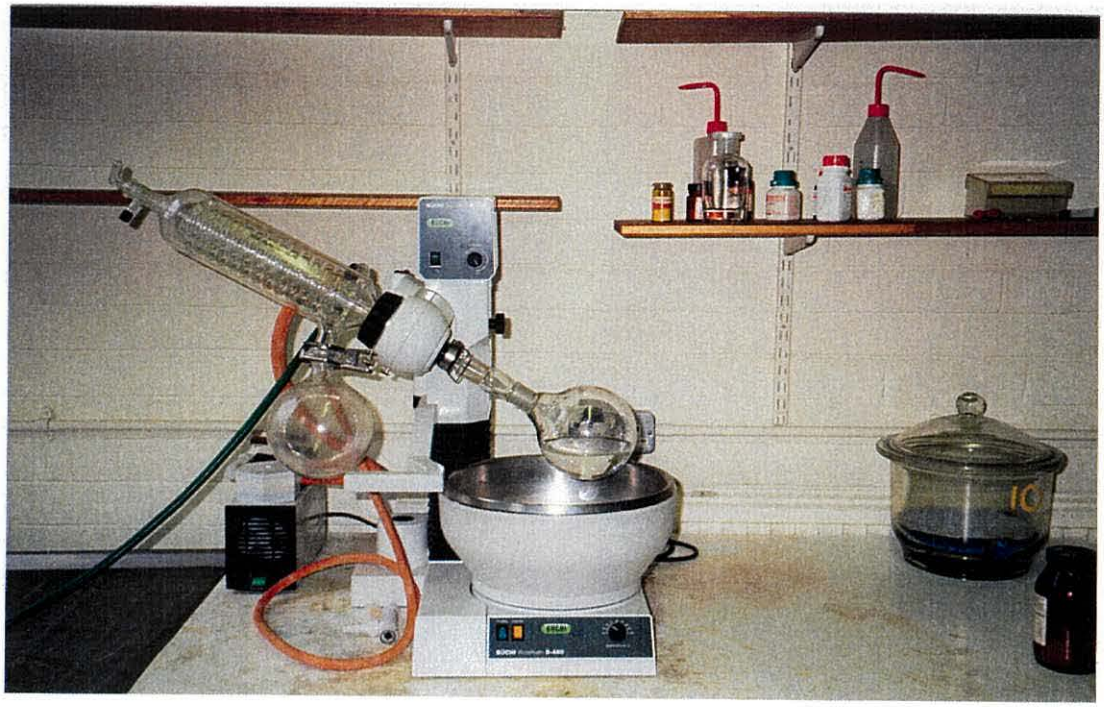
Plate 4.1. Soxhlet equipment used in extracting oil from wood samples



The extraction process for each batch of samples was run for 36 hours. In this process, the solvent is heated up to its boiling point and the resultant steam is passed through wood samples and oil is extracted after several times of re-motion. The steam is cooled down through a series of cooling systems in which cold water is kept under continuous flow. Upon cooling, the solvent drops into thimbles, which are placed just below the cooling system. Once the thimbles are full, the solvent and the extracted oil is poured into solvent container, which is subjected to continuous heating cooling and circulation.

At the end of the extraction period (36 hrs), the resulting extract was filtered using a filter paper. The solvent containing oil was then evaporated through rotary evaporator (Plate 4.2), leaving yellow clear oil behind. The resulting oil was weighed and expressed as percentage of total wood powder that was subjected to extraction.

Plate 4.2. Rotary evaporator used to evaporate the solvent to get the oil



4.2.2 Quality assessment through determination of santalol content

From the oil obtained, quantitative composition of santalol in the oil was determined on a gas chromatograph equipped with a flame ionization detector. To be able to identify the santalol in the oil samples and determine the amount, a standard santalol had to be obtained (99.5% pure) and used for comparison purposes. The standard was obtained from ''Istituto Sperimentale Per la Elaiotecnica'', Pescara in Italy under the kindness of Prof Georgio Bianchi. From the standard, seven samples were prepared with weight ranging from 0.0438 to 0.1294 g. The weights were then made up to 0.8 ml by adding ethyl ether as a solvent. The choice of the solvent was based on the ability of the oil to dissolve in it and its low boiling point compared to the compound of interest, which enables its early detection in the GS. This is important to avoid interference between compounds contained in the oil with that of solvent.

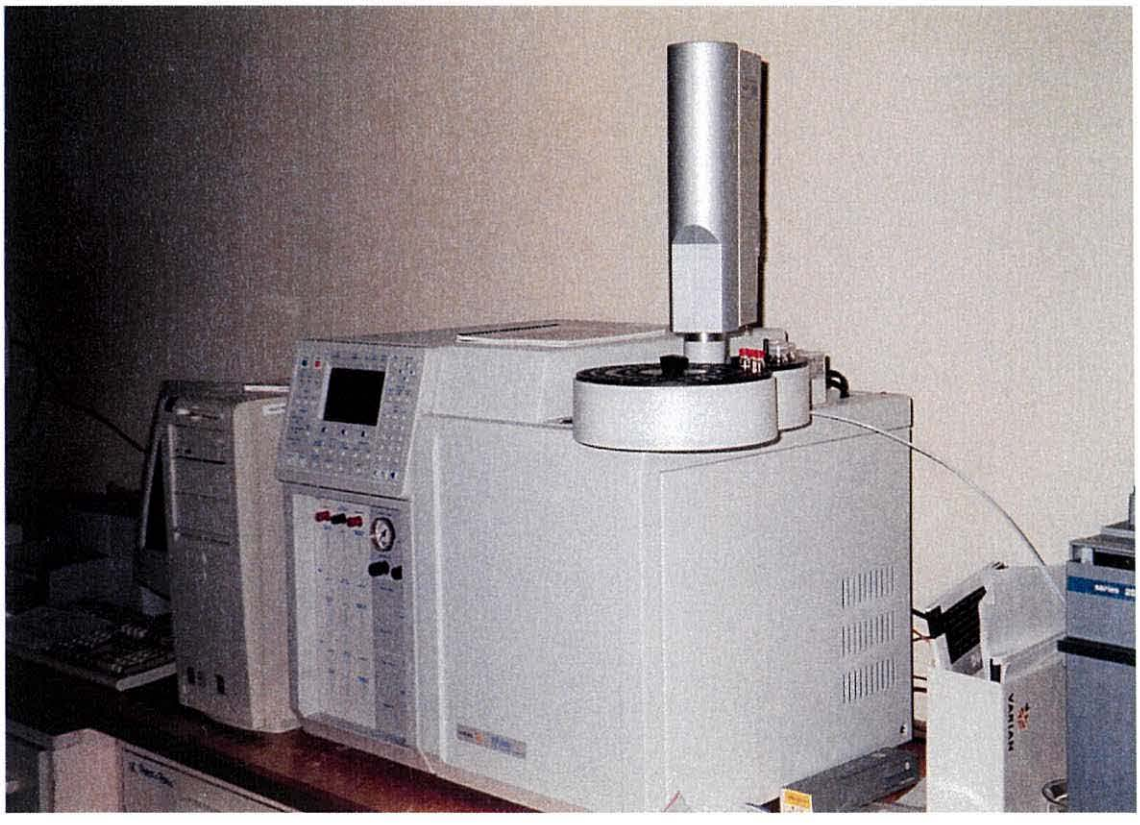
Samples were then run in the GS equipment with a starting temperatures of 40 °C held for five minutes programmed at 4°C/minute to 300 °C. The temperature was then held at this point for 20 minutes. The injector temperature was 300 °C and detector temperature was 310 °C. Helium gas was used as the carrier with a head

pressure of 12.0 psi. Plate 4.3 shows the GS equipment/machine employed for this purpose. From each standard sample that was run, the area count corresponding to santalol was obtained which was later used to plot a linear graph of oil weight against area counts. The linear equation established through this relation was:

Area cont (Y) = 4966981962.9 x Oil weight (X)

After establishing this relationship, experimental samples were run by weighing about 0.1 g and dissolving in the same solvent and amount as the standard. They were then run in the GS under the same condition as the standard. The area counts from each sample corresponding to santalol was obtained. The area counts were plugged in the equation whereby the corresponding weight of santalol required to produce the area count was obtained. The weights were then expressed as a fraction of the total weight of oil that was involved to give the percentage of santalol contained in the weighted oil.

Plate 4.3 GS apparatus/machine used in santalol identification and determination of the amount contained in the oil



The percentage after undergoing the necessary transformation, were analyzed to make comparison of quantitative and qualitative aspects of the oil among sites, between ecozones and among different portions of the trees through analysis of variance of MINITAB. Where significant differences were observed a Turkey's pair-wise comparison was employed to identify the deferring means. Allometric relationships were also explored to seek any evidence of quantity/quality variation with site characteristics of climate and soils and some tree morphological parameters.

4.2.3. Chemical composition of *Osyris lanceolata*

The aim of this experiment was to identify possible compounds contained in *Osyris lanceolata* apart from Santalol. Only samples from four trees from Sao Hill population were used for analysis due to the large expense involved. All samples were taken at the base of the trees. The wood samples were sent to the 'Istituto Sperimentale Per la Elaiotecnica, Pescara in Italy where the analysis was carried out in kindness of Prof. Georgio Bianchi.

Dried heartwood (10g), was finely chopped, treated with liquid nitrogen and crushed and then extracted with CH_2Cl_2 for three days in Soxhlet apparatus. After remotion of the solvent under reduced pressure, the extract (1.5 g) was then distilled with odor-free water for two hours giving 350 ml of distillate. The distillate was saturated with NaCl and extracted with fresh distilled diethyl ether (3 x 100 ml). The ethereal solution was dried under Na_2SO_4 and concentrated to rotary evaporator to give yellow oil used for GS and GC/MS analysis

The quantitative composition of all the essential oil was determined on a Perkin Elmer 8500 gas-liquid chromatograph equipped with a flame ionization detector (FID). A DB-5 capillary column (J&W Scientific) of 30 m length, 0.32 mm in diameter and 0.25 μm film thickness was used. Samples (1 μl) were injected in 'split' mode (split ratio 1:10) with a starting temperature of 40 °C for 5 min, programmed at 4 °C/min to 300 °C. The injector temperature was 300 °C and

detector temperature was 300 °C. Helium was used as the carrier gas with head pressure of 12.0 ρsi. The content of individual constituent were expressed as Peak area percent computed by GS system integrator.

Analysis was performed on a varian GC equipped with a Finnigan ITS 40 mass selective detector using the same capillary column and oven temperature programmed for GS. Mass spectra was acquired over 40-400 amu Range at 1 scan/sec with ionizing electron energy 70 eV, electron current 0.3 mA, ion source 200 C; the vacuum was 10-5. Helium was the carrier gas with head pressure of 8.0 ρsi.

Identification of essential oil components was done using authentic reference compounds, peak matching library search as well as published mass spectra of Adam (1995) and Joulan and Konig (1998). Identified compounds were listed and percentage associated with each was provided.

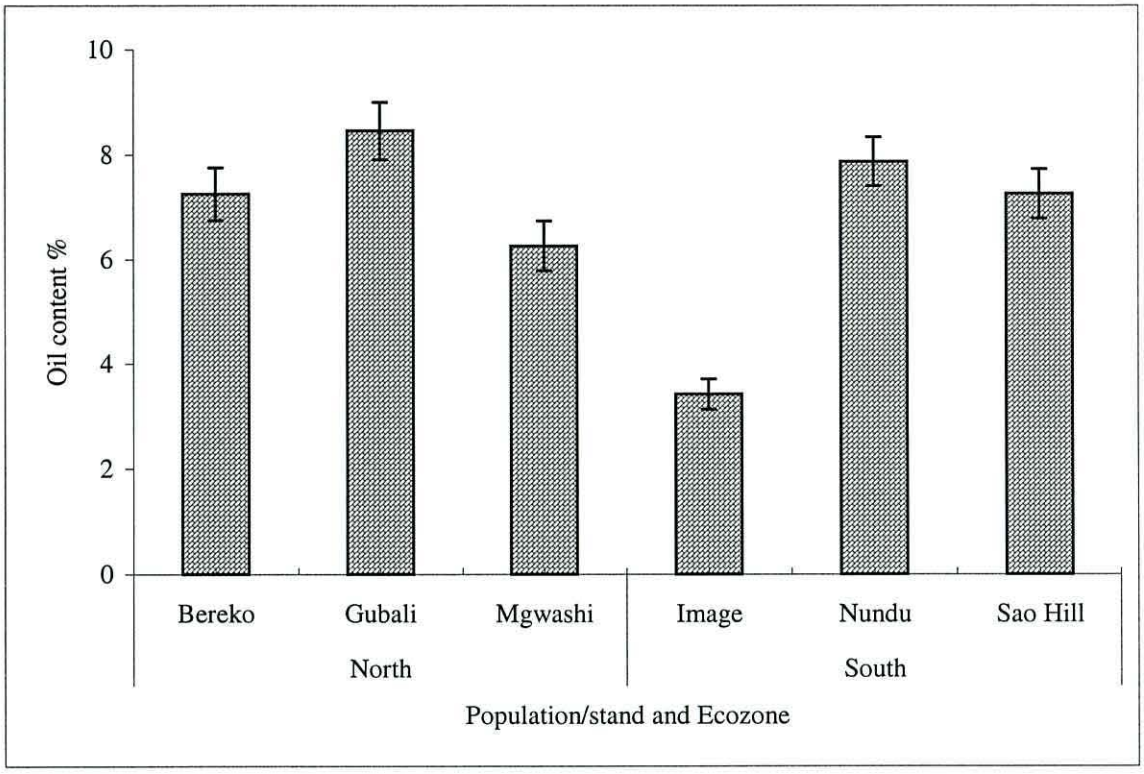
4.3 Results

4.4.1 Quantitative variation in sandalwood oil content

The result on quantitative variation in oil production among populations is presented in Figure 4.1. The results showed that sandalwood populations differed significantly ($P < 0.01$) in the amount of sandalwood oil produced. Wood from Gubali population was relatively rich in oil, giving 8.45 ± 0.54% out of the wood material that was subjected to extraction. The least content of oil was produced by sandalwood from Image population, where 3.42 ± 0.29% of oil was obtained. Nundu, Sao Hill and Bereko populations produced similar quantities of oil.

The southern and northern ecozones also differed significantly ($P = 0.04$) in the amount of oil produced. More oil was produced from sandalwood of the northern ecozone, amounting to 7.32 ± 0.32% oil. The mean production from the southern ecozone amounted to 6.18 ± 0.41% oil.

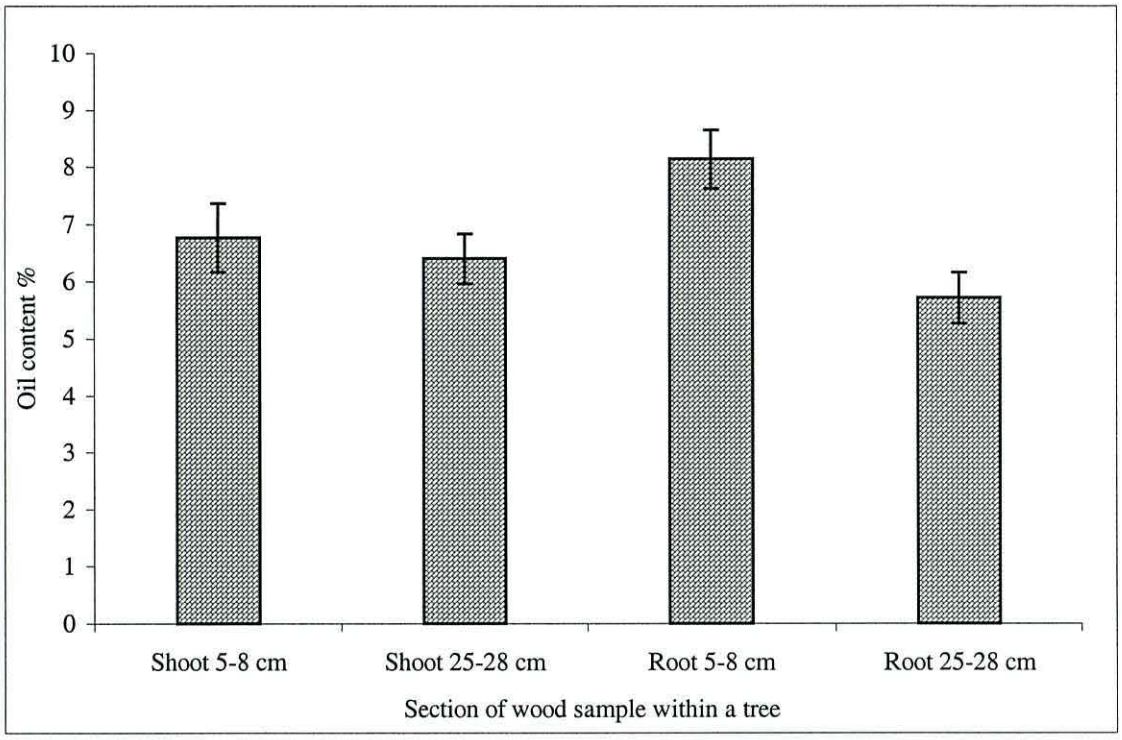
Figure 4.1 Variation in oil content in *Osyris lanceolata* from Tanzania populations



A significant variation in amount of oil existed within the northern ecozone (P = 0.02) where Gubali population produced the highest quantity (8.45 ± 0.54%). The least quantity was produced by wood from Lushoto (6.26 ± 0.48%). A significant difference was also observed among populations within Southern ecozone (P < 0.01). Wood from Nundu contained more oil (7.87 ± 0.47%) while the least content was in wood from the Image stand where the content of oil was 3.42 ± 0.29%.

Within individual trees, the amount of oil produced by the root and shoot portions did not differ significantly (P = 0.27) although the root portion seemed to have higher oil content (6.92 ± 0.39%) compared to the shoot portion (6.58 ± 0.36). However, there was significant difference in oil content between the various sections of root and stem sections (P < 0.01). The root section immediately below the ground level (5-8 cm) had significantly more oil (8.13 ± 0.52%) compared to all the other sections. The least oil content was obtained in wood samples from the root section further below (25-28 cm) where the oil content was 5.71 ± 0.44% (Figure 4.2). The two shoot sections had similar oil content.

Figure 4.2 Variation in oil content in *O. lanceolata* wood derived from different sections within a tree

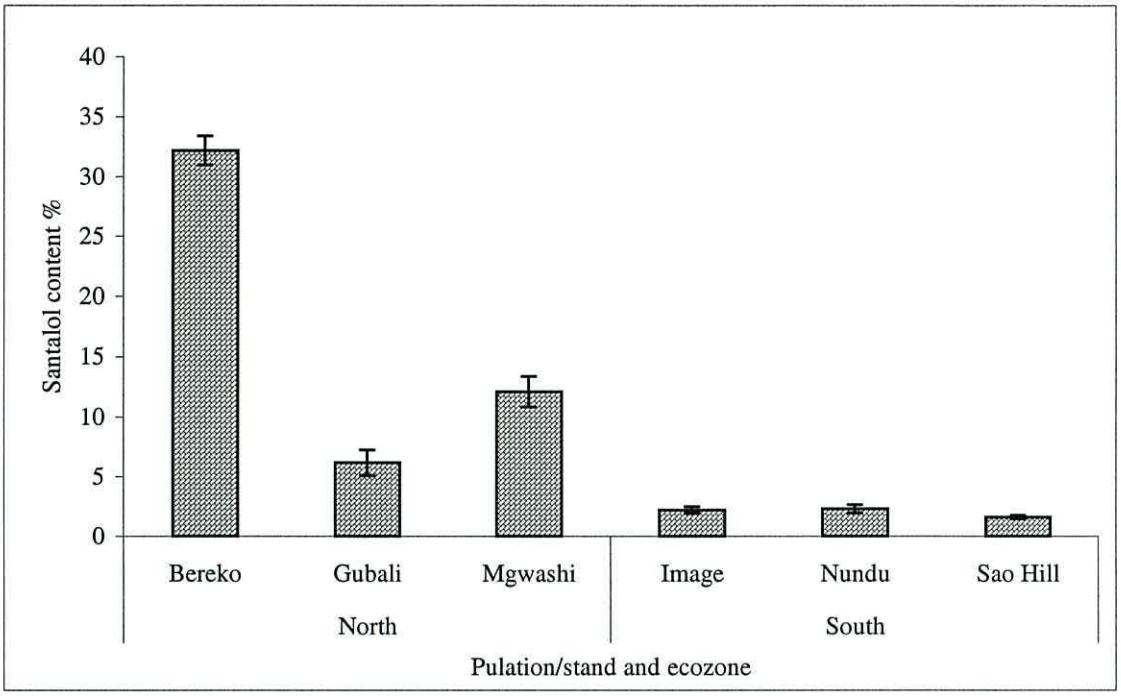


4.3.2 Santalol variation as a measure of oil quality

The result from qualitative analysis obtained through determination of santalol content in the oil extracted revealed that *Osyris lanceolata* populations differed significantly in santalol content ($P < 0.001$). The content ranged from as low as $1.6 \pm 0.2\%$ in Sao Hill population to $32.2 \pm 1.2\%$ in Bereko population (Figure 4.3).

Ecozone also differed significantly in santalol content ($P < 0.001$) with oil from the Northern ecozone containing more santalol ($16.8 \pm 2.0\%$) than oil from the southern ecozone whose oil contained $2.02 \pm 0.16\%$ santalol. Significant difference in santalol content also existed within the Northern ecozone ($P < 0.001$). The best oil quality was from Bereko that contained $32.2 \pm 1.2\%$ santalol while oil with the least santalol content was from Gubali, containing $6.14 \pm 1.07\%$. Populations in the Southern ecozone had almost the same content of santalol ($P = 0.194$), although Sao Hill oil seemed to be of inferior quality compared with oil from Image and Nundu (Figure 4.3).

Figure 4.3 Variation in santalol content in sandalwood samples from six populations in Tanzania



Within an individual tree, oil from the shoot and root system had similar content of santalol (P = 0.851). The mean content of the root portion was 9.18 ± 1.87% while the shoot portion contained 9.64 ± 1.92%. The sections within a tree also had similar santalol content although wood samples taken at the ground level seemed to have more santalol compared to those taken beyond ground level (Figure 4.4).

4.3.3 Relationship between oil quantity/quality and site characteristics

The results of correlation analysis revealed that there was no relationship between wood oil content/santalol content and site characteristics of rainfall and soil fertility.

4.3.4. Chemical composition of essential oil in *Osyris lanceolata*

The analysis of oil from Sao Hill population showed that *Osyris lanceolata* oil contains several compounds, which vary in amount (Table 4.1). Of the compounds, Lanceol forms the highest proportion 56.73 ± 2.82 % of the total oil. Santalol, which characterizes the quality of sandal oil, forms less than 1% of the total oil.

Figure 4.4 Variation in santalol content in various positions of the tree

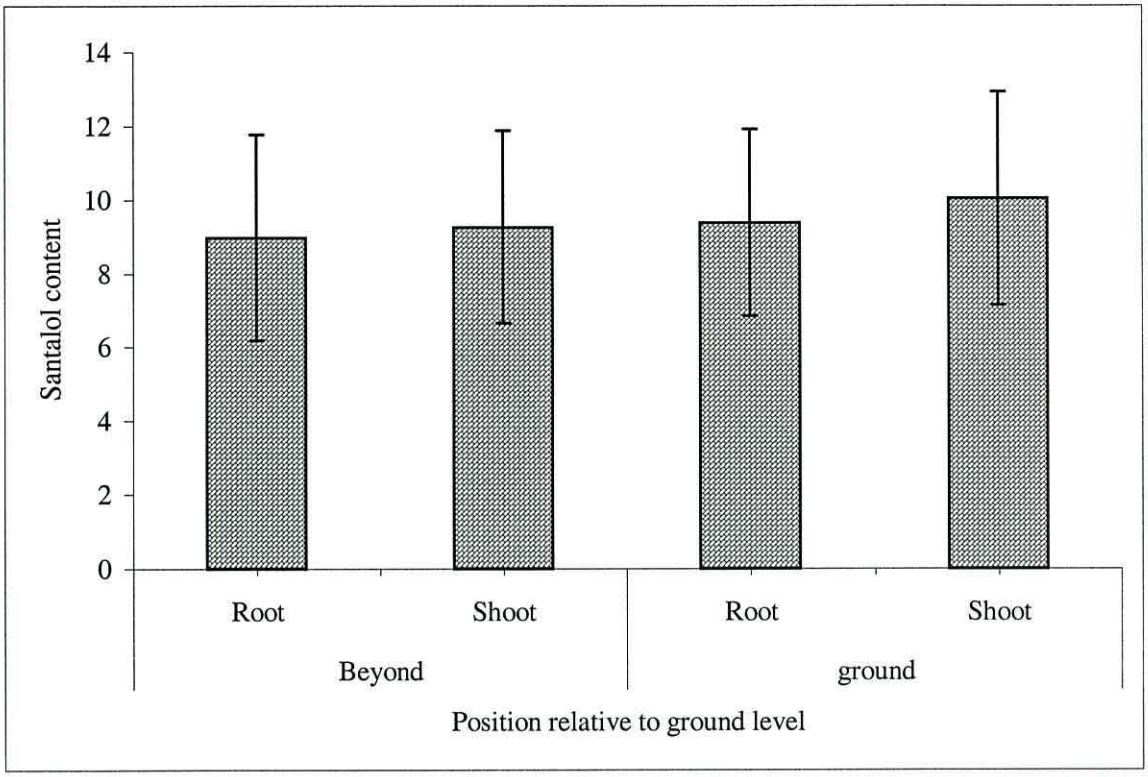


Table 4.1. Composition of *Osyris lanceolata* oil from Sao Hill stand

Compound name	% Content in oil	Std. error of mean
Z-Lanceol	56.7	2.82
Epi-α-bisabolol	5.08	0.99
Nuciferol (Z-nuciferol, E-nuciferol)	3.69	0.55
Bisabolene (Z-α-bisabolene, β-bisabolene)	3.27	1.43
Phthalate	1.46	0.44
Santalol (Z-β- santalol)	0.59	0.05
Bergamotene (α-trans-begamotene, α-cis-begamotene, β-trans-begamotene)	0.48	0.08
E,E-Farnesol	0.39	0.07
Santalene (α-santalene, β-santalene, epi-β-santalene)	0.21	0.14
Dendrolasin	0.20	0.02
E,β-Farnesene	0.11	0.08
β-sesquiphellandrene	0.10	0.06
Naphthalene	0.07	0.04
Curcumene (γ-curcumene, ar-curcumene)	0.04	0.03
Unidentifed Sesquiterpene	9.32	1.82
Unidentified compound	0.87	0.25
Unidentified acid	0.35	0.06

Other compounds forming reasonable proportions include Bisabolol, Bisabolene and Nuciferol. The respective content of these compounds were 5.05 ± 0.99, 3.27 ± 1.43, 3.69 ± 0.55%. However, the identity of some compounds including 13 Sesquiterpene alcohols that totalled to 9% of the oil was not possible due to limited facilities and reference compounds.

4.4 Discussion

4.4.1 Variations among populations

The results of the present study suggest that variation existed in quality of sandalwood oil produced among populations. There is, however, no evidence to suggest that the root system was superior to shoot system in the production and quality of sandalwood oil produced although oil content was higher in sections within the tree close to soil surface. The oil content of *Osyris lanceolata* in the Bereko stand that yielded quality oil was 7.25% (5.2-11.2%), almost twice as high as the oil content reported in *Santalum album* by Srivanisan *et al.* (1992) which is about 4.2% (2.8-5.6%). However, the santalol content of *Osyris lanceolata* oil from Bereko (32.2%) was only one third of the level in *Santalum album* oil (more than 90%).

When oil and santalol contents are considered together, *Osyris lanceolata* from the Bereko population in the northern ecozone could yield 2.4 kg of santalol from 100 kg of sandalwood, while 3.8 kg of santalol can be produced from the same amount of wood from *Santalum album*. This means although the content of santalol in *Osyris lanceolata* is general lower compared to *Santalum album*, its productivity is generally higher. Meanwhile, if long-term sustainable production of sandalwood oil is desired in the future, then trees from the Bereko population could be selected to propagate and establish plantations of *Osyris lanceolata* while looking at the possibility of improving the species for increased production and quality.

A negative correlation between rainfall and quantity of oil produced has been reported in *Santalum album* and some stresses including low soil fertility and limited rainfall have been reported to be essential for quality oil formation, as santalol is

believed to be an outcome of stress (Iyenga, 1968; Srinivasan *et al.*, 1992). However, in the present study, the reason why populations of *Osyris lanceolata* differed in content and quality of oil produced is a bit uncertain. From the result, there was no relationship between oil produced and the climate and soil of the different sites according to the correlation analysis carried out. There may be other factors responsible for this variation including environmental and genetic factors, which have not been identified in the study. These need further studies.

4.4.2. Variations within a tree

According to the results of the present study, oil content was high in sections of wood close to ground in both the root and shoot. The decrease in oil and santalol content as you move from tree base to the tip is also reported in *Santalum album* (Shankaranarayana and Parthasarathi, 1987). The difference is attributed to the fact that wood close to the base of the tree is more aged (relatively mature) and contains more heartwood compared to wood far away from the base. The more the heartwood that is contained in a tree, the more the content of oil that could be extracted (Iyenga, 1968; Coppen, 1995).

4.4.3. Composition of *Osyris lanceolata* oil

The results of the present study showed that *Osyris lanceolata* oil contained a large amount of lanceol as reported earlier by Aldizo and Naves (1954). This compound was first isolated in *Santalum lanceolatum* in 1928 by Penford (Manjarrez *et al.*, 1964) although little seems to have been reported on its use, being alone or part of other formulation. The occurrence of other compounds in this species is perhaps reported for the first time. Bisabolene that constitute 3.3 % of the total oil is known to be important in medicine as an anti-ulcer active principle compound (Yamahara, 1992). It is a component in some insecticides that forms part of the defense response targeted to control insect herbivores and possibly fungal pathogen attack (Bohlman *et al.*, 1998). Several plant species are known to contain this compound including *Senecio palmensis* (Gonzalez-Coloma *et al.*, 1995), *Abies grandis* (Bohlmann *et al.*, 1998) and *Zingiber officinale* (Yamahara, 2000).

Bisabolol constitutes 5.1% of the total oil. This compound forms an important component in a wide range of cosmetic formulations as a skin-conditioning agent at low concentrations from 0.001% in lipstick to 1% in underarm deodorants (Madhavan and Andersen, 1999). Cosmetics containing hydrogenated bisabolol are also known to have rough skin preventive role and have good skin conditioning (softening) effect apart from promoting turn over rate of the horny layer in humans (Tatsu and Noriaki, 1996). The compound is also known as an inflammatory-inhibiting sesquiterpene, playing a role in enhancing safe penetration for dermal and transdermal therapeutics (Kadir and Barry, 1991). Bisabolol is a component in hair growth stimulating cosmetics. Hair tonic/shampoo containing this compound is considered to have a preventive effect against alopecia (Tatsu and Noriaki, 1996; Noriakia and Tatsu,1996).

Another compound identified to occur in reasonable proportion is the nuciferol. The compound is one of the five major sesquiterpenic alcohols in *Santalum spicatum* oil (Piggott *et al.*, 1997). Where this chemical compound is specifically used could not be traced.

In general, the compound identified to be contained in *Osyris lanceolata* oil under this study and their uses are likely to widen the utilization and market potential of *O. lanceolata* apart from its current use.

CHAPTER V

IDENTIFICATION OF HOST PLANTS OF *OSYRIS LANCEOLATA* AND THEIR INTERACTIONS

5.1. Background information and objectives of the present study

5.1.1. Introduction

Following the rapid decline of *Osyris lanceolata* resource base due to overexploitation that has been going on in natural stands for some decades to meet the increasing demand and the obvious threat facing it (Mbuta *et al.*, 1994), the government of Tanzania opted to ban further harvesting of the species in 1990s. Since then, the government has been encouraging people, government and private firms to domesticate the species as source of income rather than relying on natural populations that has been dwindling (Mwang'ingo and Mwihomeke, 1997).

However, domestication and creation of woodlots have not been an easy task following several complications imposed by the species. One among the major constraints is its parasitic behaviour. Like many parasitic plants, this behaviour has made domestication and plantation forest complex than the known traditional silviculture (Srivassan *et al.,* 1992; Radomiljac *et al.*, 1999). Possible host plants of *Osyris lanceolata* in the whole of Tropical Africa are unidentified while studies on the potential of the possible hosts reported from some areas such as the Mediterranean and India to support its growth is nowhere reported. The parasites could sometimes display a more complex behaviour, requiring introduction of different hosts as the parasite grows as reported in *Santalum album* (Srivassan *et al.,* 1992; Radomiljac *et al.*, 1999). In this species, host plants are classified into three groups of: initial hosts (suitable at nursery stage); intermediate hosts (suitable within few years after field planting); and long term hosts (to support growth to its maturity) depending on the stage of the parasite growth at which a certain host is required (Radomiljac, 1998). How *O. lanceolata* behaves and respond when grown with various hosts is unknown and its understanding is hoped to facilitate selection of appropriate hosts. To be successfully in the domestication *O. lanceolata*, the

aspects of its parasitism need to be addressed before an attempt to raise the species at any reasonable scale is considered.

Like many other species in the family Santalaceae, *Osyris lanceolata* is a hemi-parasitic plant, and it requires the presence of a host plant for its normal growth and survival (Metcalfe, 1950; Miller, 1989; Beentje, 1994; Radomiljac *et al.*, 1999). The species is reported to be non-host specific, utilizing the roots of many plants species through haustoria connection including roots of its own (Rao, 1942a; Niranjana and Shivamurthy, 1987).

A follow up on plant development research by Herrera (1984a) from seed germination to seedling establishment revealed that seed germination could take place even without the influence of a host. The success for its establishment at this germination stage without the need of the host is a result of the big sized seeds, which has enough food reserve to initiate the germination process (Musselman and Press, 1995). However, the established seedlings rarely survive for a year without the presence of a host and in most cases seedling growth is retarded followed by their death (Rao, 1942a; Metcalfe, 1950). The parasitic nature of *Osyris lanceolata* is believed to be a strategy for survival in the limited resource environment where it is mostly found. This habit assists in getting some of its most important requirements of water and minerals. The main nutrients met through this connection include phosphorus, potassium and magnesium, which are important in plant growth (Metcalfe, 1950; Rai and Sarma, 1990).

A range of species have been identified in the Mediterranean region and India as hosts of *Osyris lanceolata* with the spectrum ranging from roots of grasses to of its own (Niranjana and Shivamurthy, 1987; Rai and Sarma, 1990; Herrera, 1988a). However, no study has been carried out in tropical East African region to identify possible host plants and while no study have reported on the potential of the identified hosts to support the growth of *Osyris lanceolata*. Besides, none of the hosts reported so far naturally occur in Tanzania and their introduction to Tanzania might necessitate prior investigation to know their effects on the growth of *Osyris lanceolata* under Tanzanian conditions

5.1.2. Parasitism in plants

Interactions between organisms in nature are the rule rather than exception. When one organism benefits to the detrimental of the other in the interaction, the association is referred to as parasitism (Douglas, 1994). Although the precise number of parasitic plants is not known, about 1% of the flowering plants ca. 3000 are estimated to be parasitic (Kuijt, 1969; Attsat, 1983). Parasitic plants form a close connection with the vascular systems of their host plants through specialized the structure known as the haustorium. They are at least partially dependent on their host for their supply of water, inorganic nutrients and organic solutes (energy) (Musselman and Press, 1995).

Parasites are classified in a variety of ways, but the most obvious method of classification depends on the point of attachment to the hosts and presence or absence of chlorophyll. With respect to the former, parasites are classified as root or shoot parasites depending on whether the haustorium /haustoria are below or above ground. About 60% of parasitic angiosperms are root parasites while 40% are shoot parasites. With regard to chlorophyll, plants are classified either as hemi-parasites or holo-parasites. Hemi-parasites contain chlorophyll and thus are able to manufacture part or fully their own food but depends on the hosts for the supply of water and nutrients. Holoparasites entirely depend on the host even for food supply. About 20% of all the species are holoparasites while the remainder are hemi-parasites (Musselman and Press, 1995). *Osyris lanceolata* is a hemi-parasite.

5.1.3 Resource exploitation by parasites

Exploitation of resources by the parasites from their hosts has been confirmed by many studies and the degree and amount of exploitation depends on the parasite requirement (Musselman and Press 1995). Lamont and Southall (1982) and Struthers *et.al.* (1986) studied the nutrient exploitation done by *Santalum spicatum* and *Amyema preissii* on *Acacia cuminata*. Reduced levels of potassium, sodium and copper were observed in parasitized plants as opposed to unparasitized. At the same time marked preferential accumulation of potassium and sodium were noted in the

parasite compared to the hosts. This confirmed the removal of nutrients by the parasite from its host. With regard to carbon (C) and C related compounds, most hemi-parasitic angiosperms are assumed to be capable of fixing atmospheric carbon dioxide due to presence of chlorophyll and thus relying on their host only for water and mineral elements. This has been thought to be the case due to the fact that the greatest contact surface is parenchymal in all haustoria giving a belief that xylem of the host contains only minerals (Kuijt, 1969). However Kramer and Kozlowisk (1979) have shown a variety of organic materials to be transferred including sugar, N-compounds and enzymes to be present in exude of parasites. Furthermore, in many hemi-parasites, the rates of photosynthesis have been found to be rather low (0.5-5 μmol $m^{-2}s^{-1}$) and are toward the bottom of the range of C3 plants. Yet, they are coupled with high transpiration rates, the net result being little net carbon gain, certainly low to support growth (de la Harpe *et al.* 1981; Press *et al.*, 1988; Press, 1995). Thus, most hemi-parasitic plants still rely on their hosts for food requirement. However, no study is reported in the literature on the relationship between *Osyris lanceolata* and its hosts.

5.1.4. Host range and preferences

The kind and type of the host that can be potentially parasitized depend among several things on the susceptibility of the host, degree of virulence of the parasite and immunity of certain plants to attack. Some parasites are intolerant to shade and will not survive under shaded condition. This eliminates some host plants that might be good hosts but their excessive shade makes them unsuitable. Mechanical properties of some hosts have also been proved to be a barrier to some parasites (Kuijt, 1969)

How a parasite gets through to the hosts so that it can make a physiological contact in natural ecosystems is believed to be through three possible ways (Kuijt, 1969; Musselman and Press, 1995). The first way is for the parasite seeds to have enough food reserve to allow the radicle to grow extensively while searching for suitable hosts. In these parasites, the seeds or disseminules are relatively large to contain enough food. This is common in all root-parasitic species. Another mechanism that

allows the parasites to get into contact with possible host is through seed dispersal by birds. Seeds are carried through the alimentary canal of the birds and upon deposition on branches it is brought into contact. The third mechanism that is strongly linked with the evolutionary history of parasitic plants is through a biochemical signal. Host plants produce biochemical exude which are sensed by the parasite seeds and ultimately they are induced to germinate. Since the chance of successful establishment through this method is small, a parasite tends to produce a vast number of seeds to increase the chance for some of them to come into contact with the host (Holmes, 1979; Musselman and Press, 1995).

While the number of species that may be defined as hosts may be many, the number of preferred hosts is usually narrow (Musselman and Press, 1995). This phenomenon is not limited to *Osyris lanceolata* but is also common in many root parasitic plants. Host plants differ in their suitability to support parasite growth. In suitable hosts, parasites tend to grow bigger and reach maturity most rapidly and reproduce. In unsuitable hosts, the parasite will be less abundant. The growth of the parasite tends to be retarded and is exposed to considerable risk by the hosts. In other hosts, the parasite occurs very rarely and as a rule develops in them only with difficulties (Holmes, 1979; Dogiel, 1964). Based on this, Holmes (1979) classified host plants as required host, suitable hosts and unsuitable hosts. Required hosts are those best suited for the parasite and are necessary for parasite survival. Suitable hosts are considered to be those in which the parasite can be supported to maturity but not at the rate comparable to required hosts. Unsuitable hosts are those in which the parasite can become established but cannot support it to maturity stage.

According to Ananthapadmanabha *et al.* (1988), host plants of *Santalum album* are categorized into three classes almost similar to that of Holmes (1979). Good hosts i.e. supporting good growth; medium i.e. supporting moderate growth; and poor i.e. supporting very little growth of sandalwood. Good, medium, or poor hosts are evaluated based on their ability to support growth (height, diameter and quantity of biomass produced) and number of haustoria produced. It had been noted that, host plants that favoured good growth of *Santalum album* have a light canopy thereby not preventing lateral and overhead light. These included *Casuarina equisetifolia, Melia*

dubia and *Acacia nilotica*. Radomiljac *et al.* (1999) in their studies on intermediate hosts of *Santalum album* observed that the growth of sandalwood seedlings was more enhanced when the parasite was attached to leguminous hosts. Similar results were reported by Rai (1990) and Taide *et al.* (1994) in the case of nitrogen fixing *Casuarina equisetifolia*. However no general conclusion can be drawn with regard to suitability of leguminous plants as hosts. Some legume hosts such as *Acacia auriculiformis*, *Leucaena leucocephala* and *Cassia fistula* have been found to be poor hosts for *Santalum album* (Rai, 1990; Taide *et al.*, 1994). However, not much work has been reported in the literature on *Osyris lanceolata*.

5.1.5. Host plant response to parasitism

The response of host plants to parasitism can be spectacular but also unpredictable. In some situations the influence may be undetectable whereas in the most extreme cases the host may undergo malformation or even die. The response of the host will not only depend on the direct effect of the parasite, but also on various environmental factors. However, the strength of the influence will be controlled by a combination of factors including the size of the parasite; the rate of growth and metabolic activity of the parasite; degree of dependency of the parasite to the host for resources; and the stage of development of the host (Kuijt, 1969; Graves, 1995).

The notable response of host plant to parasitism is decreased growth rate. This could be brought by direct interactions, in particular removal of the resources from the host that are essential for the normal development such as inorganic nutrients, carbon containing compounds and water. These in turn can predispose the host to the influence of other stresses imposed by the environment such as water deprivation, photoinhibition, chilling and heat stress. However, the extent to which the host is affected will depend on the state of the host, including its stage of development, availability of the resources and the demand at that particular time. The presence of a parasite introduces an alternative carbon sink which can disturb the normal growth form of the host, altering the allocation to root or shoot which in turn affects the rate of growth of the host (Graves, 1995).

An outstanding physiological characteristic of most parasites is their high rate of transpiration, which often exceeds that of the hosts by order of magnitude (Stewart and Press, 1990; Press *et al.*, 1988). It is at the higher end of the range observed in angiosperms. This maintenance gradient in leaf water potential towards the parasite and thus facilitate the flux of resources to the parasite. Thus, where parasites depend on hosts to meet their water requirement, the risk of damage to the hosts especially during periods of drought is increased.

Generally, the presence of a parasite can alter the rate of important host metabolic functions such as photosynthesis, respiration, the uptake of water and solutes and even the morphology of the host. The influence on these processes is often more important than direct competition for resources. Host productivity can be severely reduced by parasites induced changes in carbon dioxide utilization. In xylem feeding parasites, loss of water from the host may lead to decrease in the host stomatal conductance and consequently a fall in the rate of host photosynthesis (Graves, 1995). Carbon loss via respiration is an important part of the carbon budget of a plant, with 40-60 % of assimilates being used in respiration. Of this, about one third is involved in maintenance respiration and two thirds in fuelling growth (Johnson, 1990). Introduction of parasites leads to a slight increase in the rate of respiration, which can in turn reduce the amount of assimilate available for growth. Should the removal of carbon from the host be very large, starvation of vital processes can occur (Graves, 1995).

Morphological and architectural change in hosts is also expected as a result of parasitism. In many root parasites, the root: shoot ratio has been observed to increase in the infected plants indicating that root biomass is relatively high in infected plants. This increase slows the growth rate of the host even in the absence of other deleterious effects of the parasite. In some cases, the ratio could be so high and will not only reduce above ground assimilation, but the considerable extra mass of roots will add a significant carbon loss through respiration. Leaf growth and the growth form can be changed and hence interfering with the whole photosynthesis (Poorte and Remkes, 1990). However, large root systems can be beneficial to infected plants

especially during water deficit as these will explore a greater volume of soil to buffer the high transpiration rates of parasites (Graves 1995).

5.1.6. Nature of parasitism in particular reference to *Osyris lanceolata*

The distinguishing feature in all parasitic plants is the haustorium, an organ that functions in attachment, penetration and solute transfer. It is the physiological and morphological bridge between the host and the parasites although its function is more than a bridge (Kuijt, 1977; Stewart and Press, 1990). The origin of haustoria is still obscure, though they may have evolved from roots serving similar function of absorption, anchorage and storage (Musselman and Dickison, 1975). In *Osyris lanceolata*, the haustoria connecting the parasite to the host usually arises from the cortex, endodermis and pericycle. To enable its effective penetration, the haustoria usually undergoes several modifications depending on how easy or difficult it is in penetrating the host root tissues. Where host tissues offer great mechanical resistance, a complex anatomical structure largely consisting of wedge shaped vessel members may be formed. The sucker will then penetrate the host through mechanical force (Rao, 1942b; Metcalfe, 1950; Niranjana and Shivamurphy, 1987).

5.1.7. Objectives

This study carried out was aimed at identifying possible hosts of *Osyris lanceolata* in Tanzania through soil excavation in the field and to assess the influence of some of the hosts on early growth of *Osyris lanceolata* in the nursery as a preliminary study of assessing the interactive effects between *Osyris lanceolata* and its hosts. The objectives were addresses through these research question:

i. What species are possible hosts of *Osyris lanceolata*?
ii. Is there any preference in host selection?
iii. How does *Osyris lanceolata* interact with some of its preference host when grown together, in terms of growth?
iv. Are host plants beneficial to the early growth of *Osyris lanceolata* compared to growth without a host?
iv. How do host species react to parasitism?

5.2. Materials and methods

5.2.1. Field identification of host species of *Osyris lanceolata*

The field study was carried out in three forest reserves of Sao Hill, Image and Nundu in the Southern ecozone of Tanzania between June and July 1999. At each study site twenty trees of *Osyris lanceolata* of uniform height and diameter (Table 5.1), ten from each sex, were selected randomly. The distance between two individuals was at least 20 m. The field study involved soil excavation for visual inspection of haustorial connections between roots of sandalwood (the parasite) and those of the neighboring plants (hosts) following the method used by Herrera (1988a).

Excavation of soil was made around each tree up to a distance of 3 m radius from its base, exposing roots of both host plants and the parasite. If any evidence of very close extensive areas of contact between the roots of a host and the parasite was observed, a host-parasite relationship through haustorial connection was inferred. This was further confirmed by hand pulling to separate the roots at the point of attachment and if it was difficult to separate, the plant was declared host. No detailed structural investigation of the haustorium was made. Once declared, the distance of the host from the base of the parasite was recorded. Plate 5.1 show a close contact between *Osyris lanceolata* (slashed red) and *Rhus natalensis* as observed at Sao Hill stand.

Table 5.1 Mean tree height and diameter of *Osyris lanceolata* sampled for host plant identification at the three study sites.

Study area	Mean height (m)	Mean diameter (cm)
Image	3.5	4.2
Nundu	6.2	10.4
Sao Hill	4.8	6.3

Plate 5.1. Part of the excavated root system showing *Osyris lanceolata* in close contact with *Rhus natalensis*, which was a host

The data collected were entered into a database according to the sex of the parasite and the distance between host and parasite for further analysis. Ranking of host species was made based on their frequency to produce a list of host species for each site. Comparison was made to determine the similarity in host species between the sexes of the parasite and between sites using Sorenson Index (S_s) of similarity developed in 1948 as described by Pielou (1977) and Krebs (1998). The Index is calculated as:

$$S_s = 2a/(2a+b+c)$$

Where,

a = number of host species occurring in Sites A and B (common occurrence) or
number of host associated with both female and male parasites in each site

b = number of host species that occurs in Site B only or
number of host species associated with male parasite only in each site

c = number of host species that occurs in Site A only or
number of host species associated with female parasite only in each site.

If the Index between sites or between sexes was less than 0.50 it was inferred that there was host selectivity or preference of host between either of the sexes or dissimilarity in host species.

5.2.2. Nursery assessment of the suitability of selected host species in promoting early growth of sandalwood

The study was conduced at Iringa Zonal Tree Seed Center nursery located at longitude $35^{0}41'$ E and latitude $7^{0}46'$ S at an altitude of about 1640 m above sea level. The centre is among the three centres of the National Tree Seed Program of Tanzania (NTSP) aimed at collecting and supplying seeds for national and international afforestation programmes in addition to carrying out research related to tree seeds. The study was carried over a period of 12 months between October 1999 and December 2000.

The study was based on selected host species identified during the field study described above conducted in Image, Sao Hill and Nundu forest in June/July 1999. Four host species were selected from among all host species identified in the three study sites for this study. The criteria used in the selection of the host species were: the number of times a species was encountered as a host, the number of individuals of the host species in the population and whether the host species occurred in all the three populations or not. The selection was made from among the top ten most frequent host species that occurred in at least two of the populations. In addition, the availability of seed of the host species was also taken into consideration in the selection. Host species for which seeds are known to be difficult to obtain were excluded. The four selected host species included: *Brachystegia spiciformis*, *Dodonaea viscosa*, *Rhus natalensis* and *Tecomaria capensis*. *Casuarina equistifolia* was also included in the study based on the fact that it has been shown to be one of the most effective hosts to most parasite species of *Santalaceae* (Srinivasan *et al.*, 1992; Rai, 1990; Taide *et al.*, 1994) and the species is also known to grow and survive very well in most areas of Tanzania where *Osyris lanceolata* occurs.

Seeds of the four indigenous host species and the parasite *Osyris lanceolata* were collected from the three forest reserves in July 1999 whereas those *C. equisetifolia* were purchased from Tanzanian National Tree Seed Centre, at Iringa zone. The type of seeds and their source are given in Table 5.2. The seeds were sown in Iringa nursery beds in November 1999, with a soil mixture of local soil and sand (2:1). After germination the seedlings were transplanted in mid- December 1999 into 25 cm diameter and 30 cm depth growth containers filled with a mixture of sand, forest soil and cow manure in the ratio of 2:1:1. The forest soil was obtained from Nundu forest.

In each container, two seedlings consisting of one host and one parasite were grown. Parasites and hosts alone were also grown in separate containers (two seedlings in each) as controls. There were six treatments involved, each having 12 containers and these were replicated three times. Thus, a total of 396 containers laid out in a randomized complete block design were used for the study.

The seedlings were grown for one year and within this period they were monitored four times at an interval of three months. At each interval, three containers from each treatment and block were randomly selected to measure the height, root collar diameter and leaf area of the seedlings. Height was measured with a simple ruler, diameter with a veneer calliper and leaf area with a planmeter.

Table 5.2. Species tested for the study on suitability of host plants to promoting early growth of *O. lanceolata* at nursery level: Seed sources

Seed type	Family	Source/Origin
Brachystegia spiciformis	Caesalpiniaceae	Image Forest, Iringa
Dodonaea viscosa	Sapindaceae	Sao Hill forest, Mufindi
Rhus natalensis	Anacardiaceae	Sao Hill forest, Mufindi
Tecomaria capensis	Bignoniaceae	Nundu Forest, Njombe
Casuarina equisetifolia	Casuarinaceae	NTSP – Iringa
Osyris lanceolata	Santalaceae	Sao Hill Forest- Mufindi

As it was difficult to measure the leaf area of the needles of *Casuarina equisetifolia*, 100 needles from each *Casuarina* plant assessed were harvested at mid height, tied with a known volume of a stone and then dipped in a measuring cylinder filled with a known volume of water. The amount of water displaced, which is equivalent to the volume of the needles was then taken as approximate estimate of needle area. The seedlings were removed to inspect for evidence of haustorial connection between the roots of the parasite and the host. The shoots and roots of the seedlings were separated and weighed after drying in oven at 80°C for 48 hours.

Analysis of variance (ANOVA) through the general linear model of the MINITAB programme was used to analyse the data to find out if there were significant differences between treatments in the growth parameters measured every three months. Where significance difference was observed, Turkey's pair wise comparison was used to separate the differing means.

5.3 Results

5.3.1 Identification of sandalwood host plant species of *Osyris lanceolata*

Table 5.3 gives a list of plant species identified as hosts of *O. lanceolata* at Image, Sao Hill and Nundu forest by summarising the number of *O. lanceolata* trees in which a particular host was found to be in close contact and the total frequency of host occurrence in the site for all *O. lanceolata* trees.

The results of the present study show that host plants of *O. lanceolata* were diverse and varied among sites although some species were observed to be common to all the three study sites. At Image forest reserve, a total of 29 species were observed to have their roots in close contact with *Osyris lanceolata* and declared to be hosts. The most frequent species were *Brachystegia utilis, Brachystegia spiciformis, Rhus natalensis, Dodonaea viscosa, Ormocarpum kirkii, Combretum zeyherii* and *Clerodendron myricoides*.

Table 5.3 Host plant species of *O. lanceolata at* Image, Sao Hill and Nundu forest reserves: Total number of *O. lanceolata* tree in which a host was observed to be in close contact and total frequency of its occurrence in all trees at each site

	Image		Sao Hill		Nundu		All sites	All sites
Host plant	Trees with	freq.	Trees with	Freq.	Trees with	Freq.	Trees with	freq.
Rhus natalensis	9	15	12	30	16	50	37	95
Dodonaea viscosa	11	16	13	24	6	6	30	46
Tecomaria capensis	0	0	13	59	11	20	24	79
Aphloea theiformis	0	0	0	0	19	144	19	144
Catha edulis	0	0	3	3	13	19	16	22
Diospyros whyteana	4	5	0	0	11	17	15	22
Apodytes dimidiata	0	0	12	26	3	5	15	31
Myrsine melanophloeos	0	0	1	4	12	27	13	31
Jasminum odoratissimum	0	0	0	0	12	16	12	16
Brachystegia spiciformis	12	72	0	0	0	0	12	72
Maytenus heterophylla	0	0	11	45	0	0	11	45
Heteromorpha trifoliate	0	0	0	0	10	11	10	11
Rhamnus prinoides	0	0	0	0	10	18	10	18
Brachystegia utilis	10	34	0	0	0	0	10	34
Clerodendron myricoides	8	10	0	0	0	0	8	10
Ormocarpum kirkii	8	11	0	0	0	0	8	11
Psychotria lauracea	0	0	1	7	7	11	8	18
Ochna holstii	3	3	4	5	0	0	7	8
Schrebera elata	3	4	4	5	0	0	7	9
Flacourtia indica	3	4	4	6	0	0	7	10
Bryrsocarpus orientalis	0	0	7	16	0	0	7	16
Combretum zeyheri	6	9	0	0	0	0	6	9
Dais cotinifolia	0	0	0	0	6	9	6	9
Dombeya shupangae	2	2	4	7	0	0	6	9
Olinia rochetiana	0	0	0	0	6	13	6	13
Faurea saligna	5	6	0	0	0	0	5	6
Indigofera rhynchocarpa	5	7	0	0	0	0	5	7
Senna singueana	5	7	0	0	0	0	5	7
Aeschynomene abyssinica	5	10	0	0	0	0	5	10
Maprounea africana	5	17	0	0	0	0	5	17
Myrica africana	0	0	0	0	5	20	5	20
Dichrostachys cinerea	4	5	0	0	0	0	4	5
Erythroxylum fischeri	0	0	0	0	4	5	4	5
Myrsine africana	0	0	4	13	0	0	4	13
Euclea divinorum	0	0	0	0	3	3	3	3
Maytenus mossambicensis	3	3	0	0	0	0	3	3
Acacia hockii	3	4	0	0	0	0	3	4
Euclea natalansis	3	4	0	0	0	0	3	4
Albizia glaberrima	3	5	0	0	0	0	3	5
Mundulea sericea	3	5	0	0	0	0	3	5
Bersama abyssinica	0	0	3	7	0	0	3	7
Securidaca longependunculata	2	2	0	0	0	0	2	2
Vangueria infausta	0	0	2	2	0	0	2	2
Rytignia uhligii	2	3	0	0	0	0	2	3
Tapiphyllum cinerascens	2	3	0	0	0	0	2	3
Tarenna neurophylla	2	6	0	0	0	0	2	6
Agauria salicifolia	0	0	0	0	2	7	2	7
Markhamia obtusifolia	1	3	0	0	0	0	1	3
Hymenodictyon floribundum	1	4	0	0	0	0	1	4

At Sao Hill forest reserves, a total of 16 species were identified. Of these, the most frequent were *Rhus natalensis, Tecomaria capensis, Maytenus heterophylla* and *Apodytes dimidiata* and *Dodonaea viscosa*. Nundu forest reserve had a total of 18 species identified as possible hosts. The most frequent hosts were *Aphloea theiformis, Rhus natalensis, Catha edulis Jasminum odoratissimum, Myrsine melanophloeos* and *Tecomaria capensis*. Species that were found to be common hosts of *Osyris lanceolata* and found in at least two sites included *Rhus natalensis, Dodonaea viscosa, Tecomaria capensis, Catha edulis* and *Apodytes dimidiata*.

5.3.2. Preference of hosts by parasite

The Sorenson's index of similarity calculated as a measure of preference of hosts by the two sexes of sandalwood in the three study sites is shown in Table 5.4. The result reveals that more than 80% of hosts identified within each study site were utilized equally by both sexes. The rest of the hosts were utilized by either of the sexes. This indicates that very little preference for hosts between sexes existed in O. *lanceolata*.

5.3.3. Site specificity of host plants

There was a large difference in host species occurrence among the three sites. Less than 16, 12 and 28% of host plants identified occurred in at least two of the sites. The result presented in Table 5.5 show large dissimilarity between sites in host species occurrence according to Sorenson's index of similarity.

Table 5.4. Utilization of host plants by male and female *O. lanceolata* at Image, Sao Hill and Nundu Forest reserves

Study site in consideration	Number of host species utilized			Sorenson's index of similarity
	Male only	Female only	Both	
Image forest	2	3	25	0.91
Sao Hill forest	3	1	12	0.86
Nundu forest	1	0	17	0.97

Table 5.5. Similarity of populations in host species occurrence at Image, Sao Hill and Nundu forest reserve.

Sites compared	Number of species	Sorenson's index of similarity
Image & Sao Hill		
Species at Image only	23	
Species at Sao Hill only	10	0.27
Species at both sites	6	
Image & Nundu		
Species at Image only	26	
Species at Nundu only	15	0.13
Species at both sites	3	
Nundu & Sao Hill		
Species at Nundu	11	
Species at Sao Hill	9	0.41
Species at both sites	7	

5.3.4. Performance of *Osyris lanceolata* under different hosts

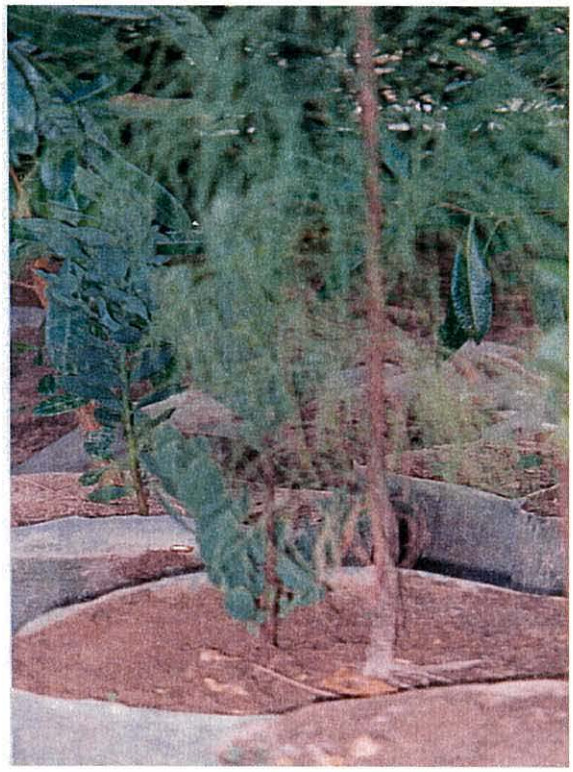
Plate 5.2 shows *Osyris lanceolata* grown with *Casuarina equisetifoilia, Dodonaea viscosa, Rhus natalensis* and *Tecomaria capensis* as hosts at the age of 12 months.

5.3.4.1 Height growth

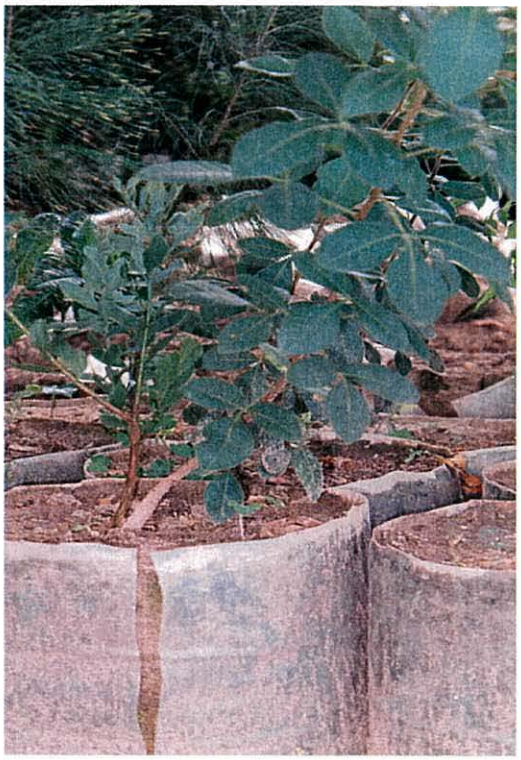
Difference in growth parameters of *Osyris lanceolata* grown with various hosts tested started to appear when plants were six months old. Height growth of *Osyris lanceolata* grown with host plants is shown in Figure 5.1. No significant differences were observed in height growth among treatments during the first three months ($P = 0.15$). Significant difference in height growth was observed during the second assessment period (six month old) ($P = 0.02$). The tallest height was obtained when *O. lanceolata* was grown with *B. spiciformis* (27.8 ± 0.9 cm) and the least (22.5 ± 0.8 cm) grown with *Dodonaea viscosa.*

Plate 5.2. ***Osyris lanceolata*** **growing with (a)** ***C. equisetifoilia*****, (b)** ***R. natalensis*****, (c)** ***D. viscosa*** **and (d)** ***B. spiciformis*** **as hosts**

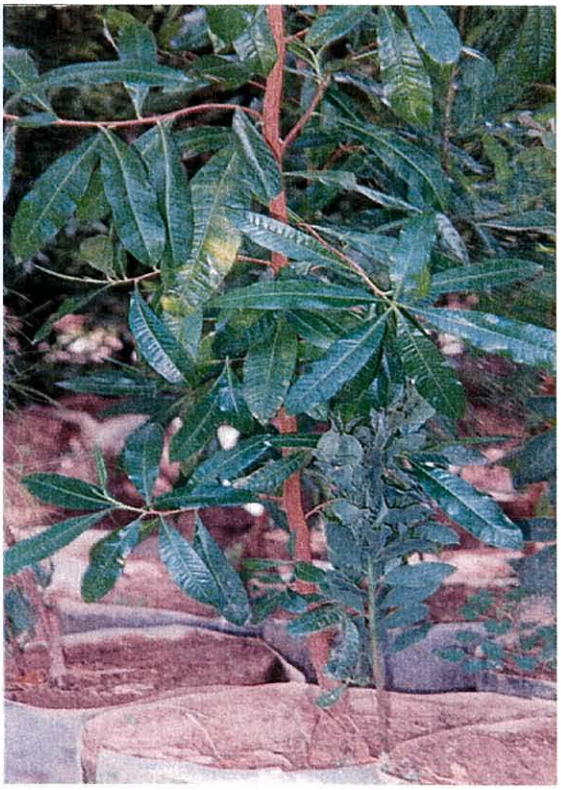
(a)



(b)



(c)



(d)

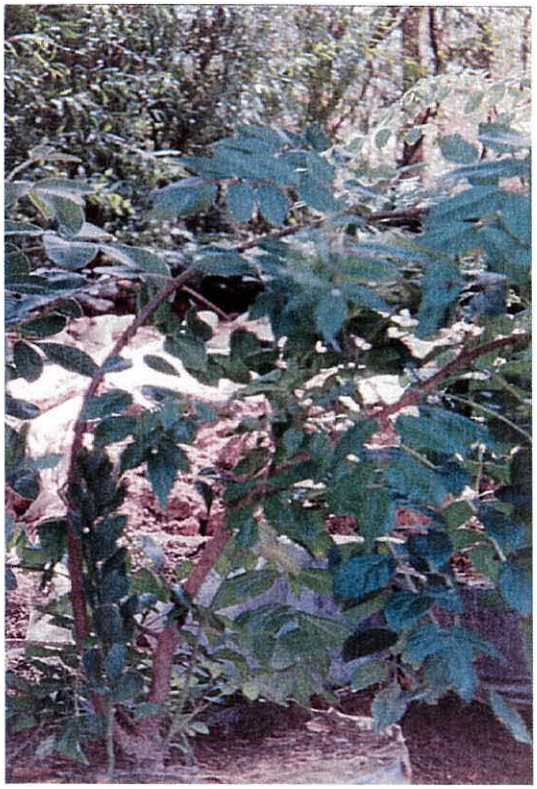
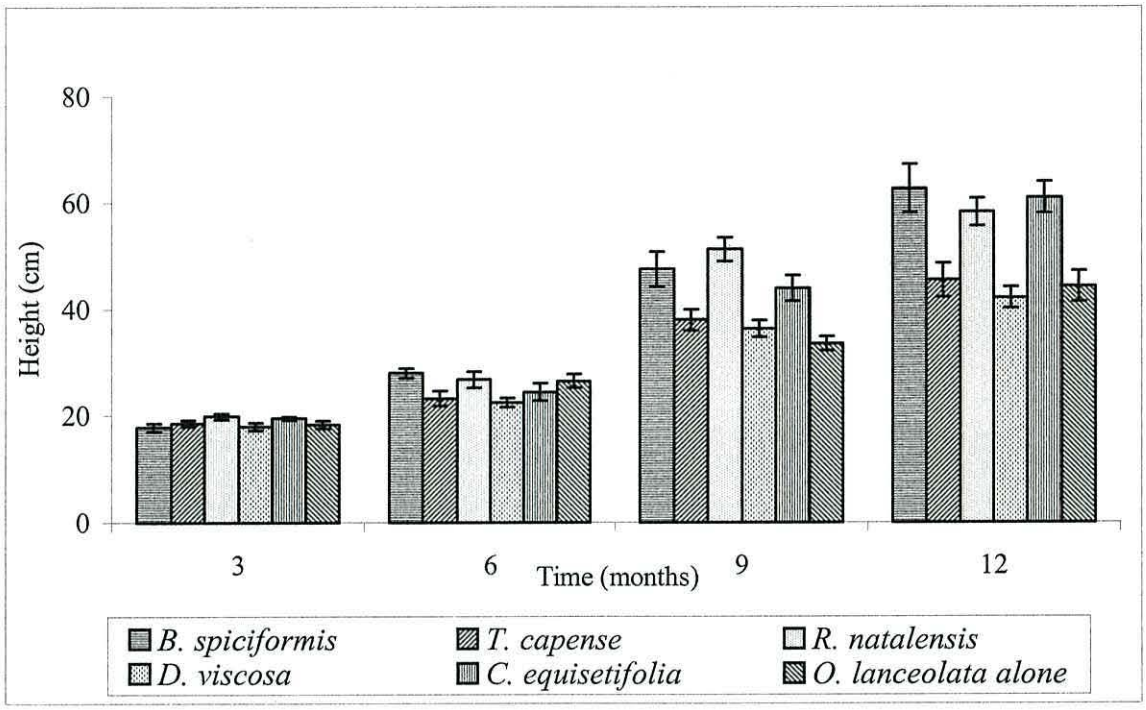


Figure 5.1 Height growth of *Osyris lanceolata* under different host plant species

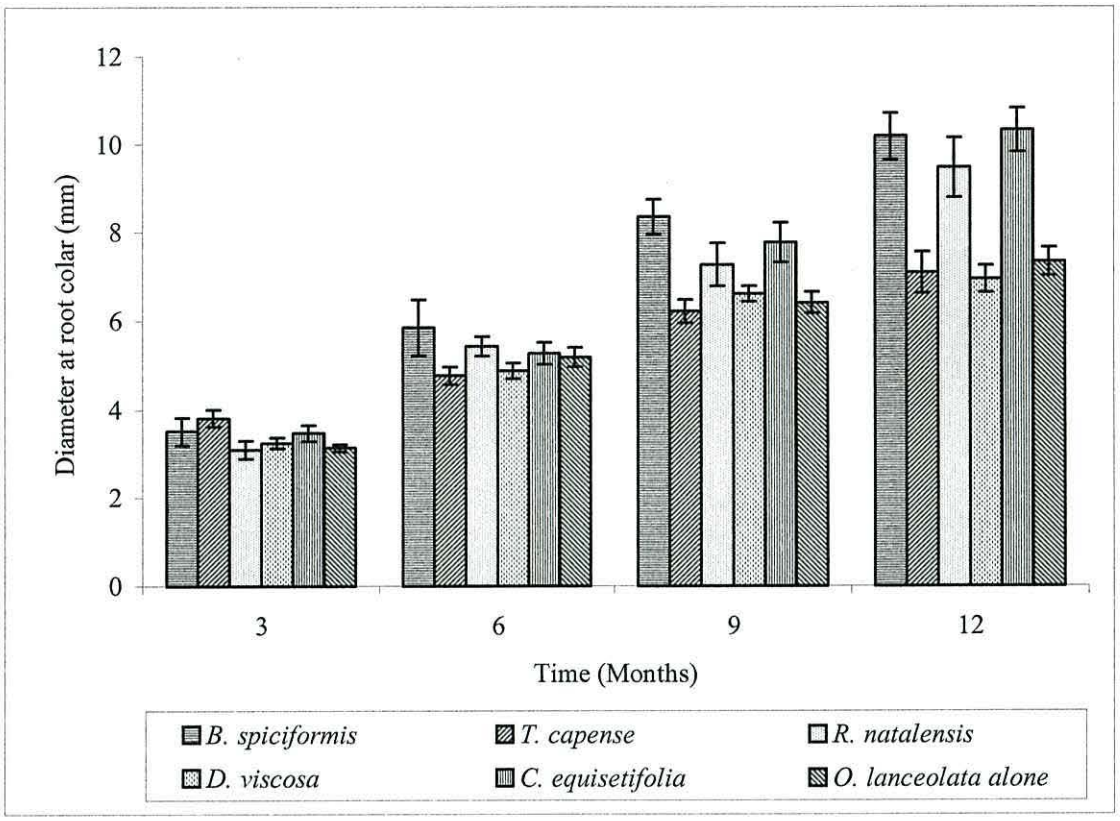


Marked difference in height growth was observed nine months after transplanting ($P < 0.01$). *O. lanceolata* grown with *Rhus natalensis* was significantly taller (51.2 ± 3.3 cm) than those grown with *Dodonaea viscosa, Tecomaria capensis* and *O. lanceolata* alone. The shortest plants were those of *O. lanceolata* grown alone i.e. control (33 ± 1.3 cm). *T. capense* and *D. viscosa* significantly suppressed height growth. After 12 months of growth, the difference in growth was even more obvious among treatments ($P < 0.01$). *O. lanceolata* grown with *B. spiciformis* was the tallest (62.6 ± 4.6 cm) but was not significantly different from *Casuarina equisetifolia* and *Rhus natalensis*. The shortest plants were those of *O. lanceolata* grown with *D. viscosa* (42.2 ± 2.0 cm).

5.3.4.2. Diameter growth

Diameter growth almost followed the same pattern as height growth (Figure 5.2). No difference in diameter growth was observed within the first six months. Nine months after transplanting, significant difference in diameter growth was observed ($P < 0.01$). Plants grown with *B. spiciformis* were bigger in diameter of (8.6 ± 0.4) mm than those grown with *T. capensis*, *D. viscosa* and *O. lanceolata* grown alone. The least diameter was in plants grown with *T. capensis* (6.2 ± 0.3 mm).

Figure 5.2. Diameter growth of *Osyris lanceolata* under different host plants

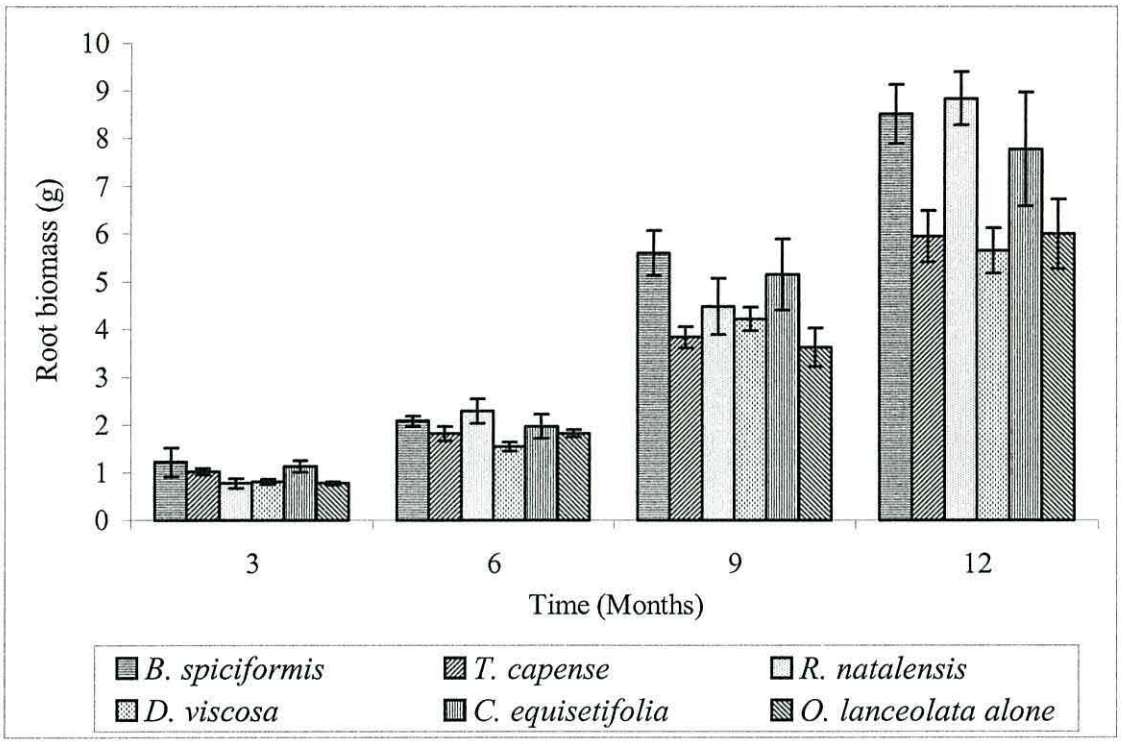


At the fourth assessment i.e. when plants were a year old, diameter growth differed significantly among treatments ($P < 0.01$). *O. lanceolata* plants grown with *C. equisetifolia* had significantly the biggest diameter growth (10.3 ± 0.5 mm) although not different from *B. spiciformis* and *R. natalensis*. Compared to those that were grown alone, with *T. capense* or *D. viscosa, O. lanceolata* grown with *D. viscosa* had the lowest value at this time, having attained a mean diameter of 6.9 ± 0.3 mm.

5.3.4.3. Root biomass

The data on root biomass are presented in Figure 5.3. No significant differences were observed among treatments in the first three months ($P = 0.09$). At six months old, significant differences among treatments in root biomass ($P = 0.03$) were observed. *O. lanceolata* grown with *R . natalensis* had more root biomass (2.29 ± 0.6 g) compared to what was attained with *D. viscosa* (1.54 ± 0.1 g).

Figure 5.3. Effect of host plants on root biomass growth of *Osyris lanceolata*

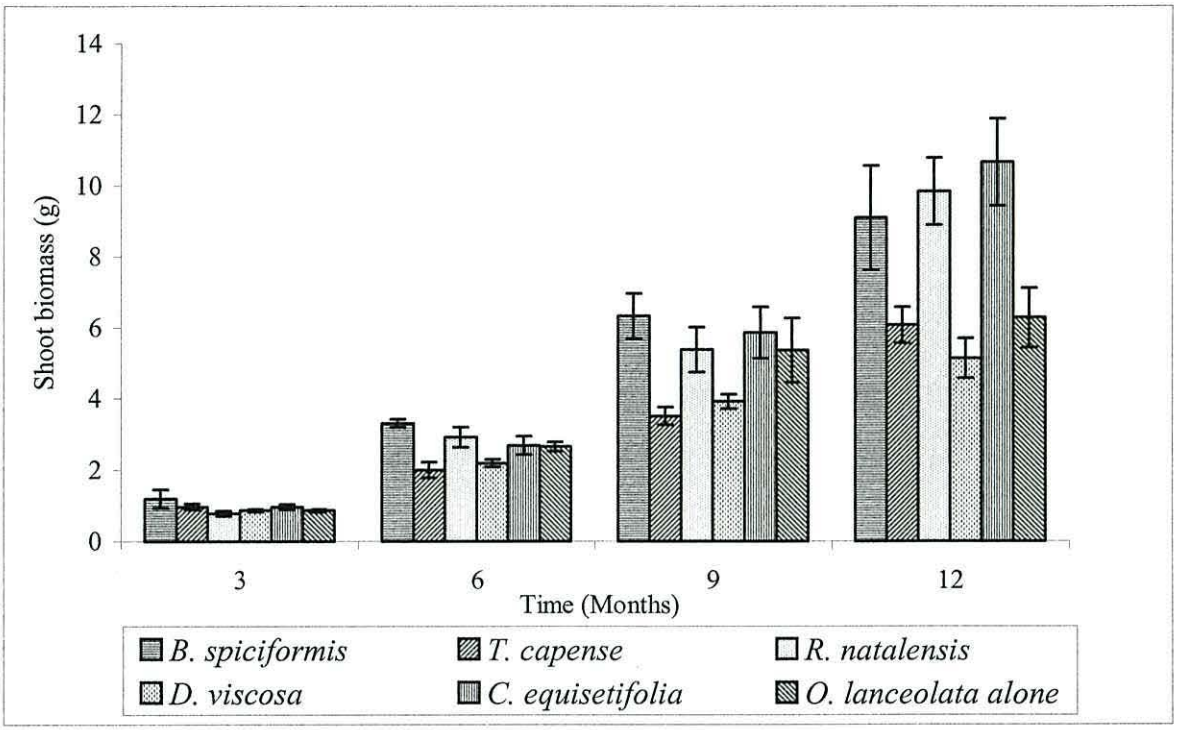


According to Tukey's comparison test, there was no significant difference between *Osyris lanceolata* grown with *R. natalensis* and the rest, including the control. At the age of nine months, significance differences in the root biomass between treatments continued to be observed (P = 0.004), however, only the difference between *O. lanceolata* grown with *B. spiciformis* and that of the control were significant (P = 0.033). *O. lanceolata* grown with *B. spiciformis* had more root biomass (5.6 ± 0.5 g) than the control that had root biomass of only 3.63 ± 0.4 g. Difference in root biomass of *O. lanceolata* became more evident when plants were one year old (P = 0.001). *O. lanceolata* grown with *R. natalensis* had a significantly higher biomass (8.84 ± 0.56 g). The least biomass was observed in *O. lanceolata* that were grown with *D. viscosa* (5.65 ± 0.47 g).

5.3.4.4. Shoot biomass and root/shoot ratio

With the exception of the first assessment done when plants were three months old, significant differences in shoot biomass among treatments was observed throughout the rest of the growth period as depicted in Figure 5.4.

Figure 5.4 Effect of host plants on shoot biomass of *O. lanceolata* at different time intervals



When plants were six ($P < 0.01$) and nine months old ($P = 0.01$), *O. lanceolata* grown with *B. spiciformis* had significantly more shoot biomass (3.30 ± 0.12 g and 6.32 ± 0.64 g, respectively). In both periods, the least biomass was found in *O. lanceolata* grown with *T. capensis* that had a mean shoot biomass of 2.00 ± 0.22 g and 3.51 ± 0.25 g at six and nine months respectively. However, according Tukey's comparison test none of the treatments had significantly more shoot biomass than what was attained by the control during these two periods. One year after transplanting, the host species continued to show difference in shoot biomass ($P < 0.01$). *O. lanceolata* grown with *C. equisetifolia* had the highest biomass (10.67 ± 1.23 g) but not different from *R. natalensis* and *B. spiciformis*. The least biomass was attained by *O. lanceolata* grown with *D. viscosa* (5.13 ± 0.56 g).

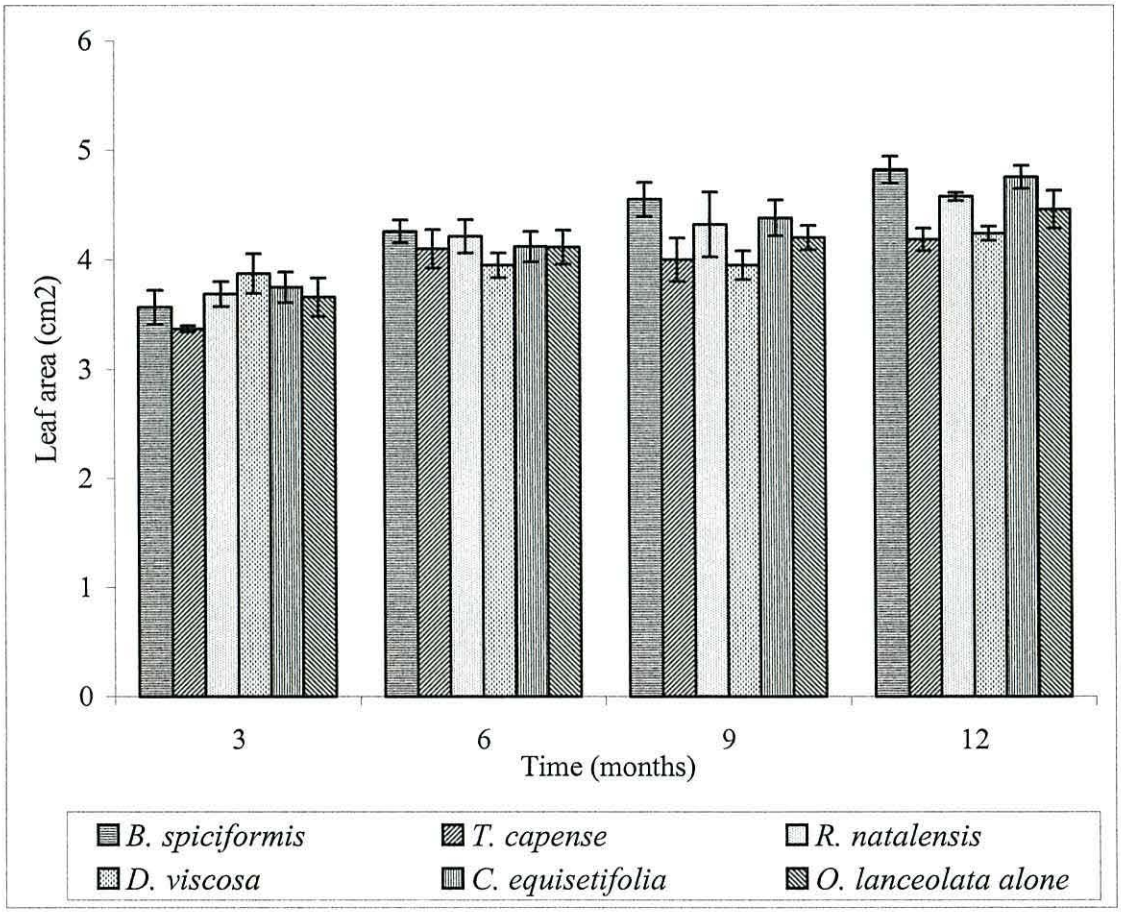
There was no significance difference among treatments in root/shoot ration during the first assessment period (i.e. at three months old). At the age of six month, significant difference was observed among treatments ($P = 0.036$), with *O. lanceolata* grown with *T. capensis* giving significantly higher ration (1.01 ± 0.17) than those grown with *B. spiciformis* (0.64 ± 0.04). The rest of the treatments had similar ratio with *T. capensis.*

At the age of nine months, the ratios differed more significantly (P = 0.004). Higher ratios were in plants grown with *T. capensis* and *D. viscosa* that had respective ratios of 1.10 ± 0.04 and 1.08 ± 0.04, and these ratios were significantly higher than that of the control (0.74 ± 0.08). At 12 months, difference between treatments in the root/shoot ratio was non significant.

5.3.4.5. Leaf growth

Significant different in leaf area was only observed when plants were 12 months old (P < 0.01). During this period, *O. lanceolata* grown with *B. spiciformis* had significantly larger leaf surface area (4.8 ± 0.12 $cm^{2)}$ compared to plants grown with *D. viscosa* and *T. capensis*. The least leaf area (4.2 ± 0.1 cm^2) was in plants grown with *T. capensis* (Figure 5.5.

Figure 5.5. Leaf area growth of *Osyris lanceolata* under different host plants



5.3.5. Effect of parasite (*O. lanceolata*) on host plant growth

5.3.5.1 Height growth

Table 5.6 summarizes growth parameter of various host plants grown with and without *Osyris lanceolata* at the age of 12 months. Host plants parasitized by *Osyris lanceolata* did not show any significant difference in height growth compared with the control/without parasite during the first nine months. Significant difference in height growth was observed during the fourth assessment (at twelve months). The height growth of *T. capensis* and *R. natalensis* were significantly suppressed by the parasite ($P < 0.01$ and $P = 0.04$, respectively). The height growth of *T. capensis* and *R. natalensis* grown with parasite were reduced by 16.8% and 10.8% respectively of their control (i.e. without parasite).

5.3.5.2 Diameter growth

No significant difference in diameter growth between parasitized and unparasitized plants was observed until when plants were 12 months old. At 12 months, parasitized plants of *T. capensis* and *B. spiciformis* were significantly reduced by 18% ($P = 0.03$) and 18.1% ($P < 0.01$), respectively of their control i.e. without parasite.

Table 5.6. Growth response of host plants with and without *Osyris lanceolata*. '' denotes species grown with *Osyris lanceolata* (Parasite)**

Species	Height (cm)	Diameter (cm)	Root biomass (g)	Shoot biomass (g)	Leaf area (cm/cm^3)
Brachystegia spiciformis	24.3 ± 0.27	4.0 ± 0.2	2.94 ± 0.06	2.72 ±0.10	4.21 ± 0.35
Brachystegia spiciformis **	23.9 ± 0.28	3.4 ± 0.1	3.41 ± 0.14	2.51 ± 0.15	3.89 ± 0.12
Casuarina equisetifolia	62.2 ± 6.1	4.7 ± 0.3	3.95 ± 0.52	6.09 ± 0.81	9.84 ± 0.0.29
Casuarina equisetifolia **	54.4 ± 3.8	4.8 ± 0.4	4.64 ± 0.31	7.04 ± 1.31	11.16 ± 0.57
Dodonaea viscosa	82.7 ± 3.2	8.0 ± 0.3	8.89 ± 0.89	20.03 ± 1.47	20.84 ± 0.73
Dodonaea viscosa **	85.4 ± 3.0	7.9 ± 0.2	10.21 ± 0.99	22.53 ± 1.39	21.0 2± 0.5
Rhus natalensis	32.5 ± 1.1	6.5 ± 0.3	3.87 ± 0.21	4.19 ± 0.23	7.99 ± 0.37
Rhus natalensis **	29.0 ± 1.2	6.1 ± 0.3	4.66 ± 0.24	4.85 ± 0.23	9.16 ± 0.51
Tecomaria capensis	54.8 ± 1.6	11.4 ± 0.5	14.04 ± 1.06	21.34 ± 1.93	8.06 ± 0.33
Tecomaria capensis **	45.6 ± 2.6	9.5 ± 0.6	12.68 ± 0.88	17.53 ± 1.41	7.28 ± 0.41

5.3.5.3. Root and shoot biomasses.

With the exception of *R. natalensis* and *B. spiciformis,* no differences in root biomass were observed between parasitized and unparasitized plants. 12 months after transplanting, Root biomass of parasitized *R. natalensis* and *B. spiciformis* was significantly increased by 16.7% (P 0.03) and 13.6% ($P < 0.01$) respectively of their control (i.e. without parasite). With regard to shoot biomass, parasitized and unparasitized plants did not show any significant difference throughout the growth periods of 12 months.

5.3.5.4 Leaf area growth

Leaf area between parasitized and unparasitized plants did not show any significant difference with the exceptional of *C. equisetifolia*. At 12 months, leaves of parasitized *C. equisetifolia* was significantly reduced by 17.1% ($P < 0.01$) of those grown without *O. lanceolata* (parasite).

5.4. Discussion

5.4.1 Host plant identification and their intensity of utilization

Host plant species identified in the three sites studied varied a great deal in terms of the number of species and intensity of their utilization by *O. lanceolata*. The variability observed could be the result of non-selectivity of *O. lanceolata* to hosts as reported by Rao (1942a). More host plants (almost twice) were observed at Image forest reserve compared to either Sao Hill or Nundu. This might be attributed to differences in climatic and soil conditions among the sites. Image generally receives less amount of rainfall compared to the other two sites. Thus water deficit is more critical at Image forest than at the two sites. The soils of Image area are also relatively poor in nutrient status. As a consequence, a wide range of plant species are utilized as hosts by *O. lanceolata* at Image. Similar observations have been reported in most parasitic plants growing in poor soils (Lamont and Southall, 1982). Many

host species at Image enable *O. lanceolata* to meet its water and nutrient requirements. They could be serving as buffers between the parasite and the physical environment by assisting the parasite to obtain potentially limiting resources of water and nutrients adequately without competition.

The high frequency of occurrence of some of the host species observed in the three study sites suggest that although *O. lanceolata* is non-host specific, it may have host preference. Where suitable hosts were available, *O. lanceolata* tended to be attached to them more than to others. At Image Forest Reserve, *Brachsytegia spiciformis* and *Brachsytegia utilis* were the most utilized species while at Sao Hill Forest, the most utilized species were *Tecomaria capensis, Mayetenus senegalensis* and *Rhus natalensis*. At Nundu *O. lanceolata* was observed to concentrate around *Aphloia theiformis* and *Rhus natalensis*. The reasons for such preferences are still unknown. However, it has been shown in other plant species that host plants tend to release some chemical exudates which are sensed by the parasites. Upon sensation, roots of the parasites tend to grow toward the host that have released the chemical signals (Kuijt, 1969; Musselman and Press, 1995).

The similarity indices obtained between sexes of *Osyris lanceolata* in host selectivity indicate that males and females are similar in their proportional use of host plants at the three sites. Similar observations are reported by Herrera (1988a) in the Mediterranean.

5.4.2 Influence of host plants on growth of *Osyris lanceolata*

Host plants were observed to have a varied influence on growth of *Osyris lanceolata*. The effect varied from growth promotion to suppression in most growth parameters assessed. This suggest that selection of host species is important at nursery level if *Osyris lanceolata* has to be raised in plantation. The effect started to appear after the first three months. This shows that *O. lanceolata* can support itself during the early stages of germination and growth. This is possibly due to its large sized seeds that have enough reserve to support a small plant as suggested by Kuijt

(1969) and Musselman and Press (1995). However beyond three months, the rate of photosynthesis was rather low to support growth as reported in many hemi-parasitic plants (de la Hape *et al.*, 1981; Press *et al.*, 1988; Press, 1995) and thus a host is needed.

Generally, *Brachsystegia spiciformis, Rhus natalensis* and *Casuarina equisetifolia* had positive effects on growth of *Osyris lanceolata* while *Dodonaea viscosa* and *Tecomaria capensis* seemed to have a suppressive effect. The suppression of growth shown by *Dodonaea viscosa* and *Tecomaria capensis* could be the result of excessive competition for light between them and *Osyris lanceolata*. The growth of the two host species was rather fast compared to *Osyris lanceolata* and as a result they were able to cast heavy shade on *Osyris lanceolata,* consequently retarding its growth. Excessive shading has been reported to be detrimental to the growth of *Santalum album* (Radomiljac, 1998; Rama Rao, 1911), another hemi parasitic plant of the family Santalaceae. On the other hand, poor growth of *Osyris lanceolata* under *Dodonaea viscosa* and *Tecomaria capensis* could also be due to less competitive ability of *Osyris lanceolata* or the resistance or inability of the hosts to supply nutrients to the parasites. There are similar reports in the literature on poor growth of root hemi-parasites attached to some hosts such as *Olax phylanthi* attached to *Amaranthus caudatus* and *Portulaca oleracea* (Tennakoon and Pate, 1996). This poor growth is suggested to be due to minimal uptake of nitrogen brought by large proportion of nitrate accumulating in the host's xylem sap. This in turn reduced the intake of heterotrophic carbon. Radomiljac (1998) observed that *Acacia hemignosta* and *Crotolaria retusa* were unable to increase survival and growth of *Santalum album* and the main attributing factor was failure or inability of the host species to supply nutrients and moisture to the parasite.

Although the reasons why *Brachystegia spiciformis, Rhus natalensis* and *Casuarina equisetifolia* promoted growth of *Osyris lanceolata* are uncertain, the light crowns of these species, which may have allowed more light to penetrate and become available to *Osyris lanceolata*, could be one of the reasons. Rama Rao (1911) had similar opinion when suitability of various hosts to *Santalum album* were studied. Host

plants that favor good growth of parasites usually possess light crown thereby not preventing both lateral and overhead light for the parasite. For *Brachystegia spiciformis* and *Rhus natalensis,* their ability to promote good growth of *O. lanceolata* could be attributed to the high root/shoot ratio found in parasitized plants of both species. The high ratio indicates that these species have the ability of increasing their biomass that will assist them in exploring greater volume of soil to meet their water and nutrient requirement as well as the parasite (Graves, 1995). While this seems to be beneficial under water deficit and nutrient deficient soils, the extra root biomass created is usually done at the expense of above ground assimilation that in turn reduces the growth rate of the host.

The observed good performance of *Osyris lanceolata* under *Casuarina equisetifolia* could be linked to its nitrogen fixing ability as observed by Rai (1990) and Taide *et al.* (1994) in pot cultures studies of *Santalum album.* Also Radomiljac *et al.* (1999) reported that nitrogen fixing plants of *Sesbania formosa, Acacia trachycarpa* and *Acacia ampliceps* were better hosts compared to non nitrogen fixing hosts in *Santalum album.* Many other parasitic plants such as *Olax phyllanthi* (Tennakoon and Ehleringer, 1996) and *Phoradendron californicum* (Ehleringer *et al.*, 1985) have been shown to perform well with nitrogen fixing host plants. Good performance of parasites with nitrogen fixing host plants is related to reduced competition for nitrogen between the hosts and the parasites. While the parasite will be utilizing most of the soil nitrogen and extracting some from its host, the host will meet most of its nitrogen requirement through fixation.

5.4.3 Response of host plants to parasitism

As competition between plants species is always expected whenever two or more plants are grown together, planting of *Osyris lanceolata* as a parasite with a host cannot be exceptional. Differences between parasitized and unparasitized host plants were mainly observed when plants were 12 months old. It is likely that competition during the early stages of growth is minimal as the parasite drains little resource from its host to meet its requirement. The need for host becomes important as the

parasite size increases (Rao, 1942a,b). The damage imposed depends on the host size and vigour (Graves, 1995) The observed reduction in height and diameter growth in *Tecomaria capensis* when plants were 12 months old could be the result of competition for nutrients or excessive withdrawal of resources by the parasite. This deleterious effect is also reported in *Sesbania formosa*, a good intermediate host of *Santalum album* (Radomiljac, 1998). Thus *Osyris lanceolata* may have reduced the growth of *Tecomaria capensis* by acting as an additional sink for *Tecomaria capensis* and reducing it capacity to fix carbon (Tuohy *et al.*, 1987; Graves *et al.*, 1989).

The higher root biomass observed in parasitized than in unparasitized host plants of *Rhus natalensis* and *Brachystegia spiciformis* is in general agreement with what have been observed in *Nicotiana tabacum* parasitized with *Orobanche ramosa* (Ernst, 1986), *Sorghum bicolor* parasitized with *Striga hermonthca* (Graves *et al.*, 1989), and *Acacia ampliceps* parasitized with *Santalum album* (Radomiljac, 1998). As suggested by Graves (1995), this architectural change in the host is a strategy of hosts to try to explore more area and get enough nutrients to support itself and the parasite. In most cases, the above ground assimilation is reduced in favour of below ground assimilation. This automatically reduces shoot growth of the hosts while at the same time more energy is spent in respiration.

CHAPTER VI

STORAGE AND PRE-TREATMENT OF SEEDS OF *OSYRIS LANCEOLATA*

6.1 Background information and objectives of the present study

6.1.1. Introduction

Osyris lanceolata, which is an important source of income to several rural communities of Tanzania, has been declining rapidly due to intensive and uncontrolled harvesting for perfumery and production of sandalwood oil. Yet no attempts have been made to reverse this trend and assure sustainable supply of the resource (Mbuya *et al.*, 1994). The market of sandalwood has ever been rising year after year due to an ever-increasing demand for sandalwood products. The species is, thus, under serious threat unless measures are taken to replenish it either through natural regeneration or planting (Rai and Salma, 1990; Srinivasan *et al.*, 1992).

While individuals and private firms have been encouraged to propagate the species as part of their income generating activities, there has been a serious lack of basic information on silviculture of the species to guide and support this development. In addition to its parasitic behaviour (Kuijt, 1969; Mbuya *et al.*, 1994) that makes plantation more complex than traditional silviculture as in many parasitic plants (Srivassan *et al.,* 1992; Radomiljac *et al.*, 1999), problems associated with seed supply due to storage difficulties (Mbuya *et al.*, 1994) and the resultant poor germination (Msanga 1998) add to this complexity. Trials to propagate the species through seed have never been very successful, always necessitating a large amount of seeds to be sown to attain a small amount of targeted seedlings. Germination is reported to be poor, hardly reaching 60% in a very sporadic way over prolonged period of more than six weeks (Mbuya *et al.*, 1994; Msanga, 1998). The seed coat covering the embryo is generally thin but hard and possibly impermeable which is speculated to hinder satisfactory germination. To the moment, no trials to overcome this problem has ever been carried out or documented although application of pre-sowing treatments such as soaking seeds in water and scarification are thought to be

some of the possible in resolving the problem (Msanga, 1998). The treatments are considered important so as to improve the germination capacity of seeds and possibly reduce the time taken for germination to take place and thus allow easy propagation through the use of seeds.

Supply of sound viable seeds at proper time has also been a problem as the rate of predation and pathogenic attack in the species is high (Personal observation). The beetle *Dismegistus sargumeus* De Geer that feed mainly by sucking the juice of the fruits, lays eggs on flowers of the species. These eggs are enclosed inside during fruit formation and upon their hatching, the born larva eats the internal contents and escapes through tiny holes. Apart from embryo consumption by larva, the feeding mechanism of the insect might be introducing some pathogens or toxic compounds ultimately kills the embryo within the seed.

This bring into attention the importance and need for storing seeds to ensure supply whenever they are needed and as buffer to cover years of poor seeding. However, the recalcitrant behaviour of the seeds complicates the whole storage exercise if not making storage impossible (Mbuya *et al.,* 1994). This further necessitates searching for appropriate methods of storing such seeds so that their viability can be prolonged for at least short-term supply of up to a year.

6.1.2 Plant propagation by seeds and its importance

Plants can be propagated either sexually (though seeds) or asexually (through vegetative material) (Bewley and Balack, 1978; Kijkar, 1992b; Kantari 1993a). However, the use of seeds is considered to be the most efficient and economical for most species provided genetic variability is controlled within acceptable limits (Hartmann and Kester, 1997; Adjers and Srivastava, 1993). In some species it is the only means as vegetative methods are either not possible or uneconomical (Hartmann and Kester, 1997). Further, seeds are easy to transport from where they are collected to most destination without loosing viability easily compared with vegetative material such as cuttings, which can easily be subjected to undesired effect of desiccation (Willan, 1985; Bewley and Black, 1978). Through seeds, it is easy to retain variability

existing in a population and this is important especially when the species faces disasters such as disease outbreak (Longman, 1976).

While propagation through seeds is the most frequently used and economical method for most species, knowledge of the seed biology is essential for successful seed production and handling (Bonner *et al.*, 1994). Many problems are known to be associated with seeds of some species and among them is the rapid loss of seed viability due to storage difficulties and inability of some seeds to germinate at the desired level or total failure attributed by dormancy problems (Willan, 1985). Failure to store seeds is critical in some species, as this can make seeds unavailable at the time when they are needed for regeneration. The problem is more pronounced in recalcitrant seeds as these need special care from the time they are collected, processed and stored (Roberts, 1973; Nikolaeva, 1977; Meyer and Polyjakoff-Mayber, 1989; Bewley and Black, 1994).

6.1.3 Storage of seeds and maintenance of viability

Seed storage involves preservation of viable seeds from the time of collection until they are required for sowing, the objective being to delay deterioration or decrease its rate. The period of storage may range from few months to several years depending on the purpose of storage (Bonner *et al.*, 1994). The importance of storing seeds comes from the fact that, only rarely does the appropriate sowing date coincide with the best date for seed collection. More often seeds are stored to wait for the sowing season to come (Roberts, 1972; Hartmann and Kester, 1997). The fact that some species bear abundant seed crop at an interval of several years, makes storage to be important so as to preserve seeds for years of poor or no seed production. Sometimes collection and storage of seeds is done as a means of conserving the genetic resources. Under this circumstance, seeds may be stored for several years (Justice and Bass, 1979; Willan, 1985). However like any other living organisms, seeds in the store tend to loose viability with time and ultimately end up with death. The rate of loss of viability and longevity is largely determined by the seed characteristics, quality of seeds at the time of collection, their handling between collection and storage, genetics and the condition in which they are stored (Hartmann and Kester, 1997; Bonner *et al.*, 1994).

With regard to seed characteristics, two major groups of orthodox and recalcitrant are recognised on the basis of basic physiology and condition under which they can be stored. Orthodox seeds are tolerant to desiccation and can be dried to low moisture content of less than 10%, and successfully stored at sub freezing temperatures for long periods (Roberts, 1973; Bonner *et al.*, 1994). They are easy to store provided sound mature seeds, free from mechanical damage, fungi, insects and which have suffered no physiological deterioration during processing are used (Stein *et al.*, 1974). The safe storage moisture content for most of them is 4-8 %. Low temperatures are more effective but, the cost of maintaining it needs to be weighed against the viability that need be achieved at the end. Many hard seeded seeds keep well at room temperatures for reasonable time. However, where storage is done for genetic resource conservation temperatures of -18 °C is recommended (IBPGR, 1976).

On the other hand, recalcitrant seeds cannot survive drying below relatively high moisture content often in the range of 12-50 % and cannot be easily successfully stored for long time (Roberts, 1973; Chin, 1988; Redhead and Hall, 1992). A big variation in temperature requirement of recalcitrant seeds is known to exist, although their trend is similar to that of orthodox species within certain limits i.e. the lower the temperature, the longer the period of longevity (Willan, 1985). However, most recalcitrant seeds can not tolerate freezing temperatures (Chin *et al.*,1981; Ngulube and Mkandawire, 1997).

Other seed characteristics well known to influence longevity of seeds are seed structure and chemistry. Thick and hard seed coats restrict moisture uptake and gas exchange while most seeds with thin seed coats allow too much of both, making their life span short. Seed chemistry has also an influence on longevity as oily seeds tend to be harder to store than starchy seeds (Bonner *et al.*, 1994). Genetics of the species is also an important factor as some seeds of species can naturally live longer than others.

Within a species, longevity of seeds in the store is influenced by seed characteristics such as provenance effect brought by environmental stress, seed condition and handling before storage; genetics; and storage environment.

6.1.3.1 Provenance effect

Storage longevity varies among provenances of the same species. Weather condition, photoperiod, mineral nutrition and soil moisture are the most common attributes to this variation. Of these, weather condition prevailing in different localities of species occurrence is believed to be the most important. Warm and moist weather encourage immediate germination of seeds and seeds from these areas tend to behave in the same way even when they are stored. Seeds of cold dry areas have certain dormancy character, enabling them to stay for sometime to wait for conducive season for germination to come than those from warm moist areas (Justice and Bass, 1979).

6.1.3.2. Seed condition

Even under ideal conditions, seeds will loose viability if they are defective from the start. Fully mature ripened seeds retain viability longer than the seeds collected when immature. Maturity of seeds is important as it allows certain biochemical compounds that are essential for preserving viability to be formed. These chemicals may have not formed if proper maturity is not reached (Harrington, 1972; Stein *et al.*, 1974). Damage to the seeds during extraction, cleaning and drying processes has to be avoided, as damaged seeds tend to loose viability faster. Mechanical injuries that cause cracks or bruises to the seed coat provide an entry for pathogens that in turn destroys the seed. Other seed conditions that can prolong the viability of seeds in the store include freedom from physiological deterioration, freedom from fungi and insects (Justice and Bass, 1979; Willan, 1985).

Of the seed conditions, perhaps the moisture content of seeds during storage is the most influential factor affecting seed longevity. Deterioration of seeds increases as moisture content increase due to increased respiration rate (Justice and Bass, 1979). For effective storage aiming at reducing ageing, the seed moisture content needs to be as low as possible. Moisture content of 4-10% has been proved to be effective for general storage (Bewley and Black 1978; Redhead and Hall, 1992, Bonner, 1984). Recalcitrant seeds follow similar rule within certain limits. However, the critical

moisture content is the minimum to which it is allowable to dry rather than the maximum content for prolonged storage. Critical point varies a lot between species and is too dependent on the temperature at which seeds are stored. The effective moisture content for storage of most recalcitrant seeds is in the range of 12-50 % (Roberts, 1973; Redhead and Hall, 1992). Yet, this range is risky as fungal attack could easily occur, especially when temperatures are high (Willan, 1985).

Once the appropriate storage moisture content is established, avoidance of fluctuations caused by relative humidity outside the container is important as this can result in seed deterioration. Use of moisture proof containers that allow aeration is recommended for storage of recalcitrant seeds (Stein *et al.*, 1974).

6.1.3.3. Storage environment

Regardless of whether seeds are orthodox or recalcitrant, seeds are subject to ageing and eventually death. The speed of ageing and deterioration is greatly affected by the condition of storage mainly dictated by seed moisture content, temperature and storage atmosphere (Justice and Bass, 1979; Hartmann and Kester, 1997). Loss of viability during storage may be associated with several reasons such as loss of food reserve caused by respiration, accumulation of toxic or growth inhibiting by-product of respiration, loss of activity of enzyme systems, lipid peroxidation leading to production of free radicals which react with and damage other components in the cell (Roberts, 1972; Villiers, 1973). Of these, release of free radicals following lipid peroxidation is considered to be the first effect of ageing in seeds (Villiers, 1973). However, loss of seed viability is largely governed by the rate of respiration and any measure aiming at reducing it without damaging the seeds are considered effective in extending seed storage longevity.

Storage temperature ranks second to seed moisture content in influencing the seed storage longevity. Reduced temperatures lower the rate of respiration and in turn lengthen the life of seeds (Hartmann and Kester, 1997). It can also be used to offset the effect of high moisture content (Chin and Roberts, 1980; Farrant, 1988). As a rule of

thumb, within a range of 0-50 °C, the life span of seeds is doubled for each 5 °C lowering temperature (Harrington, 1972). While this is applicable for most orthodox seeds, recalcitrant seeds behave only within limits. Many species such as cacao and mango are killed if temperature is reduced below 10 °C (King and Roberts, 1979). Also lowering of temperature in seeds stored with moisture content between 20-50 % need great care as the risk of chilling injury is introduced which can lead to early death of seeds (Bonner, 1990). Roberts and King (1980) recommended the critical temperature for storing seeds with moisture content above 20 % to be 0 °C to avoid the chilling effect. It follows that, within a certain moisture content, temperatures have to be adjusted in such a way it is neither too low to cause chilling nor too high to encourage early germination of seeds.

Storage atmosphere is related to oxygen availability. As loss of viability is governed by the rate of respiration, exclusion of oxygen from the seed's surrounding environment is crucial (Roberts 1972; Tompset, 1983; Willan, 1985). In most cases this is done by replacing oxygen with other gases such as carbon dioxide, nitrogen or by use of a partial or complete vacuum. While this is true for most orthodox seeds, some oxygen has been proved to be essential for storing recalcitrant seeds at relatively high moisture content (King and Roberts, 1979). As a result containers used to store recalcitrant seeds are selected in such a way that aeration will be maintained. Cloth, hessian bag containers (Ngulube and Mkandawire, 1997) and polythene bags (Stein *et al*, 1974) have commonly been in use.

6.1.4 Dormancy in seeds and their possible remedy

Germination of viable seeds requires exposure to favourable environmental conditions. Among these include adequate water supply, suitable temperatures, good composition of gases and in some cases light may be of importance (Meyer *et al.*, 1973; Nikolaeva, 1977). However, seeds of many species have been reported not to germinate at all or delay to germinate and do so irregularly even if supplied with favourable conditions for germination. Such seeds are termed dormant and they tend to germinate only after some sort of pre-treatment is applied to them (Mugasha and Msanga, 1987; Meyer and Polyjakoff-Mayber, 1989).

Seed dormancy presents various problems in nurseries. The long period involved in raising seedlings needs more labour and tends to tie up the nursery space unnecessarily, consequently increasing the cost of seedling production (Mugasha and Msanga, 1987). Dormancy reduces the efficient use of seeds as many seeds need to be sown to get a small amount of seedlings while in some cases no seedling at all can be obtained, adding to the cost of collecting or purchasing additional seeds. Further, dormancy causes irregularity in seeds germination resulting into nurseries with stock of variable age and size which is a problem in field establishment as small pieces of land spread over time will be required to be planted at different times (Nwoboshi, 1982; Willan, 1985; Kijkar, 1992a). Thus the mechanism of seed dormancy need to be known so that methods of overcoming it are designed to ensure timely germination and uniform growth of the seedlings. Dormancy in seeds is classified into exogenous (seed coat /pericarp) and endogenous (morphological /physiological) dormancy. Sometimes a combination of the two is classified as the third type (Waering and Saunders, 1971; Nikolaeva, 1977). In the dry tropics, the most common dormancy is the one associated with seed coat (Willan, 1985).

6.1.4.1 Seed coat/exogenous dormancy

Seed coat/exogenous dormancy is mainly associated with physical, chemical or mechanical dormancy. In physical dormancy the seed coat/pericarp tends to be impermeable to water or gases and sometimes to both (Nwoboshi, 1982). Without imbibition and gaseous exchange to take place, renewal of embryo growth and germination are impossible (Bewley and Black, 1994; Meyer and Polyjakoff-Mayber, 1989). This is common especially in many legumes such as *Leucaena, Albizia, Ceratonia, Albizia* and some *Acacia* species (Amen, 1963; Willan, 1985; Duangpatra, 1991).

The impermeability of the seed coats to water and gases are normally caused by a layer of palisade-like macrosclereid cells, especially thick-walled on their outer surfaces and coated with a layer of waxy cuticular substances. In seeds with small openings (strophiolar cleft) that permit the entry of water and gases, the entry may be plugged

with cork-like filling consisting of suberin, thus rendering the seed impermeable (Rolston, 1978; Dell, 1980). The methods to overcome physical dormancy are designed at softening, puncturing, wearing away or splitting the seed coat to increase moisture uptake and gas exchange. Thus rubbing the seed coat with sandpaper, filing and drilling a small hole on the seed coat, cracking or chipping the seed and partial removal of the seed coat are common practices (Maghembe and Msanga, 1988; Onyekwelu, 1990; Nwoboshi, 1982; Tietema *et al.*, 1992). Other alternatives include soaking of seeds in cold or hot water for various periods, the use of hot wire, and soaking seeds in concentrated acids (Duangpatra, 1991; Redhead and Hall, 1992).

Chemical dormancy is related to the presence of inhibitors in the pericarp or seed coat. Some of the main inhibitors that have been identified in many fruits and plant tissues include B-inhibitor complexes (di-nitophenols, salycic and oxybenzoic acids), abscisic acid, Coumarin, cyanide, azide, fluoride and hydroxylamine (Guan *et al.*, 1988; Walton, 1980; Meyer and Polyjakoff-Mayber, 1989; Hartmann and Kester, 1997). These inhibitors tend to interfere with normal metabolic activities by interfering on certain pathways such as respiration of tissues that are essential for initiation of growth. Thus to overcome this `dormancy, pre treatments are usually aimed at leaching away the inhibitors and liquid pre treatments such as water and peroxides have been found to be useful (Khan, 1977).

The other type of seed coat dormancy is mechanical and is associated with the resistance of the seed coat or pericarp to allow the growth of the embryo. The seed coats tend to be tough, thick and sometimes woody requiring great pressure by the seed embryos to break and germinate (Duangpatra, 1991; Msanga and Kalaghe, 1992; Yadav, 1992). Several methods have been used to overcome this dormancy, but normally splitting, cutting or wearing away the seed coat have been useful (Msanga and Kalaghe, 1992; Sadhu and Kaul, 1989). Other methods that have been used include use of chemical such as sulphuric acid (Chamshama and Downs, 1984; Sagwal, 1986; Sadhu and Kaul, 1989; Tietema *et al.*, 1992); soaking of seeds in water for various periods (Redhead and Hall, 1992; Adjers and Srivastava, 1993). Ideally most treatments aimed at overcoming physical dormancy removes mechanical dormancy as well.

6.1.4.1. Embryo/endogenous dormancy

This is related to the morphology or physiology of the seeds (Willan, 1985; Meyer and Polyjakoff-Mayber, 1989; Duangpatra, 1991) and is sometimes referred as embryo dormancy. Morphological dormancy is due to the incomplete maturation of the embryo by the time seeds or fruits are shed from the trees (Nwoboshi, 1982). The time required for maturation in most woody perennials varies from one to three months, although in certain species five to six months may be necessary. This time allows the embryos that have not completed development to mature and important anatomical changes to take place that allows germination (Hartmann and Kester, 1997; Meyer and Polyjakoff-Mayber, 1989; Bonner *et al.*, 1994). This time also allow complete differentiation of cells that must precede any germination (Duangpatra, 1991). The method employed to overcome morphological dormancy include collection and sowing of seeds when maturation has taken place (Nwoboshi, 1982; Bhardway and Chakraborty, 1994) and/or subjecting seeds to a period of moist warm pre-treatment before the embryo develop sufficiently for germination to take place (Bonner, 1984; Willan, 1985).

Physiological dormancy is associated with decreased activity of the embryo. It is also related to biochemical changes that must take place in seeds before germination could commence. However, little is known concerning these changes (Nikolaeva, 1977; Meyer and Polyjakoff-Mayber, 1989). To overcome this dormancy, the period of after-ripening may be necessary. During this period, the composition of the storage material in the seed may alter, the permeability of the seed coat may change and substances promoting germination may appear or the inhibitory ones may disappear (Nwoboshi, 1982; Meyer and Polyjakoff-Mayber, 1989). Other common methods that have been in use include seed stratification for different days (Nikolaeva, 1969) and use of chemicals such as gibberellic acid, citric acid, kinetin, hydrogen peroxide, indole acetic acid and ethylene (Viendra, 1990; Viendra, 1992; Bonner *et al.*, 1994). Stratification have been found to trigger off biological changes which transform complex food substances into simpler forms utilised by the embryo when it renews growth and activate enzyme systems necessary for seed germination (Nikolaeva, 1969; Bonner *et al.*, 1994). Maithan *et al.* (1989) showed this to be applicable in seeds of *Aesculus indica* that had germination problems

6.1.4.3. Combined and secondary dormancy:

Sometimes a combination of seed coat and embryo dormancy occurs in the same seed (Meyer and Polyjakoff-Mayber, 1989; Bewley and Black, 1994). A number of species in the *Rosaceae* family possess a combination of physical or mechanical and physiological dormancy (Gordon and Rowe, 1992; Willan, 1985). In this case breaking one type of dormancy may be insufficient to enhance germination unless it is followed by the second pre-treatment. Thus the method to be used needs to be adjusted accordingly. On the other hand, secondary dormancy results from some action, treatment or injury to the seeds during collection, handling or sowing. Exposing seeds of some species such as *Pinus taeda* to excessive temperatures and moisture show delayed germination (Bonner, 1984).

6.1.5. Objectives

This study presents the results of storage experiment carried out to evaluate how possible seeds of *O. lanceolata* can be stored at least for short term supply by manipulating seed moisture content and storage temperature that are known to be the major factors controlling the lifespan of recalcitrant seeds. It also aimed at assessing the influence of various pre-sowing treatments that can enhance germination and early growth of seedlings in the nursery as the first step towards successful domestication of the species. To meet these objectives three research questions were set:

i. Can the viability of *Osyris lanceolata* be extended beyond what is achieved under normal processing and storage conditions?
ii. At what critical seed moisture content and storage temperature can the life span of *Osyris lanceolata* be maintained for a long time and how long is the period through which reasonable viability can be maintained?
iii. Are seed pre-sowing treatments effective in improving germination and early growth of seedlings compared with sowing without application of these treatments?

6.2. Material and methods

6.2.1 Experimental sites

The study was carried out at the Iringa Zonal Tree Seed Center using seeds material collected from Sao Hill Forest Reserve. See Chapter One for detailed description of the study sites and location of experimental site.

6.2.2 Experimental procedures

6.2.2.1 Seed storage and longevity experiment

This experiment was carried out to assess the optimal moisture content of seed and storage temperature that could reduce the rate of loss of seed viability and thus prolong the life span.

Ripe fruits of *O. lanceolata* were collected in Sao Hill forest population from 15 mature trees that were spaced at a mean distance of 35 meters between individuals. This was done to ensure representation of the species as much as possible (Simons, 1997). After collection, fruits were transported to Iringa Zonal Tree Seed Center in plastic containers where further processing was done. Upon arrival at the Tree Seed Center, fruits were soaked in tap water for 24 hours to facilitate easy removal of the pulp. Water was changed every 6 hours to avoid fermentation that is harmful to the seeds. The pulp was removed by running the fruits into concrete mixture and squashing by hand. The seeds obtained were slowly dried in shade to the desired moisture content (15, 20, 25 and 30%). To arrive at the desired moisture content, a periodic test through reweighing of seeds and oven drying was adapted as described by (Kumar *et al.*, 1996) with some modification. In this method, four replicates of 20 seeds per sample were dried for one hour at 130 °C in an oven to achieve one level of moisture content. The moisture content (MC) was then expressed as a percentage of the fresh weight.

$$M.C = \frac{\text{weight of fresh seeds–weight of dry seeds}}{\text{weight of fresh seeds}} \times 100$$

Once seeds attained the desired moisture content, their viability was assessed through a simple cut and inspection test. Thereafter they were treated with captan (a fungicide) to prevent them from fungal attack. The seed batch was then divided into four portions. One of each portion was then stored at the intended storage temperatures. The packaging material used to store seeds was polyethylene bags of 4 mm thickness. These have been recommended to be ideal for restricting moisture exchange yet allowing some oxygen to get in which is important in storage of recalcitrant seeds (Stein *et al.*, 1974; Pukittayacamee *et al.* 1995).

The experimental design adopted was a factorial combination of four seed moisture contents and four storage temperatures making a total of sixteen treatments. The four levels of moisture content tested included 15, 20, 25 and 30%. These were chosen based on recommended range for storage of most recalcitrant seeds (Roberts, 1973; King and Roberts, 1979; Willan, 1985; Redhead and Hall, 1992). The storage temperatures assessed included (-1) – (+1), 3-5, 8-10 and 13-15 °C. One refrigerator was used for each temperature treatment. All the four refrigerators were then placed in germination room where the relative humidity was maintained constantly.

Each of the sixteen treatments had a total of 3000 seeds. At the time of each assessment (done at four weeks interval), about 300 seeds from each treatment was drawn and divided into four replicates for the seed viability test. From each replicate 50 seeds were tested. Loss of viability was assessed through a topographic tetrazolium test, in which seeds were initially soaked in water for 24 hours. After this period, seeds were sectioned longitudinally through the endosperm and embryo and immersed in 1% 2,3,5-triphenyl tetrazolium chloride solution in darkness for 24 hours. The staining pattern of the embryo was then studied under a dissection microscope to determine viable seeds. Only embryos that stained red at both the growing points were declared viable as done for *Aporusa lindleyana* by Kumar *et al.* (1996). Viable seeds were then expressed as a percentage of 50 seeds that were assessed.

Data was analysed by plotting viability trends over time and employing analysis of variance to compare viability among treatments at each assessment time. Regression

analysis was applied to see how much of the variation observed in viability could be attributed to storage temperature or seed moisture content. Before subjecting data to analysis, all percentage data were transformed into arcsine values as recommended by Sokal and Rohf (1995) and Zar (1996).

6.2.2.2 Seed pre-sowing treatments and seedling growth

The effectiveness of five seed pre-sowing treatments in enhancing germination and early growth of *O. lanceolata* was tested by subjecting seeds to various treatments which included (1) soaking seeds in tap water for 12 hours; (2) soaking seeds in boiled water and left to cool for 12 hours; (3) seed nicking by hot wire; (4) total removal of seed coat; and (5) a control (untreated). After treating the seeds as described above, they were sown in nursery bed using sand as a growth medium. Upon germination, germinates were potted into polyethylene tubes containing a mixture of sand, forest soil and cow manure in the ratio of 2:1:1. The forest soil was obtained from the sandalwood growing stands. Seedlings were then left to grow for six months after which they were harvested and assessed Plate 6.1 shows germinating seedling, 14 days after transplanting.

Plate 6.1 Germinating *O. lanceolata* seedlings. 14 days from transplanting bed

The experimental design adapted was a completely randomized design. Each treatment was replicated four times and each replication contained a total of 50 seeds. Thus a total of 1000 seeds were used for this experiment.

In germination assessment, seeds were declared to have germinated once the plumule emerged from soil and grown to 5 mm. Germinating seeds were recorded daily until no further germination took place in any of the treatments, (up to 112 days). In addition, dormancy period was also recorded i.e. days taken for seeds from sowing to completion of germination.

At the termination of the germination experiment, all seeds that did not germinate were removed and tested for viability through a cutting test and visual inspection and scored as dead or alive. Alive seeds together with germinated seeds were added together to determine viability. For seedling that were potted, raised in the nursery and ultimately harvested, the data collected included height, root collar diameter, length of primary root and total seedling biomass. To determine seedling biomass, the plant material both root and shoot was oven dried at 80 °C for 48 hours before weighing.

Total number of seeds that germinated in a particular treatment was expressed as cumulative germination percentage (GP). Other germination attributes derived included germination value (GV), germination energy (GE) and dormancy periods. Germination value was calculated following a method of Djavanshir and Pourbeik (1976). The method estimate germination energy as:

$$GV = \Sigma DGS/N \times GP/10$$

Where:

GV = Germination Value

DGS = Daily Germination Speed,

N = Number of counts since commencement of germination,

GP = cumulative Germination % at the termination of experiment,

10 = a constant,

ΣDGS = sum of individual Daily Germination Speed till the end of germination.

For germination energy, the criterion used by Allen (1958) was adapted. The criterion estimate germination energy as a number of days required attaining 50% of germination capacity through recording rate of germination. The general linear model of the MINITAB statistical package was then employed to analyze the data for evidence of significance differences in all parameter assessed and derived among treatments. If significant difference was found Tukey's pair wise comparison was used separate the differing means.

6.3. Results

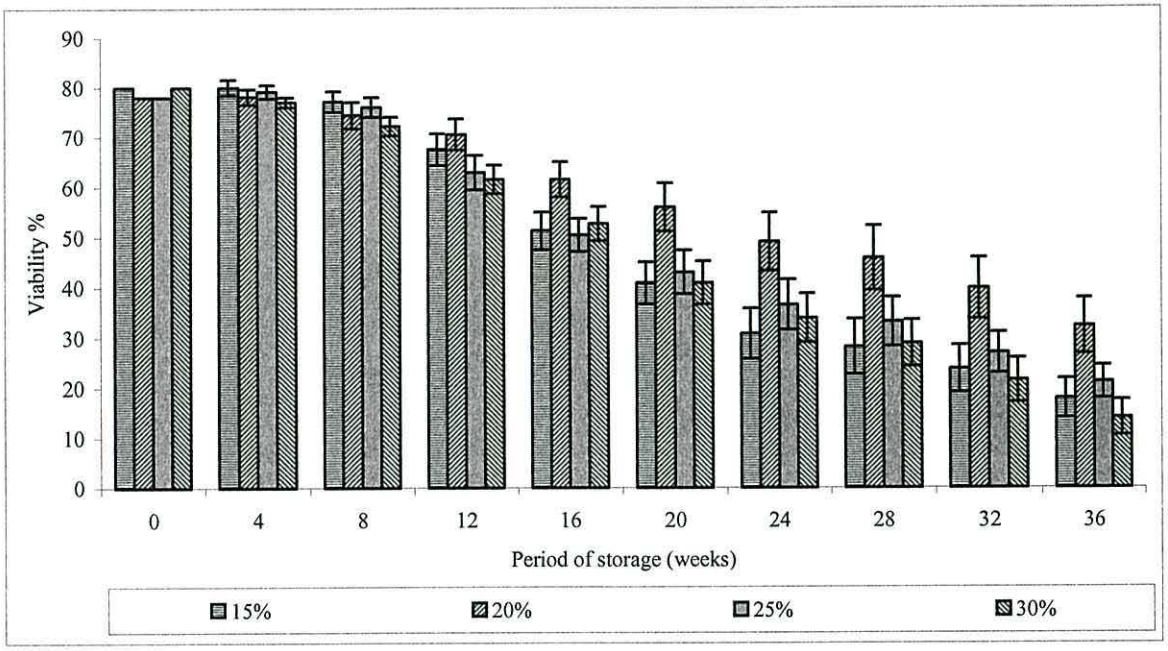
6.3.1 Storage of *Osyris lanceolata* seeds

6.3.1.1 Effect of initial seed moisture content on retention of seed viability

High levels of seed viability of seeds were maintained during the first 8 weeks, regardless of the moisture content at which seeds were stored (Figure 6.1). Significance difference among treatments started to be observed at week 12 ($P < 0.01$). Seeds stored with moisture content of 20% retained significantly higher viability (70.5%) than those stored with 25% (62.9±3.2%) and 30% (61.5±2.9) moisture content, but not significant different with those stored with 15% (67.5 ± 3.5%). From 16th week until termination of the experiment i.e. at the end of 36 weeks, seeds with 20% moisture content were able to maintain significantly higher viability ($P < 0.001$ at 16, 20, 24 28, 32 and 36 weeks) than any other moisture content treatment. At week 36, seeds stored with 20% moisture content had a viability of 32.3 ± 5.5%). The least viability was obtained in seeds stored with 30% moisture content (14±3.6%).

Considering the whole seed storage period of 36 weeks, the rate of loss of seed viability was estimated to be 2.0, 1.3, 1.8 and 2.0 % per week for seeds stored with initial moisture content of 15, 20, 25 and 30%, respectively.

Figure 6.1. Effect of initial seed storage moisture content on seed viability of *O. lanceolata* at different time of assessment

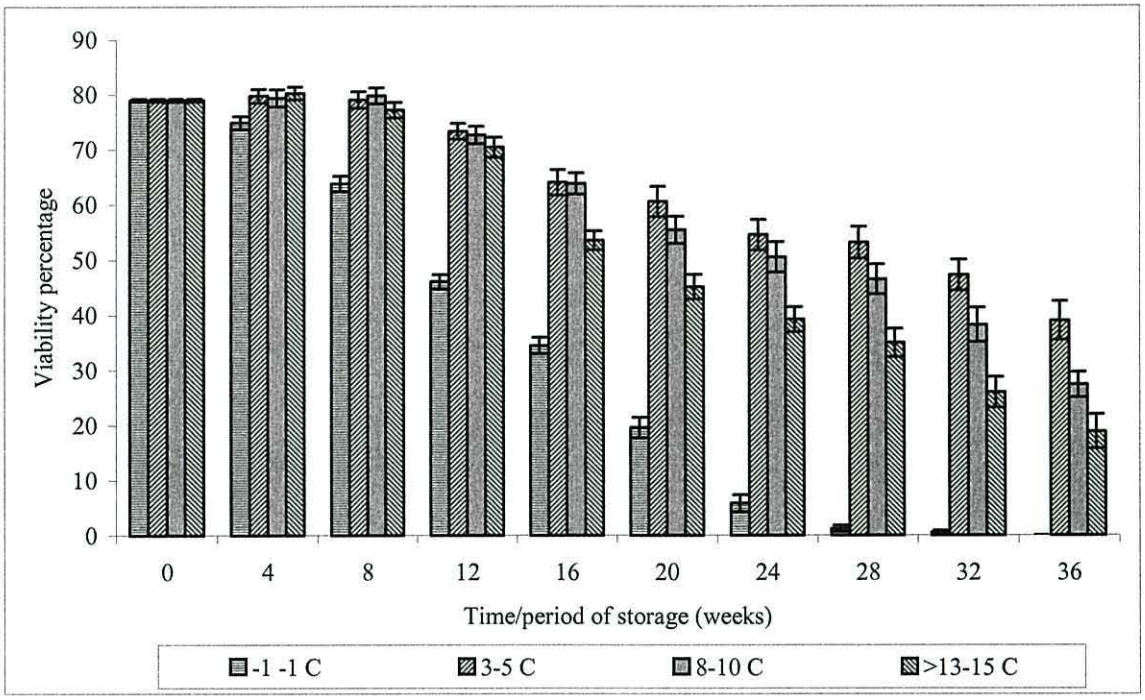


6.3.1.2 Effect of storage temperature on viability retention in stored seeds of *O. lanceolata*

Significant loss ($P < 0.01$) in seed viability due to difference in storage temperatures was observed within the first four weeks. In this week, seeds stored at -1-1 °C had significantly lower viability (74 ± 1.3%) as depicted in Figure 6.2. Viability in other treatments involving storage at 3-5, 8-10 and 13-15 °C, viability remained virtually the same for the first eight weeks.

At 12 weeks of storage, viability was significantly reduced in the three treatments compared to the original viability although no significance difference was evident among them. In seeds that were stored at -1-1 °C, the viability was reduced to 46.1±1.2% at 12 week. The drop continued abruptly until week 28, when almost no viable seed remained in the treatment. Significant differences among the other three temperature treatments (excluding -1-1 °C), began to be observed from 16th week (Figure 6.2). During this week, the three treatments differed significantly ($P< 0.01$) with more viable seeds being observed in seeds stored at 3-5 °C with a mean viability of 64.4 ± 2.3%. The least viability retention was observed in seeds stored at room temperature that had a mean viability of 53.0 ±1.7%.

Figure 6.2. Effect of storage temperature on seed viability of *O. lanceolata* at different times of assessment

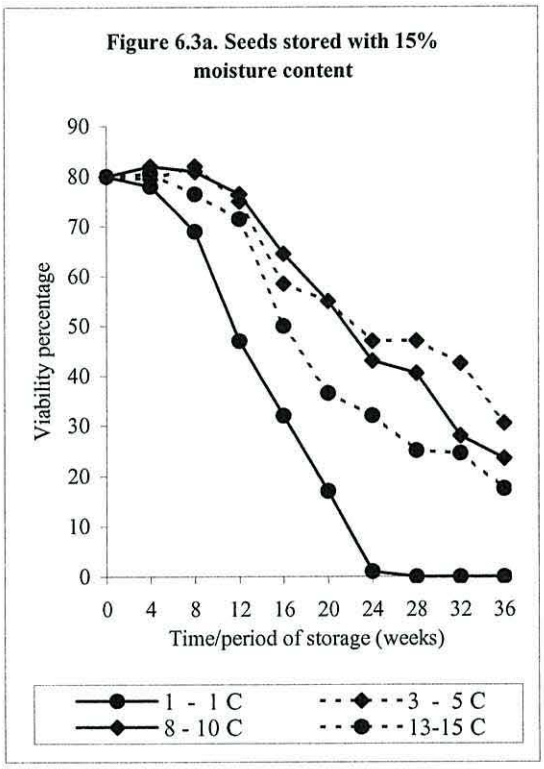


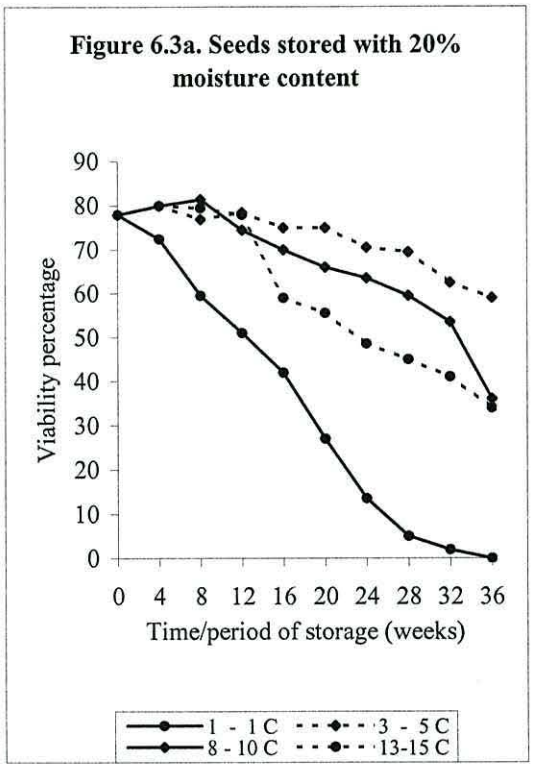
At the termination of the experiment (36 weeks), significantly ($P < 0.01$) high viability (39 ± 3.5%) was observed in seed stored at 3-5 °C while the least (0%) was in seeds stored at -1-1 °C. The rate of loss in seed viability brought by different temperature treatments was estimated to be 2.5, 1.2, 1.5 and 1.9 % per week for seeds stored at -1-1 °C, 3-5 °C, 8-10 °C and 13-15°C, respectively.

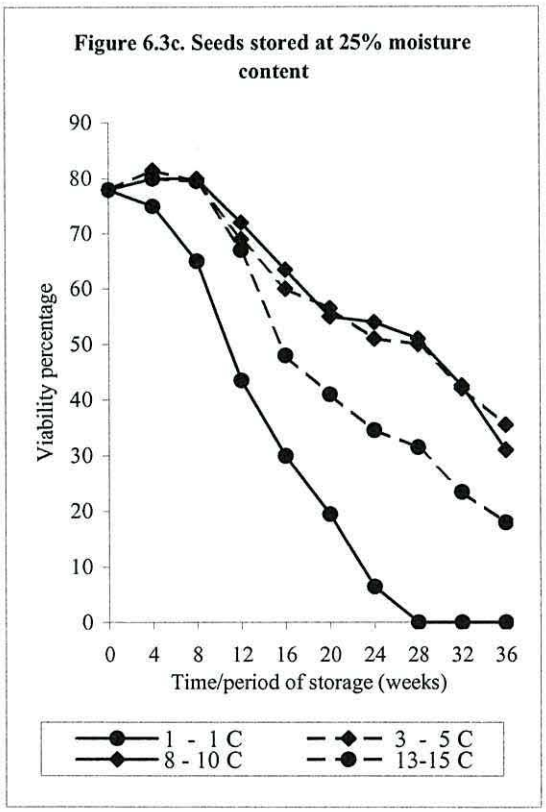
6.3.1.3 Synergistic effect of moisture content and temperature on retention of seed viability

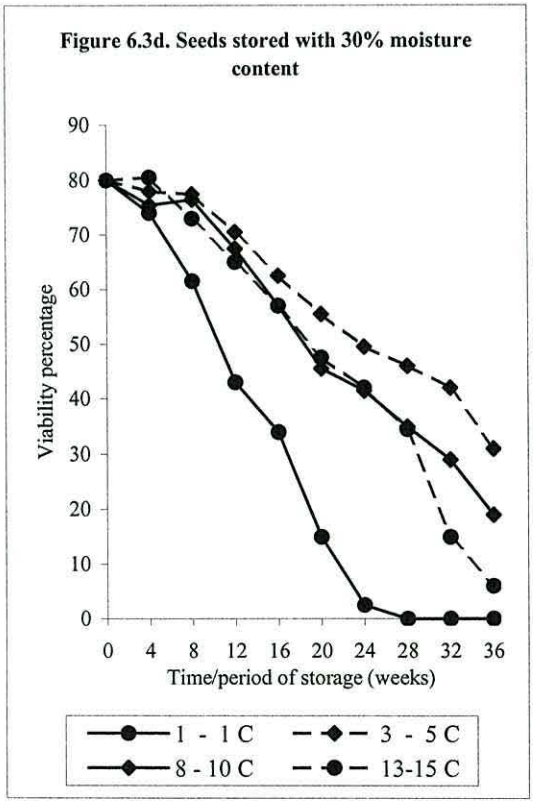
The synergistic (simultaneous) effects of moisture content at which seeds are stored with and temperature are presented in Figure 6.3a-d. Significant synergistic effect started to be observed from 28th week onward. At 28, 32 and 36 week, seeds dried to moisture content of 20% and stored at 3-5 °C had mean viability 69.5±2.2 ($P = 0.04$), 62.5±1.0 ($P = 0.04$) and 59±2.6% ($P < 0.01$) respectively higher than what was retained in any other temperature and moisture treatment synergists. The rate of loss of seeds at this condition was estimated to be 0.5% per week. The highest rate of seed viability loss was estimated to be 2.7% per week observed in seeds stored with initial moisture content of 15% and temperature of –1-1 °C.

Figure 6.3a-d. Effect of storage temperature on rate of loss of seed viability for seeds stored with particular initial moisture content









6.3.2. Pre-sowing treatments and their influence on seed germination and early growth of seedlings

6.4.2.1 Influence on seed germination and viability percentage attained

Germination of seeds in general was slow and sporadic (Figure 6.4). Significant differences among treatments ($P < 0.01$) were observed in their ability to improve germination percentage of seeds (Figure 6.5). Seeds subjected to complete removal of the seed coat had significantly higher germination percentage (66.5±3.8) compared with control seeds, seeds soaked in cold water and those treated with hot wire. The least germination was attained in seeds treated by hot wire with a mean germination 38±3.4%.

Viability test done at the termination of the experiment (102 days) revealed that, some seeds were still viable in some treatments (Figure 6.5) and the difference among treatments was significant ($P < 0.01$). Seeds in which complete removal of the seed coat was employed had the highest viability (66.5±3.8%). However this treatment differed only with seeds that were subjected to hot wire treatment that had the least viability of 38.5±3.3 %.

Figure 6.4 Germination trends of *O. lanceolata* seeds subjected under different pre-sowing treatments

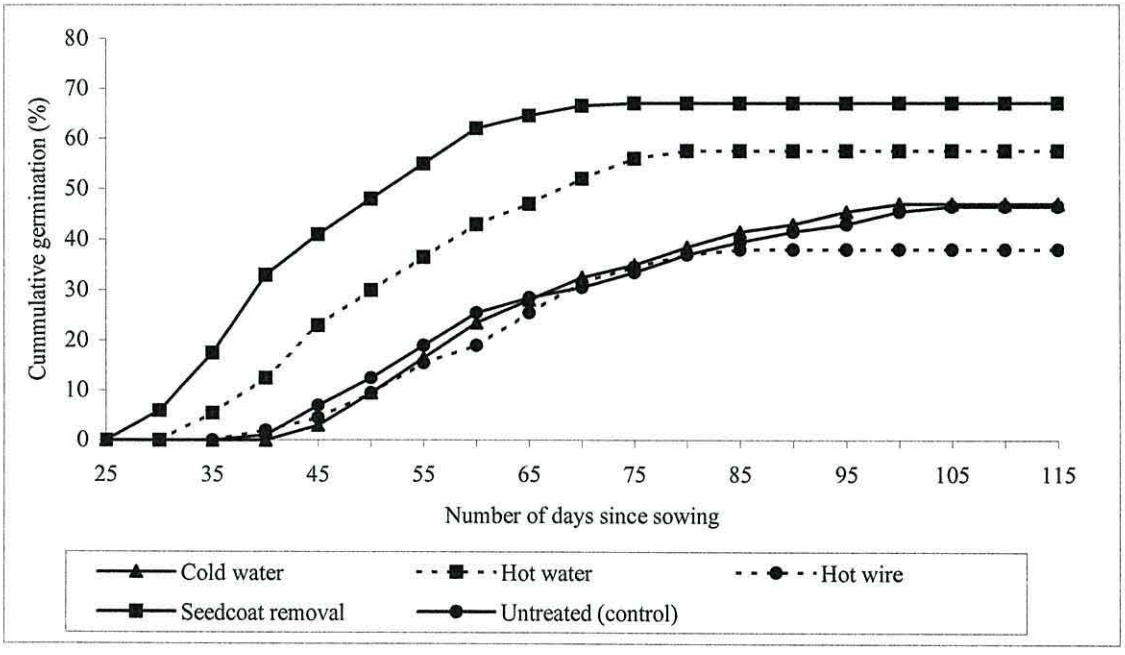
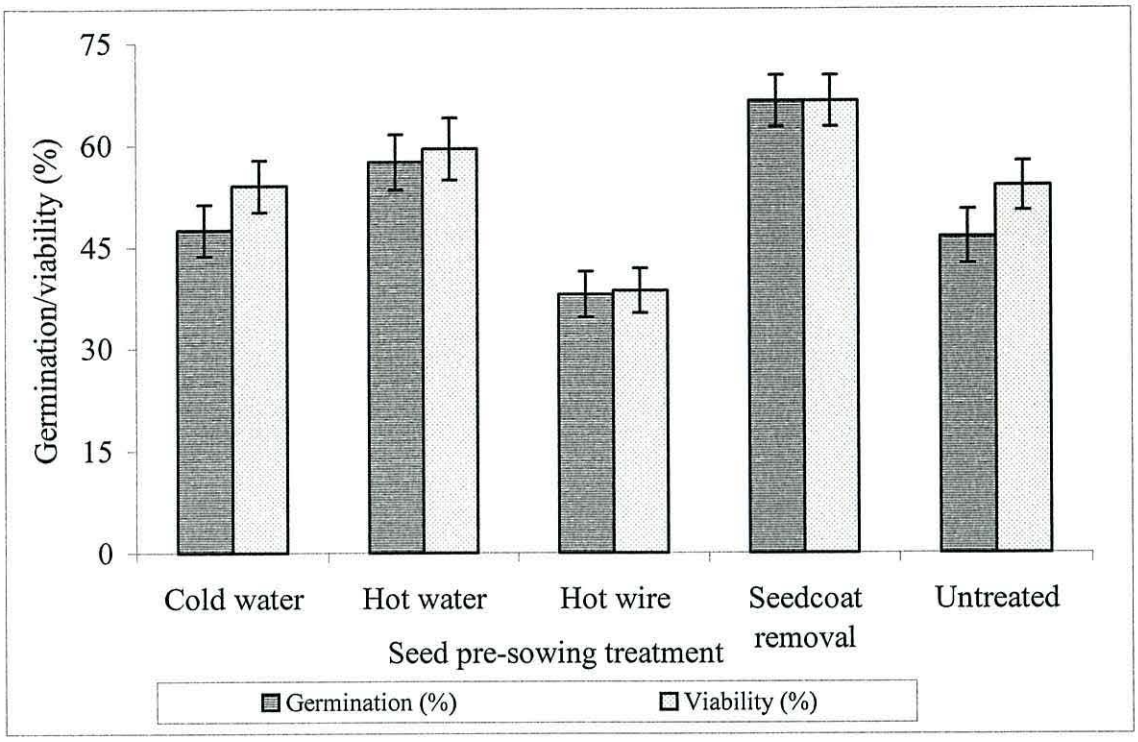


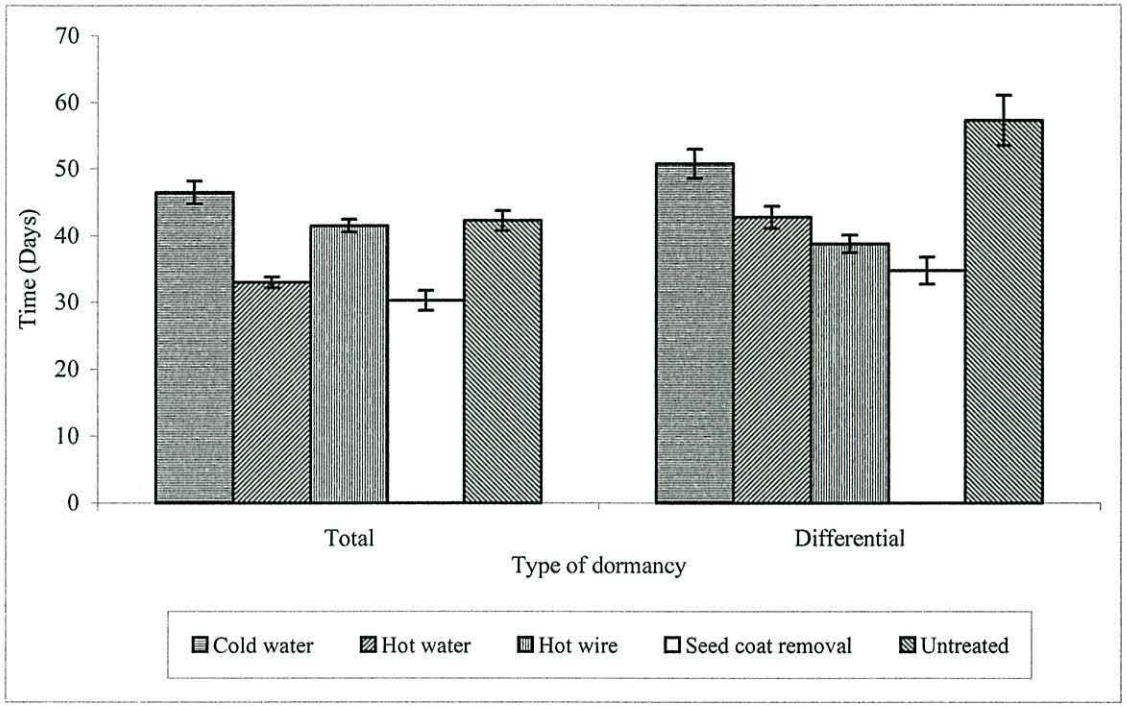
Figure 6.5. Effect of seed pre-sowing treatments on germination and viability percentage on seeds of *O. lanceolata*



6.3.2.2. Effect on dormancy period

The first seed germination was observed on the 27th day after sowing while the last germination was on the 104th day. The time at which germination commenced differed significantly among treatments (Figure 6.6). Seeds subjected to hot water treatment and those in which complete removal of the seed coat was employed had significantly shorter dormancy period (30.3±1.5 days) compared to untreated, cold soaking and hot wire treatments. Seeds soaked in cold water had the longest total dormancy period of 46.5±1.7 days. The interval between commencement and completion of germination also differed significantly among treatments ($P < 0.01$). Seeds subjected to complete removal of the seed coat and hot wire had significantly shorter interval than those untreated or soaked in cold water. The shortest interval was in seeds subjected to complete removal of the seed coat (34.8±2 days) while the longest interval was 57.3±3.8 days observed in untreated seeds that acted as a control.

Figure 6.6. Effect of pre-sowing treatments on total and complete dormancy in seeds of *O. lanceolata*



6.4.2.3 Effect on germination energy and value

Germination energy and germination value followed a similar trend as cumulative germination percentage. Significantly more germination energy ($P < 0.01$) and value ($P < 0.01$) were observed in seeds whose seed coats were completely removed with half of the total number of seeds that germinated (33.2%), germinating within 40.5±1.7 days. The mean germination value attained with this treatment was 2.98±3.8. The least germination energy was observed in seeds pre-treated by soaking in cold water that had half of its total number of seeds that germinated (23.2%), germinating in 62±1.9 days. The least germination value was in seeds pre-treated by nicking with hot wire, that had a mean value of 0.49±0.12.

6.3.2.4 Effect on seedling growth: height, diameter, root elongation and total biomass

Seedling growth aspect for height and diameter are presented in Figure 6.7 while root elongation and total plant biomass are presented in Figure 6.8. Growth of

seedlings varied according to the pre-sowing treatment applied. Significantly variation was observed in height growth ($P<0.01$). The best height was observed in seeds that were pre-treated by complete removal of seed coat (34.0±1.1 cm). Untreated seeds had the least height growth (23.1±0.8 cm).

Diameter growth also varied among treatments ($P < 0.01$). Better diameter growth was in seeds treated by soaking in hot water (5.4 ± 0.1 cm) while the least was in seedling in which seeds were soaking in cold water (4.6 ± 0.1). Significant variation ($P < 0.01$) was also observed in root length. Longer roots (33.0±1.3 cm) were observed in seeds that were treated by nicking with hot wire while untreated seeds had the shortest roots (27.5±1.2 cm).

Similarly, significant variation was observed in the seedling total biomass ($P < 0.01$). Seedlings whose seeds were treated by soaking in hot water had a total biomass of 5.18±0.19 g, significantly higher than what was attained in seedlings whose seeds were untreated or soaked in cold water. The least total biomass was in seedlings whose seeds were pre-treated by soaking in cold water (4.07±0.24 g).

Figure 6.7. Height and diameter growth of *Osyris lanceolata* seedlings from seeds subjected to different pre-sowing treatments

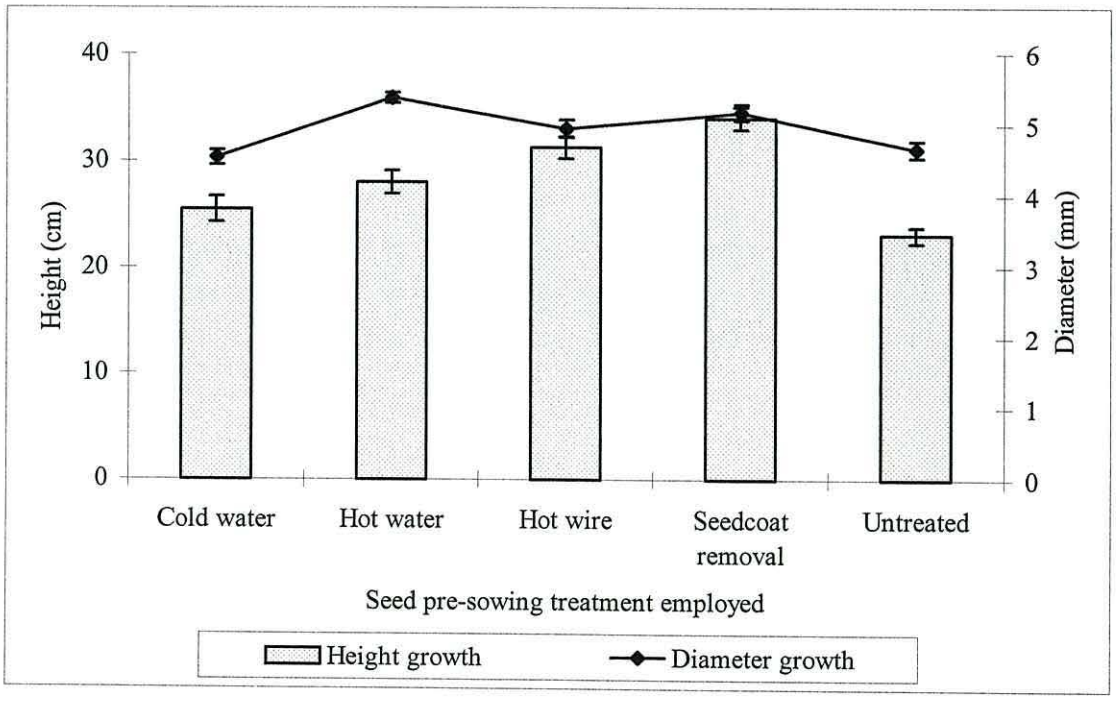
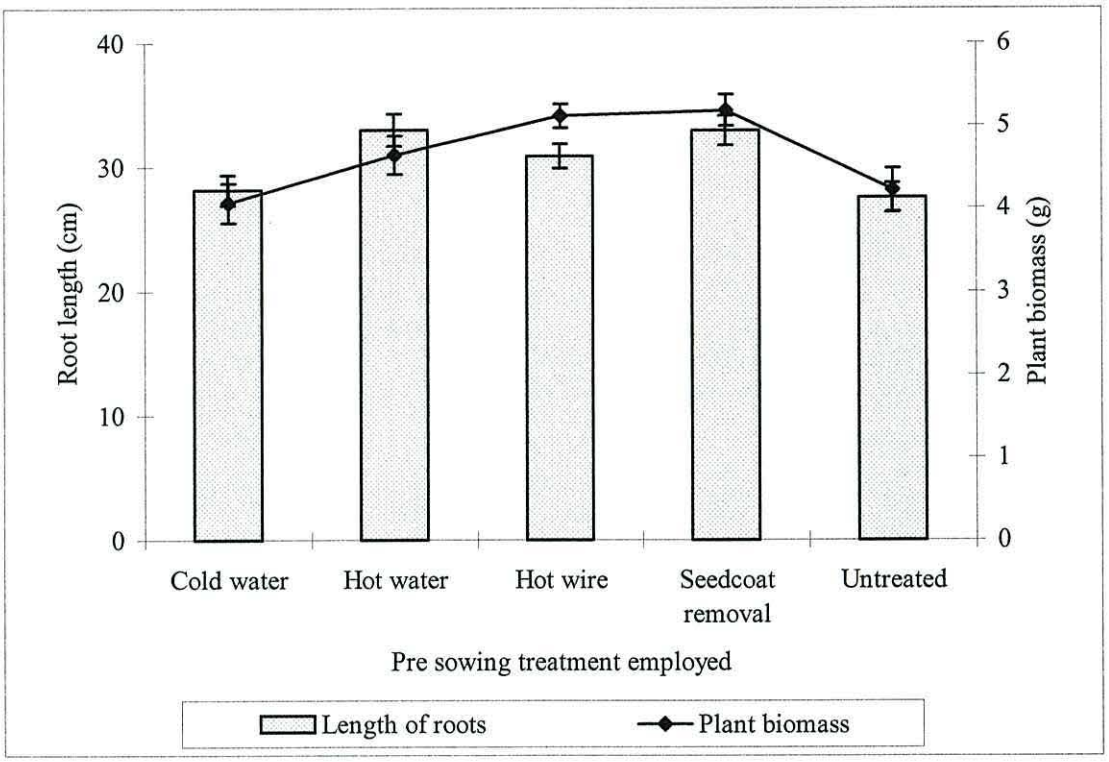


Figure 6.8. Root growth and biomass attained in *Osyris lanceolata* seedlings from seeds subjected to different pre-sowing treatments



In general seedling whose seeds were treated by hot wire nicking, soaking in hot water and complete removal of the seed coat had significantly the same height, diameter, root length and total biomass and differed significantly in most growth aspects with seedlings whose seeds were either soaked in cold water or sown untreated.

6.4 Discussion

6.4.1 Storage and retention of seed viability in *Osyris lanceolata*

Amount of water contained in seeds (moisture content) and the temperature at which seeds were stored influenced the retention of seed viability as reported in several other recalcitrant seeds (Roberts, 1972; Willan, 1985). In general the best viability of seeds was achieved in *Osyris lanceolata* seeds when seeds were stored at 3-5 °C with initial seed moisture content of 20%. While low storage temperatures are known to be

beneficial in storage of most seeds and can be used to offset the effect of high moisture content (Chin and Roberts, 1980; Farrant *et al.*, 1988), storing seeds of *O. lanceolata* below 1 °C was detrimental as a rapid decline in viability was observed. This is not surprising, as most recalcitrant seeds are well known to be sensitive to low temperature. However, the degree of sensitivity is quite variable among species (King and Roberts, 1979; Chin *et al.*, 1981) with some seeds such as those of *Shorea tarura* (Sasaki, 1976), *Uapaca kirkiana* (Ngurube and Mkandawire, 1997) being unable to tolerate temperatures below 4 °C. Others such as *Theobroma cacao* (Hor *et al.*, 1984), *Aporusa lindleyana* (Kumar *et al.*, 1996) and most dipterocarp species (Tompset, 1992) have been proved to be unable to withstand even temperature below 15 °C.

The massive death of seeds observed in *O. lanceolata* below 1 °C is likely to be a result of the chilling effect that is unfavourable for storage of most recalcitrant seeds. According to Roberts (1972), Bonner (1990) and Tompset (1992), the cause of death in recalcitrant seeds at low temperatures is due to the freezing of its content and this damage is most pronounced in seeds stored with moisture content of more than 14%. Also the intolerance to low temperatures could be related to geographical origin of seeds. It is understood that, most tropical seeds are killed at sub ambient temperatures (Sasaki, 1976) while seeds of temperate origin such as *Quercus* sp. can germinate at 2 °C after having stayed in cold storage for eight months (Chin, 1988). However, the exact reason as to why recalcitrant seeds are killed at sub-ambient temperatures is not clear. It is believed that, the mechanism operating in orthodox seeds could also be extended to explain the death of recalcitrant seeds (Chin, 1988).

According to Simon *et al.* (1976), low temperatures cause protein denaturation while Wolfe (1978) described the death of seeds due to low temperatures as a result of decline in fluidity of the membranal lipids which occur during chilling. This results into changes in membrane thickness and permeability, which in turn affect the membrane-bound enzymes. Boroughs and Hunters (1963) and Hor (1984) hypothesized some of the possible reasons for viability decline in recalcitrant seeds of cocoa with declining temperature to be due to the presence of some temperature-dependent, rate-limiting reactions. These are suppressed at low temperatures causing

lethal metabolic disruption and thus killing seeds. A number of physiological, biochemical and ultra-structural changes have been detected including three fold increase in leachate conductivity and lower leucine incorporation in the cell membrane systems. It is also hypothesized that low viability could be the result of liberation of some toxic material owing to the cold induced changes in the membrane permeability that interfere with the normal functioning of the seed's metabolic activities.

High temperatures, at or above 13 °C were also observed to rapidly reduce the viability retention of seeds in *O. lanceolata* especially when storage extends beyond 12 weeks. The effect of high temperature is well known in both recalcitrant and orthodox seeds and according to the rule of thumb of Harrington (1972), the lifespan of seeds is halved for every 5 °C rise in storage temperature within zero and 50 °C for most species. Similar observation is reported in short lived seeds of *Azadirachta indica* in India where storage of seeds at ambient temperature of 33.8 °C reduced viability to 8% in three months compared to 90% in freshly collected seeds (Ponnuswany *et al*, 1991). This loss of viability was not only related to high temperature but was also related to the rapid decline in moisture content from 30.8% to 15.5% which occurred, contributing to the desiccation injury. However, seeds of other recalcitrant species such as *Shorea ovalis* (Sasaki, 1980) and most dipterocarp (Tompset, 1992) have been proved to thrive well above 15 °C and store well at the respective temperatures of 21 °C and 15-21 C. This suggest that, storage temperature in recalcitrant seeds is quite variable. Many prefer temperatures below 15 °C but above freezing points, but some higher than this (Sasaki, 1980). In general, high temperatures are lethal in storage as they tend to increase respiration rate that spends seed's reserved food, which in turn shortens their lifespan (Justice and Bass, 1979; Hartmann and Kester, 1997). It also increases the activity of microorganisms, which in turn can destroy the stored seeds (Willan, 1985).

The effect of the amount of water in seeds during storage is similar to that of temperature and usually the two are so interdependent making difficulty to explain one within the other. Generally, the lower the moisture content, the longer the

maintenance of seed viability (Roberts, 1972; Justice and Bass, 1979; Chin, 1988; Gordon, 1992). Like many recalcitrant seeds *O. lanceolata* follows this rule within certain limits. However, drying and storing seeds with moisture content below 20% seemed to be detrimental, similar to drying and storing seeds with moisture content exceeding 25%. The effect is more pronounced in seeds that had to be stored for more than 12 weeks. This complies with what is reported in many recalcitrant seeds whereby drying seeds below certain critical moisture content (referred as the lowest safe moisture content) tends to kill seeds (Willan, 1986; Tompset, 1987, 1992). This critical moisture content is relatively high in recalcitrant seeds and usually is within the range of 12-30%. Yet it varies a lot among species (Roberts, 1973). In *Trichilia dregeana* (Choinski, 1990), *Aporusa lindleyana* (Kumar *et al.*, 1996) and *Aisandra butyracea* (Dhar *et al.*, 1999), the critical moisture content below which viability was observed to begin declining was 30, 30 and 25% respectively. In dipterocarp species (A*latus, Tuberculatus and Intricatus*), viability loss is reduced and longevity increased as seeds are dried from 20% -6% (Tompset, 1987). Similarly recalcitrant seeds of *Michelia compressa* can tolerate moisture contents below 10% (Lin and Wu, 1995).

It has been always difficult to explain why seeds are killed below a certain critical moisture content, but some possible hypothesis have been put forward to explain this. Seeds of many tropical plants possess a high concentration of phenolic compounds and oxidases built within the cells. Upon dehydration below certain limits, the cell membranes are damaged releasing these compounds. The released compounds are then oxidized and protein/phenol complexes are formed with subsequent loss of enzyme activity and ultimate death of seeds (Loomis and Battaille, 1966). Farrant *et al.* (1988) hypothesized that, recalcitrant seeds behave as imbibed orthodox seeds when they are first shed and normally start germination immediately. As usual, germinating seeds that are undergoing cell division and vacuolation are more sensitive to desiccation and any attempt to dry them below some limits leads to their death. The recent hypothesis of why recalcitrant seeds are intolerant to desiccation is that of Tompset (1992) who modified the hypothesis of King and Roberts (1979). It is suggested that a reduction in

moisture content below critical levels causes loss of membrane integrity and nuclear disintegration, consequently killing seeds. It further suggested that, there is a possibility of death of seeds occurring rapidly at or below critical moisture content or viability loss occurs at a rate, which is negatively correlated with moisture content over wide range of moisture contents.

The effect of storing recalcitrant seeds with high moisture content is generally similar to that of storing orthodox seeds. That is, deterioration increases as moisture content increases (Justice and Bass, 1979). The rapid fall in seed viability of *O. lanceolata* stored with moisture content of 25% or higher may be related directly to the higher respiration rate. Seeds with higher moisture are known to have high respiration rate which in turn deprives the stored food of seed causing rapid loss of viability (Willan, 1985). High respiration rates also release more energy and usually overheating may contribute to loss of viability unless proper aeration is permitted. As the packing material used is believed to have enough aeration to prevent overheating, this is unlikely to be the reason in viability loss observed in *O. lanceolata*.

The loss of viability in seeds stored with high moisture content could also be related to the storage temperature employed. Low temperatures tend to offset the effect of high moisture content in most orthodox seeds, though excessive moisture in recalcitrant seeds exposes seeds to the risk of freezing damage caused by ice formation (King and Roberts, 1979). While this could be a good reason for seeds stored with relatively high moisture content, it does not clearly tell as to what happened in seeds stored with low moisture content at low temperature of (-1) (-1) ^{o}C) in which seeds ended up with loss of viability. Possibly the desiccation effect at low moisture content was the most important factor rather than low temperatures. High moisture content is also related to increased possibility of pathogenic attack since microorganisms such as fungi and bacteria are encouraged (Willan, 1985). However having taken care of pathogenic attack especially fungi by treating the seeds in the present experiment with captan, microorganisms may not be the cause of loss seed in viability.

6.4.2 Effect of pre-sowing treatments on seed germination and early growth of seedlings

Complete removal of the seed coat and soaking seeds in hot water before seed sowing offered the best cumulative germination percentage, germination energy and germination value in *O. lanceolata.* They also shortened the dormancy periods. Complete or partial removal of the seed coat to enhance germination have been reported to be successfully in many species with hard seed coats such as *Vangueria infausta* (Msanga and Kalaghe, 1992), *Robinia pseudo-acacia* (Sadhu and Kaul, 1989), *Trichilia emetica* (Maghembe and Msanga, 1988) and *Tetrapleura tetraptera* (Onywekwelu, 1990). Likewise, the use of hot water has improved germination in many leguminous species such as *Acacia polyacantha*, *Sesbania rostrata* (Asenga and Otysina, 1996) and many other hard seeded coat species (Redhead and Hall, 1992; Adjers and Srivastava, 1993). The improvement observed in these species is usually related to improved permeability of the seeds to moisture and air and/or overcoming the mechanical resistance imposed by seed coat to the embryo growth (Nikolaeva, 1977; Nwoboshi, 1982; Bewley and Black, 1978, 1994; Meyer and Poljakoff-Mayber, 1989). These phenomena are likely to have operated in *O. lanceolata* seeds.

Both physical and mechanical dormancies seem to operate together as observed in *O. lanceolata* as evidenced by the seeds where hot wire treatment was employed to improve permeability but ended up with poor germination as in untreated seeds. Although the treatments might have improved permeability, the resistance of the seed coat to rupture did not allow germination to take place. This is was further evidenced by the fact that, no seed was observed to be alive in this treatment at the termination of the experiment, showing that, seeds died even after favourable conditions of air and moisture were supplied. Msanga and Kalaghe (1992) reported similar observations in *Vangueria infausta* seeds, where partial removal of the seed coat improved permeability but was insufficient to overcome the mechanical dormancy exercised by the seeds. This necessitated its complete removal, which ultimately improved germination.

The poor performance of cold water soaking and untreated seeds is related directly to the failures of the treatments to improve permeability and/or removal the mechanical resistance imposed by the seed coat. These treatments were insufficient to soften the seed coat to allow entry of gas and water at a reasonable rate as evidenced by the presence of some viable seeds at the termination of the experiment that failed to germinate. The death of most seeds also suggests that, there was some access of water and moisture, although at a slow rate, that caused initiation of germination. But due to the presence of the hard seed coat surrounding the seeds, the embryo failed to rupture and germinate leading to the death of most seeds.

Considering the growth of seedlings after germination, the best results were achieved in seeds treated by complete removal of the seed coat in terms of height, diameter growth and overall biomass. The reason is that, seeds in this treatment started to germinate almost 10 days earlier than in other treatments. However, the observed difference in height growth between seedling from seeds soaked in hot water and those from in which the seed coats were completely removed that started germinating almost at the same time is hard to explain. It is likely that soaking seeds in hot water leached out some chemical compounds that are essential for accelerating growth in plants. However, little can be said since there is no strong evidence reporting this to be occurring in seeds of any species.

CHAPTER VII

VEGETATIVE PROPAGATION OF *OSYRIS LANCEOLATA*

7.1. Background information and objectives of the present study

7.1.1. Introduction

Due to the difficulties in seed germination and problems associated with seed storage reported in *Oysris lanceolata* brought by the respective dormancy and recalcitrant character (Msanga, 1998; Mbuya *et al.*, 1994), the use of vegetative means was considered and evaluated as an alterative to supplement or substitute the use of seeds. In addition vegetative propagation offers an opportunity to improve *Osyris lanceolata* through selection as recommended in many species that are considered to face threat of loosing some genotypes of interest (Teklehaimanot *et al.*, 1996). To the moment, no work has been carried out in Tanzania or elsewhere to explore the potential of using vegetative means in propagating *O. lanceolata.*

Parts of plants such as stems, roots, bulbs, leaves and tissues have been used for many years in multiplying plant species (Leakey, 1981, Hartmann and Kester, 1997; Baker, 1992; Kijkar 1992a; Kantari, 1993a). The techniques employed include the use of cuttings, grafting, budding, air layering and specialized stems and roots (Longman, 1981; Leakey, 1985; Lamb *et al.*, 1998). The method to be used depends on the response of the plant as plants respond differently to the method used. While some plants can be propagated better by one method, others can be propagated using several methods. For those with more than one method of propagation, the economy of the method and the usefulness or value of the plant produced is considered in the choice (Hartmann and Kester, 1997). Many advantages of vegetative propagating exist. Some of the most important ones include:

- maintenance of clones as it involves mitotic cell division that duplicates the genotype thus perpetuating the desired character of any single plant;
- multiplication of plants that produce seeds which are difficult to germinate but can be propagated more economically by vegetative parts;

- propagation of plants that produce seedless fruits or non-viable seeds such as is the case of some orange varieties and figs;
- avoidance of long juvenile period which may tie up the nursery space unnecessarily (Krishnamoorthy, 1981; Hartmann and Kester, 1997; Kantari, 1993b).

In tree breeding, vegetative propagation takes the advantage of rapid genetic gain to develop uniform stands with predictable and desirable attributes of growth and form (Fielding, 1963; Redhead and Hall, 1992). Other advantages include production of uniform stock plants which are desirable in some characters such as resistance to drought, cold, parasites and diseases; control of growth form, combination of clones in the same plant by budding or grafting (Longman, 1976; Hartmann and Kester, 1997).

7.1.2. Vegetative propagation through stem cuttings

In comparison with other vegetative propagation techniques such as budding, grafting, and tissue culture, which are rather difficult and require special technical knowledge, the use of cuttings and possibly marcoting/air layering are the simplest, easiest and cheapest methods (Krishnamoorthy, 1981; Kantari, 1993a). Cuttings are made from vegetative portions of the plants such as stems, modified stems (rhizomes, tubers, corms and bulbs), leaves or roots (Lamb *et al.*, 1998). In forestry the most widely used type of cutting is the stem. These are easy to prepare and they are not readily perishable, making them easier to transport over a relatively longer distances. In addition, they require little or no special equipment during rooting (Kantari, 1993a; Hartmann and Kester, 1997).

However, to be successful in the use of cuttings, formation of roots is the critical factor as the shoot is already present. The capacity of stem cuttings to form roots is assessed by the percentage of cuttings that initiate roots, the number of roots per rooted cutting and the speed with which the roots emerge and grow. However, rooting ability varies among species. The genetic component of this variability may sometimes be attributed to lack of endogenous auxins, phenolics or other rooting co-factors. The variability may also be attributed to lack of enzymes or their activators for synthesis of auxin-phenol complexes and the presence of inhibitors or the presence of enzymes that oxidize or degrade auxins or their co-factors (Leakey, 1985).

Within a plant species, several factors have been observed to be important for successful root formation. The most important ones include: juvenility factor or age of the stock, state of cutting material to be used (physiology of the donor), position of the stem from which the cuttings are made, time in a year at which cuttings are collected, treatment of cuttings and control of environmental conditions of moisture/humidity, light, temperature and the type of rooting media (Krishnamoorthy, 1981; Leakey, 1983; Hartmann and Kester, 1997; Kijkar, 1992b; Kantari, 1993a; Longman, 1993).

7.1.2.1. Juvenility factor (age of the stock) in vegetation propagation

Cuttings taken from the plants in the juvenile development phase often form new roots easier than those taken from the plants in the adult phase (Libby *et al.*, 1972; Libby and Hood, 1976; Hartmann and Kester, 1997; Kantari, 1993a). This has been observed to be the case in many species such as *Dalbergia sissoo* (Gupta *et al.*, 1993), hybrid *Acacia* (Kijkar (1992a), *Acacia mangium* (Darus 1990), and *Parkia biglobosa* (Teklehaimanot *et al.*, 1996, 2000).

The decline in percentage rooting in cuttings as plants become older is postulated to be associated with the presence of anatomical features such as sclerenchyma that obstruct adventitious root formation. These are always present in older stock as observed in *Acacia mangium* by Darus (1990). Also it is postulated that, the reduced rooting potential as a plant ages may be a result of lowering phenolic levels which are believed to act as auxin co-factors or synergists in root initiation (Girouard, 1967; Darus, 1993). Hess (1965) added that in some species, the difficulty in rooting is not only due to age of the stock but also due to natural presence of some inhibitors or absence of some rooting co-factors. For this reason, several techniques have been developed to assure the availability of juvenile shoots so as to make possible vegetative propagation even for the hard to root species. These include felling the donor tree to allow young shoot to come out, hedging the trees or severe pruning (Libby *et al.*, 1972; Kijkar, 1992b; Kantari, 1993a).

7.1.2.2. State of cutting material to be used (physiology of the donor)

Physiologically, the cutting material to be used should have a high content of carbohydrate, as this might be critical in hard to root species (Haissig, 1984; Kantari, 1993a). The nutrition of the plant stock exerts a strong influence on the development of roots and shoots from the cutting (Hartmann and Kester, 1997; Kijkar 1992b; Longman, 1993). Initial carbohydrate content provides the cutting with adequate energy reserves for optimal rooting under conditions of limited photosynthesis. Low rooting capacity of the cuttings due to limited food reserve is to some extent influenced via absence or reduced root respiration (Haissig, 1984). This has been observed to be the case in most *Citrus, Dipterocarpus*, and *Hibiscus* species (Jouhari and Rahman, 1959; Stoltz and Hess, 1966). In some species it has been observed that leaving a few leaves on the cutting (1- 2) enhances rooting due to continued production of food through photosynthesis, while total removal lowers the success. Failure to root is associated with rapid depletion of carbohydrate reserve through respiration with no extra production (Geary and Harding, 1984; Darus 1993).

However, the amount of leaves to be left need to be considered as excessive leaves may lead to their death following excessive transpiration before roots are formed to support it (Aminah 1992; Longman, 1993; Darus 1993).

7.1.2.3 Position of the plant part from which cuttings are made

Cuttings taken from young branches on a tree tend to root better than those from old branches (Dunberg, 1977). The reason for this is the fact that young branches of the plant are more juvenile compared with old one that are normally preparing for flower development (Kleinschmit and Schmidt, 1977; Alden *et al.*, 1977). On the other hand, within the same shoot, cuttings taken from the basal position of the shoot root better than those taken from the terminal position in many plants (Srivastava and Mangil, 1981; Singh *et al.* 1984; Mwang'ingo, 1997). However, this is not to be generalized as some species such as *Shorea macrophylla,* rooting has been found to be better with cuttings from the middle position (Lo, 1985). In *Azadirachta spp* (Kijkar, 1992b) and

Carallia brachiata (Kumar *et al.*, 1993) only terminals have been found to give good rooting. The better performance of basal cuttings compared to other parts within a shoot is explained by Viartz (1979) and Orourke, 1940 cited by Hartmann and Kester (1997) as due to uneven distribution of inhibiting substances in different parts of shoots. Their concentrations generally decrease with the position away from the terminals where most of them are manufactured. Further the numbers of pre-formed root initials in some plants tend to decrease from the base to the tip of the shoot. Consequently the rooting of basal portions tends to be considerably higher than terminals.

Another reason for differential performance between basal and terminal cuttings could be due to differences in the nutritional status (Viartz, 1979; Haising, 1986). Cuttings with higher C/N ratio contain more food materials especially carbohydrates than those with lower C/N ratio. This ratio is generally lower at the top than at the base of a single stem cutting from which the cuttings are made. This also could explain why basal cuttings in some plants tend to perform better than terminals. On the other hand, terminal cuttings are observed to be better than basal portions in some species (Lo, 1985; Kumar *et al.* 1993). This is likely to be due to high concentration of endogenous root-promoting substances arising in the terminal buds. The closer the terminals are to the manufacturing industry, the better they are in concentration of the rooting-cofactors, making them root better than basal (Leakey and Mohammed, 1985; Hartmann and Kester, 1997). The better performance of terminal portions of the shoot could also be related to juvenility factor and lower level of lignification. Terminal cuttings are younger and possess cells, which are less differentiated and thus they possess more cells that are capable of becoming meristematic and form roots easily as they are not too specialized (Leakey, 1985; Lo, 1985; Kijkar, 1992b).

7.1.2.4 Time in a year at which rooting of cuttings is initiated

Time of year at which the cuttings are collected can also influence the success of stem cuttings in rooting especially in hard to root species (Nanda and Anand, 1970; Joshi *et al.*, 1992; Kantari, 1993a). Rooting of cuttings initiated prior to flowering or commencement of active growth usually performs better than those taken during these

periods. This is associated with the high levels of stored food at that time and/or low levels of flower stimulus antagonistic to rooting. Also it is associated with elimination of competition for materials necessary for both rooting and flower formation (Johnson, 1970 cited by Hartmann and Kester, 1997; Kantari, 1993a; Joshi *et al.,* 1992).

Nand and Anand (1970) and Cheffins and Howard (1982a) pointed out that the level of endogenous regulator substances and nutritional status of plants is high during some periods of the year. Thus cuttings harvested when these compounds are high tend to root easily compared to those collected at other times. It is generally recommended that cuttings of deciduous species should be collected in winters when plants are leafless while for the evergreen species, cuttings should be obtained at various times in relation to such flushes i.e. after flushes of new growth but before flowering commences. These are the periods associated with high levels of endogenous regulators and nutritional status of the parent stock (Cheffins and Howard, 1982b; Hartmann and Kester, 1997; Kantari, 1993a; Forestry Commission, 1995).

7.1.2.5 Handling and treatment of cuttings

Collection and handling of the cuttings and application of synthetic growth substances is another factor that can affect rooting in various ways. Collection of the cuttings is generally recommended to be done in the early mornings on cloudy days and be kept in cool moist place to maintain their turgidity (Loach, 1977; Kantari, 1993a). The rooting capacity of cuttings is frequently related to the water balance and usually dehydrated cuttings give poor rooting (Leakey, 1983). As such removal of excessive leaves, shoot tips and vegetative buds to discourage vegetative growth that will decrease the transpiration rate and promote root initiation may be of importance in most species. Furthermore, the free upper ends of the cuttings are preferably to be sealed with petroleum jelly or dipped in molten paraffin wax to discourage water loss from the cuttings by evaporation via the free cut surfaces (Krishnamoorthy, 1981; Aminah, 1992; Gupta *et al.*, 1993). However, how much leaves need be removed is questionable as in some species, maintaining some leaves is important as they play a major role in food manufacture and thus aiding in the overall rooting process (Leakey, 1985; Longman,

1993). Thus, an optimal balance between photosynthetic gains and transpiration rate need to be considered in estimating the amount of leaf to be retained. Longman (1993) suggests a total leaf area of about 50 cm^2 to be optimal to replenish the dwindling carbohydrate reserve while minimizing transpiration.

The use of root promoters such as Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA) and Indole Acetic Acid (IAA) have for a long time been proven to be effective in increasing the capacity of cuttings to produce adventitious roots (Okaro and Omokaro, 1975; Howard and Harrison-Murray, 1985; Leakey, 1985; Darus, 1993). However plants with high amount of auxins and rooting co-factors naturally, tend to root more easily without or with little application of axuins than those with low amounts (Farmer, 1966; Hansen *et al.*, 1978; Hineslay and Blazich, 1980; Hartmann and Kester, 1997). Many plants have been shown to respond well to the application of auxins such as IBA, NAA and IAA. Some of these include *Triplochiton scleroxylon* (Okoro and Omokaro, 1975; Leakey *at al.*, 1990), *Acacia* hybrid (*A. mangium x A. auriculiformis*) (Kijkar, 1992a), *Perrila frutescens* (Badola, 1993), *Woodfordia floribunda*, *Coriaria napalensis* and *Debregeasia hypoleuca* (Chauhan *et al.*, 1994), *Parkia biglobosa* (Teklehaimanot *et al.*, 1996, 2000).

In general, hormones hasten the initiation of roots, the number of roots formed on the cuttings, length of the roots and the ability of the cuttings to be rooted over a wide period of time (Krishnamoorthy, 1981; Hartmann and Kester, 1997). However, the amount of hormone to be applied and the effectiveness expected varies depending on how easy or difficult the species is to rooting (Hartmann and Kester, 1997; Kijkar, 1992b; Joshi *et al.,* 1992). Also it depends on the season of application as auxins commonly interact with the season of treatment (Howard and Harrison-Murray, 1985; Leakey, 1985).

The mechanism on how auxins and rooting co-factors operate is explained in various forms. They might be initiating morphological changes on the tissues responsible for root formation at the base of the cuttings or they may be increasing transportation of photosynthates and other products such as endogenous growth regulators from the

leaves to tissues involved in root formation (Howard and Harrison-Murray, 1985; Darus, 1993). Auxins enhance starch hydrolysis (Haissig, 1974) and several enzymes have been identified to increase in activity during primordium development, suggesting the involvement of both embden-meyerhof-parnas pathway of glycolysis and the pentose phosphate pathway in root initiation (Haissig, 1982).

7.1.2.6 Control of environmental conditions during rooting of cuttings

Control of environmental conditions and rooting medium are important in the success of rooting, otherwise much variability in rooting and in some cases, no rooting will take place (Darus, 1993; Kijkar, 1992b; Kantari, 1993a). The most important environmental conditions to be taken into account include water relation, temperature, light and rooting medium. Low water potential of the cutting is always associated with reduced rooting capacity and rooting has been shown to suffer whenever leaf water potential falls to about –0.8 to –1.0 MPa. Below these points, there is a linear relationship between declining leaf water potential and decreased rooting (Loach, 1977). As such, maintenance of large number of leaves in a cutting may encourage excessive water loss from the cutting before root formation takes place. This could result into death of the cuttings. If leaves are to be maintained, then the vapour pressure of water around the leaves need to be maintained nearly to that of intercellular spaces within the leaves (Aminah, 1992; Darus 1993). To assist this, mist spray chambers have been in use in many places to obtain optimal conditions. Maintenance of a relative humidity above 70 % is in most cases optimal for many species (Lepisto, 1977; Kijkar, 1992a, 1992b; Darus 1993; Kantari, 1993b). However, where advanced mist facilities are lacking, non-mist propagators have been in use accompanied by frequent watering and proper shading (Leakey *et al.*, 1990; Longman 1993; Lamb *et al.*, 1998).

Daytime air temperatures of about 21 to 27 °C with night temperatures of about 15°C are suggested to be satisfactory for rooting cuttings of most species. However, some species root better at lower temperatures (Hartmann and Kester, 1997, Kijkar, 1992b). Temperature influences cambial activity and could therefore be expected to be important in propagation especially during winter. However, excessive higher temperatures need to be avoided as they may promote increased respiration, which will

deprive rapidly the little food reserve available to the cuttings (Leakey, 1985). Also higher temperatures favor development of buds in advance of roots thus increasing water loss from the cuttings (Hartmann and Kester, 1997).

Evidence from rooting studies shows that, light influences formation of adventitious roots in stem cuttings of many plants (Elliason, 1971; Hartmann and Kester, 1997). Cuttings from most conifers and deciduous trees of temperate and tropical origin such as *Pinus sylvestris* (Hansen *et al.*, 1978), *Populus and Salix* (Eliasson and Brunes, 1980) and *Triplochiton scleroxylon* (Leakey, 1985) root more readily when the stock plant is kept in light levels well below the photosynthetic saturation point.

The mechanism by which light intensity influences rooting are not well known. However, studies on the effect of light on inhibitor content of root suckers by Elliason (1971) suggest that, levels of certain natural growth inhibitors are higher in plant tissues grown in the high light intensity than in etiolated environment. Leakey (1985) had similar observation in *Triplochiton scleroxylon* and related the high rooting ability of cuttings from stock plants grown at low irradiance with the low content of starch that is believed to inhibit rooting. Even rooting of stem cuttings where the bases are in the dark within a rooting medium stimulates rooting due to etiolating effect. Due to this fact, light intensity in an attempt to root stem cuttings of most species had been controlled in various ways. Among these is the use of black shade nets or nylon materials (Kantari, 1993b; Kijkar, 1992a; Longman, 1993). Longman (1993) recommends the light intensity of about 15-25 % of full light to be enough for non-mist propagators while for mist propagators 30-50% is considered to be appropriate.

The type of rooting media is also of considerable importance. Rooting medium needs to accomplish three main functions i.e. hold the cuttings in place during the rooting period; provide moisture to the cuttings and to permit penetration of air to the bases of cuttings. Thus a medium needs to be light in weight, loose structured, hold water well, easily available and inexpensive. Several rooting mediums have been in use including coconut husks, sawdust, river sand, vermiculate and sphagnum and have shown reasonable success (Kijkar, 1992b; Darus, 1993).

7.1.3 Vegetative propagation though marcotting/layering

Similar to stem cuttings, marcotts/layers have been in use to multiply plants in some species. This technique, which is considered as a modification of rooting stem cuttings, adventitious roots are initiated on the stem while it is attached to the mother tree. The rooted marcots is later detached, transplanted and become a separate plant on its own (Hartmann and Kester, 1997). The technique has been important especially in hard to root clones and has been in use in production of horticultural important rootstock and other plants of economic value (Rom and Carlson, 1978). The technique is also valuable in enabling production of relatively small number of large sized plants of special cultiva in an outdoor environment with a minimum propagation facilities (Hartmann and Kester, 1997).

Like most of the vegetative means, layering is considered as one of the potential propagation method that could be used in improving the species by taping the superior genotype in a species with a diverse genetic base like what is observed in *Parkia biglosa* (Teklehaimanot *et al.*, 2000) and the reported observations in *Osyris lanceolata.*

7.1.3.1 Physiological base of layering and its success

The physiological basis of regeneration by layering is similar to that of cuttings. However, layering explores several advantages of which cannot be achieved by cuttings, making the overall technique more successful at small scale especially in hard to root species (Ruviv and Reuven, 1984). The continued attachment of the stem to the mother plant during rooting allows continuous supply of water, minerals, carbohydrate and hormones through the intact xylems and phloem. This flow is important especially in plants that take a long time to initiate roots. Such plants are likely to die if they had to be detached from the parent plant as in cuttings that subject the stem to excessive loss of water. As water flow is not interrupted, layering takes the advantage of the possibility of maintaining as more leaves than cuttings. Maintenance of leaves in marcotting keeps on the photosynthesis process of the stem on. The manufacture food increases the capacity of the layer to produce adventitious root (Haissig, 1984).

The other advantage taken by layers is the accumulation of photosynthates and hormones around the rooting zones enhanced through continued photosythesis and girdling process. As in stem cuttings, rooting ability of stem is improves by its nutrition status. Better nutrition accelerates rooting as the rooting ability is increased (Haissig, 1984; Longman, 1993). Application of auxins to the girdled part of the stem is generally effective in enhancing rooting as with stem cutting propagation (Sparks and Chapman, 1970, Vieitez, 1974).

Exclusion of light through burying of layers in the soil or tying with a rooting medium create the etiolating effect which have been proved to useful in many species with rooting problems. The effect and mechanism in which reduced light favour rooting is similar to the one reported in stem cuttings. Reduced light is essential for rooting (Elliason, 1971; Howard *et al.*, 1985).

Another physiological condition that contributes to success with layering is the reinvigoration and possibly rejuvenation of the layered shoot that arise. Girdling stimulates formation of new shoots from the base of the plant next to the root system. Thus layering technique such as mount layering/stooling in which shoots are produced by severely pruning back stock plants takes the advantage of this stimulation to produce new plants (Howard *et al.,* 1985).

As observed in cuttings, the season at which layers are initiated influences the overall rooting with great success being attained by raising marcotts/layers in the season associated with high accumulation of carbohydrate and other substances essential in promoting root formation (Cheffins and Howard, 1982b; Forestry Commission, 1995; Hartman and Kester, 1997).

7.1.3.2 Techniques of propagation by layering

Several layering technique exists, but the most commonly used system to layer plants include: simple layering, which involves bending of an intact shoot to the ground and buried with soil to form adventitious roots (Rom and Carlson, 1987); compound/french layering that resemble simple layering but instead of producing one

shoot like in simple layering, several shoot are produced by laying the branch horizontally to the ground level (Hartman and Kester, 1997); Serpentine layering which is suitable for plants producing long flexible shoots such as vines. The technique involves covering and uncovering horizontal shoot at alternate points to produce roots at different internodes; Air layering involves girdling the stem or removal of a strip of bark and tying the debarked park by rooting medium to initiate roots. Other commonly used methods include mount layering/stooling (Duarte and Medina, 1977, Garner, 1988), trench layering and drop layering (McDonald, 1986). Of the layering techniques, mound layering for producing fruit under stock and air layering for some tropical fruits and trees are of most commercial importance at the moment (Hartman and Kester, 1997).

7.1.3.3. Air layering/marcotting in propagation

This technique that utilizes aerial part of the plant without necessarily bending the stem, involve girdling the stem or removal of strip of bark and then tying the exposed points with rooting media has proved to work in many plant species including pines (Mergen 1955; Barnes, 1974;) and *Parkia biglobosa* (Teklehaimanot *et al*, 2000). The width of the girdled part normally depend on the kind of the species. However, 2-3 cm girdle width is commonly used in many plants although the recommended width is around three to four times the diameter of the stem involved. Where necessary, the exposed part is scraped to ensure that phloem and cambium are removed completely to prevent pre-mature healing before roots are initiated (Broschat and Donselman, 1981). Once girdling has been done, the exposed points are enclosed with a medium which has the potential to hold moisture and is well aerated. Excessive moisture that is reported to be detrimental as marcotts can be likely to be subjected to rotting (Wyman, 1952) need to be avoided. Growing the marcotts in a reduced shade to about 50% is generally preferred to reduce water stresses (Broschat and Donselman, 1981).

As pointed out before, application of auxins enhances rooting in marcotts. The amount of auxins and how is to be applied vary depending on how easy or difficult the species is to initiate roots. The general recommend procedure in some species is

to soak the rooting medium into a dilute solution of hormone about 60 ppm) before tying to the marcotts (Wells, 1986). On the other hand application could be done by injecting a known concentration of hormone (2-5 ml) as reported in *Parkia biglobossa* (Teklehaimanot *et al.,* 2000).

Air layer are generally removed from the trees upon root initiation. Declaration of this stage is important as further success depends on it. Marcotts have to be removed when fibrous secondary roots appear. Earlier adventitious roots that are normally thick with little fibres have little ability to absorb water once a marcott is detached and planted and can subject it to death due to stress. In general removal of the marcotts have to be done when growth is not active (Hartmann and Kester, 1997). Reduction of aerial parts particularly leaves is important during transplanting as the marcott goes through acclimatization process. Within the first few weeks, marcotts should preferably be placed in the propagator where some control to maintain reasonable relative humidity can be done to avoid water stress (Teklehaimanot *et al.*, 2000). Once marcotts have acclimatized, further rearing can be continued until when they are considered ready for field planting.

7.1.4 Objectives of the present study

The present study was carried out to investigate the possibility of raising *O. lanceolata* through vegetative means to supplement and provide an alternative to the use of seeds. The success to this technique was also intended to serves as the fastest means in capturing the best genotypes remaining so far before they become extinct. Initial consideration was given to the use of stem cuttings and marcotts. These two techniques are known to be the easiest and cheapest methods compared to other vegetative means that need specialised technical expertise (Hartmann and Kester, 1997). The specific objectives were:

i. To assess the effect of seasons of collecting cuttings and initiation of marcotts on rooting and successive establishment of cuttings and marcotts
ii. To assess the effect of nodal position of cuttings on rooting, and
iii. To assess the effect of Indole-3-butyric acid (IBA) and Naphthalene Acetic acid as root promoters in promoting rooting of cuttings and marcots.

To fulfil these objectives the following research question were asked:

i. Can *Osyris lanceolata* be raised successfully through vegetative means using cuttings or layers? If so, to what extend are the two techniques useful ?
ii. Does seasonality have an influence in determining rooting and ultimately shooting success of cuttings and marcotts? If so, what is the best season for collecting cuttings and initiating marcotts.
iii. Does the source position on the stem cuttings important in influence the rooting success of the cuttings?
iv. Does rooting success increase with external application of auxins? If so, do the two auxins, IBA and NAA, differ in their ability to induce rooting? Which concentrations of them are more effective in promoting rooting?

7.3. Materials and methods

7.3.1. Vegetative propagation by stem cuttings

The study was carried in Tanzania between December 1999 and February 2001. Stem cuttings for vegetative propagation experiment were collected from mature and healthy trees at Sao Hill Forest Reserve and planted at Iringa Zonal Tree Seed Centre nursery, Tanzania. In order to maintain their humidity, cuttings were collected in the mornings, wrapped in white polyethylene sheeting material and put in damp hessaian sacks. Then they were transported to the experiment Iringa Zonal Tree Seed Centre where further processing and preparation for planting took place. After preparation, cuttings were soaked in rooting promoting hormones of IBA and NAA and the controls in distilled water for 12 hours before they were planted.

Three factors were investigated: season of cutting collection, nodal position of cutting (basal and terminal) and application of auxins (IBA and NAA). The seasons assessed were December, February, June and September. December is at the beginning of the rainy season when *O. lanceolata* starts producing new leaves. February is in the middle of the rainy season when flowering of *O. lanceolata* takes place. June is at the end of the rainy season, which is associated with the end of peak fruiting period.

September falls within the dry season, two months before the rain starts. Nodal position was assessed to determine the effect of part of the branch used in its effectiveness in promoting rooting of stem cuttings. Each of the stem cutting harvested, which was 30 cm long and 5-10 cm diameter, was sectioned into two equal parts (basal and terminal part), making sure that each part contains at least two nodes. All the leaves on the lower part (basal) were completely removed while the leaves on the upper part (terminal) were trimmed to 2-4 leaves, which formed an approximate leaf area of 10-20 cm^2. For the hormone treatment, both IBA and NAA were applied, each at three concentrations of 50, 100 and 150 ppm. The control set was treated with distilled water only.

The cuttings were initially raised in a non-mist propagator (Plate 7.1) for root initiation. The non-mist propagator used was a modification of non-mist propagator of Leakey *et al.* (1990) and Longman (1993). The four sides were constructed using earth bricks. The lid, which covered the top of this brick structure, was made of white translucent polyethylene sheet, which was fastened on all the four sides to a wooden frame. Underneath the polyethylene sheet, a black net was laid to reduce the light intensity to about 20% of full sunlight. The propagator was 15 m in length, 2 m width and 0.75 m height. Inside the propagator the floor area was divided into 1 m long and 0.9 m wide sections. These were filled with gravel and stones up to about 20 cm depth and the top 15 cm depth was filled with sieved river sand. To avoid excessive heat during daytime, the non-mist propagator was constructed under a shade of thatched roof structure.

Within the non-mist propagator, cuttings were raised in plastic containers measuring 15 cm long and 30 cm wide filled with rooting medium of sterilized and sieved river sand. The containers were perforated at the bottom to allow easy drainage of water. Watering of the cuttings was done every two days in the morning. For the rest of the time, mist spraying was applied to maintain the relative humidity inside the propagator as high as possible. This was done every day at 11.00 am, 2.00 pm and 11.00 pm and whenever the propagator was opened for inspection.

Plate7.1 Non-mist propagator used for root initiation in *O. lanceolata* stem cuttings

The experiment was a two-factor randomised design, with two nodal positions of cuttings (basal and terminal) and two types of hormones of IBA and NAA each at three levels of concentrations (50, 100 and 150 ppm). There were four replications of each treatment combination arranged in completely randomised design. Ten cuttings per treatment were used, making a total of 560 cuttings for the whole experiment in one season. The experiment was repeated four times to cover the four seasons of December, February, June and September.

During each season's experiment the cuttings were removed from the non-mist propagator after 4-6 weeks and assessed for rooting. The cuttings, which rooted and survived were transferred to larger polyethylene containers 30 cm long and 25 cm wide filled with a mixture of sand, forest soil and animal manure in the ratio of 2:1:1. Five grams of NPK was added to each container. The pots were then placed in the nursery and raised for three months, a period considered to be long enough for cuttings to be ready for planting out. At the end of the three months of stay in the

nursery, the cuttings were harvested and assessed. The data collected included: percentage survival (shooting); number of roots per cutting; length of roots per cutting; and the total root biomass per cutting based on oven dry weight at 80 °C.

7.2.2. Vegetative propagation by marcotting/air layering

The experiment was carried out at Sao Hill Forest and the rooted marcots were later transferred to the project's nursery at Sao Hill. Two factors were investigated in this experiment: season of initiating rooting of marcots and application of hormones. The seasons and hormones assessed were similar to the stem cutting experiment (December, February, June and September and IBA and NAA at the three concentrations of 50, 100 and 150 ppm, respectively).

24 mature trees of O*syris lanceolata* were randomly selected in the forest. On each tree 20 branches measuring 5-10 cm in diameter were randomly selected. On each branch, one newly produced shoot was randomly selected. On each shoot, a ring of bark of about 3 cm wide was removed to expose the cambium. The exposed cambium was surrounded with damp decomposed sawdust (mainly of pine) and wrapped in white translucent polyethylene sheeting tied on both sides with fine threads. Out of the 20 ring barked shoots on each tree, hormone was applied to half of them while the remaining half were controls which were injected with distilled water only. On each of the ten marcots treated with hormone, 5 ml of one of the rooting hormones at one concentration was injected. Plate 7.2 shows initiated marcots while attached to their mother trees.

The experiment was a completely randomized design. On a single tree, one type of hormone at one concentration and a control without hormone was applied, each to ten marcots. This gave a total of 20 marcotts on a single tree. Each treatment (one hormone at one concentration and a control) was then repeated four times on four trees, thus utilizing a total of 24 trees for all treatments during each season experiment.

Plate 7.2. Initiated marcotts still attached to their mother tree

Marcots were left to stay on the mother trees for a maximum of eight weeks to allow them to initiate roots. They were watered every two days in the morning and inspected for evidence of rooting. After eight weeks, all marcots were removed from the mother trees and assessed for rooting percentage. The rooted marcots were then potted in polyethylene tubes measuring 30 cm long and 25 cm wide filled with a mixture of sand, forest soil and animal manure in the ratio of 2:1:1. Five grams of NPK was added to each container. They were transferred to the Sao Hill project nursery where they were raised in a similar way as the stem cuttings for a further three months.

At the end of the three months of stay in the nursery, the marcots were harvested and assessed. The data collected included: percentage shooting and rooting; number of roots per shooted marcot; length of roots per shooted marcot; and the total root biomass per shooted marcot based on oven dry weight at 80 °C.

7.3. Results

7.3.1. Vegetative propagation by stem cuttings

7.3.1.1 Rooting in non-mist propagator and shooting in the nursery

Rooting of stem cuttings varied a lot among seasons, with some seasons giving no success at all. Successful rooting was achieved with cuttings raised in December, June and September while no single stem cutting raised in February rooted or survived. For this reason, February was excluded from the results. Figure 1a-c shows the effect of season, nodal position and auxin application of rooting and subsequent shooting success.

The number of stem cuttings that rooted under non-mist propagator varied significantly ($P < 0.01$) among the three seasons analysed (December, June and September). Cuttings raised in September gave the highest rooting percentage of 38.6 ± 1.9%. Nodal position of stem cuttings had no influence on rooting when considered alone, however, its interaction with season had a significant influence ($P = 0.008$). Basal cuttings of September had the best rooting success (40 ± 2.9%) and the least was achieved in basal cuttings of December that had a rooting success of 14.6 ± 2.1% (Figure 7.1a).

Types of auxin varied significantly in influencing the number of cuttings that rooted ($P = 0.008$) with IBA being superior to either NAA or the control. The various concentrations of auxins also significantly differed in their effectiveness ($P = 0.001$). There was an interactive effect between auxin concentration and season ($P = 0.001$). Application of 50 ppm IBA to stem cuttings collected and raised in September had the highest rooting success (55.0 ± 5.6%). The lowest rooting was in stem cuttings that were treated with 100 ppm IBA, collected and raised in December with a rooting success of 15.0± 4% (Fig 7.1b).

Figure 7.1a-c. Effect of season, nodal position and auxin on rooting and shooting success in stem cuttings of *Osyris lanceolata*

Figure 7.1a. Effect of position of stem cutting on rooting success under non-mist propagator

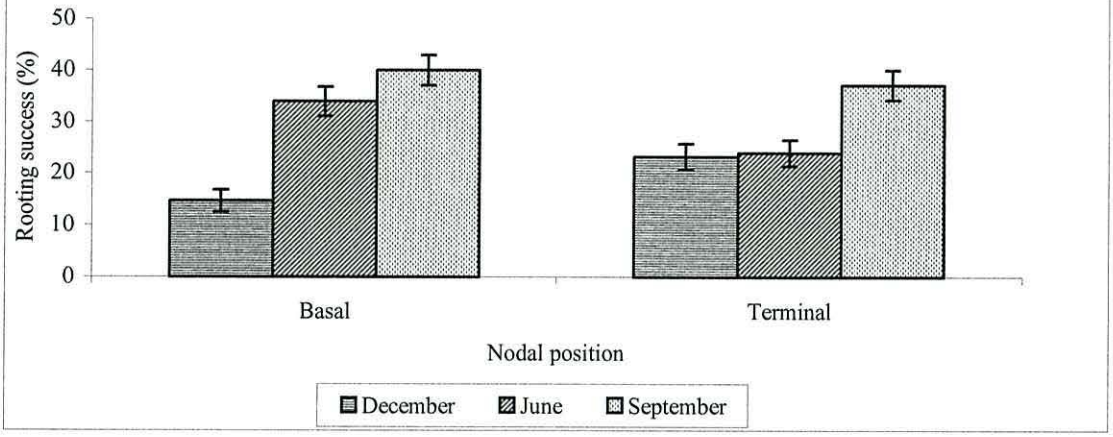


Figure 7.1b. Effect of varried concentrations of auxins on rooting success at different seasons of raising under non-mist propagator

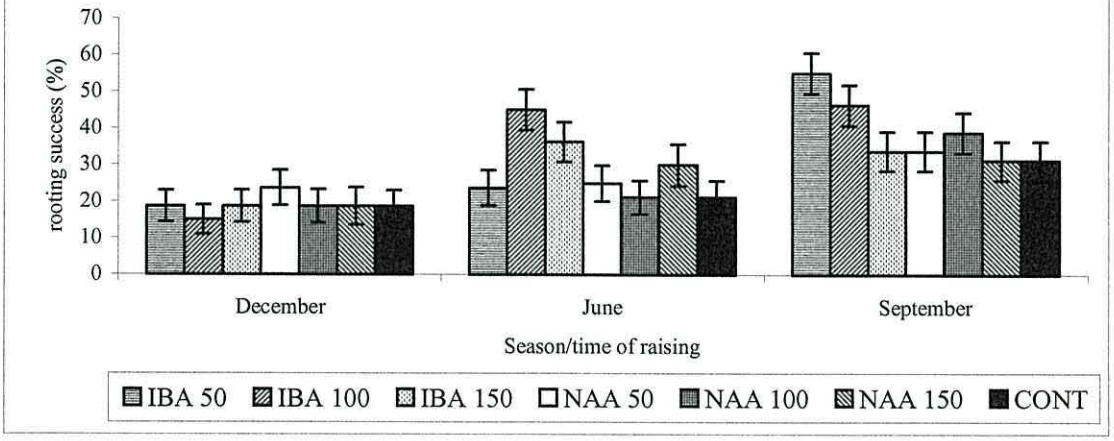
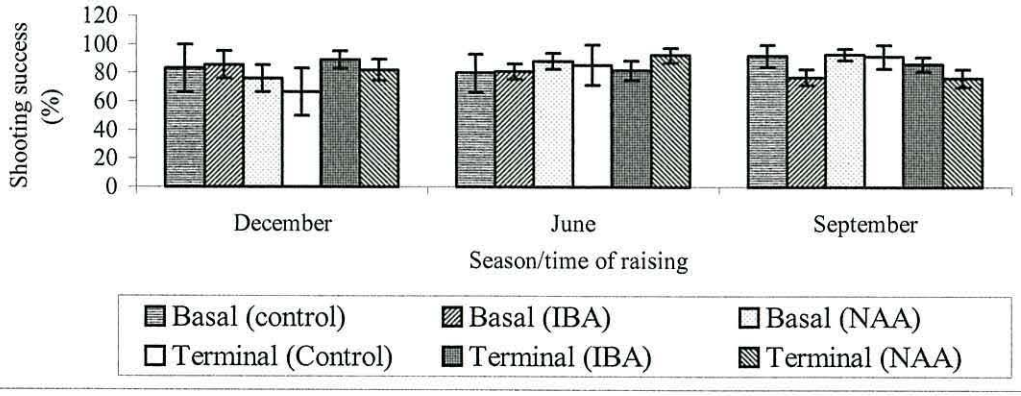


Figure 7.1c. Effect of position of stem cuttings and axuins type on shooting and establishment of the rooted cuttings at the Nursey



Within each season, nodal position had a significant influence on cuttings raised in December (P = 0.027) and June (P = 0.008), but had little effect on September raised cuttings (P = 0.432) as shown in Figure 1a. In December, terminal cuttings had better rooting (23.2 ± 2.5%) than basal cuttings (14.6 ± 2.1%) while in June, rooting was better with basal cuttings (33.9 ± 2.8%) compared to the terminals (23.9 ± 2.8%).

A significant difference was also observed among type of auxin for cuttings raised in June (P = 0.02) and September (P = 0.03), but not in December (P = 0.72). A significant difference also existed among auxins concentration in June (P< 0.01) and September (P = 0.02) cuttings. In June, stem cuttings treated with 100 ppm IBA had better rooting (45 ± 5.6%) compared with the control, 50 ppm IBA and 100 ppm NAA. The least rooting was observed in the control set that gave a mean rooting of 21.2 ± 4.6 %. During September, better rooting was attained with stem cuttings treated with 50 ppm IBA (55 ± 5.6%) compared to those treated with 150 ppm NAA (31.3 ± 5.2%). There was no significant difference between the rest of the auxin concentrations including the control, although control had the least rooting of only 32.5 ± 5.4% (Figure 7.1b). No interactive effect between nodal position and auxin concentration was observed for any season.

Shooting or survival of the stem cuttings in the nursery depended on what was achieved in the non-mist propagator. Most cuttings that rooted in the non-mist propagator continued to survive in the nursery and ultimately producing shoots as a sign of their successful establishment. Thus there was no significant difference between seasons, nodal positions and type of auxins in the percentage of rooted cutting that produced shoots in the nursery. Yet, the highest shooting attained was 93.1 ± 3.9% from basal cuttings raised in September and treated with NAA while the least was in terminal cuttings of December treated with the control that had a mean shooting of 66.7 ± 16.7% (Figure 1c).

However, considering shooting as a proportion of the total cuttings that were initially raised in the non-mist propagator, the outcome was similar to rooting i.e.

shooting differed among the seasons ($P < 0.01$) with September being the best although nodal position as well as the type and/or concentrations of auxins had no significant effect when considered alone. However, nodal position had an interactive effect with season ($P < 0.01$). Stem cuttings of basal origin raised in September had the best shooting (34.3 ±2.8) while the least was in basal cuttings of December that had shooting of only 11.8 ± 1.9%.

7.3.1.2. Number of roots produced per rooted cutting.

Figure 7.2a-c. Presents the number of roots produced per cutting. The number of roots produced per cutting varied significantly among seasons ($P < 0.01$). Cuttings raised in June had more roots (12.4 ± 0.2) compared to those raised in September (11.8 ± 0.2) but not significantly different to those of December (Figure 7.2a). Nodal position did not have significant effect ($P = 0.43$). Likewise, no significant difference was observed due to either types of auxin ($P = 0.803$) or auxin concentration ($P = 0.58$). However, a significant difference existed in the interaction between type of auxin and season ($P = 0.05$). Application of IBA in June produced more roots (12.4 ± 0.3) compared with the control treated cuttings that were raised in September that had a mean root number of 10.6 ± 0.5 (Figure 7.2b).

Within each season, nodal position, type of auxins and/or their varied concentrations had various influences on the number of roots produced only in September but not in December and June. In September, differences in number of roots were observed among the type of auxins ($P < 0.01$) with IBA having more roots than the control but similar to NAA. The various concentrations of auxins also differed significantly in their effect ($P < 0.01$) and had an interactive effect with nodal position ($P = 0.03$). Basal cuttings treated with 50 ppm IBA produced more roots (13.8±0.4) compared to basal cuttings treated with 100 ppm IBA (10.9 ± 0.5) and terminals treated with the control (9.4± 0.7) and 50 ppm NAA (10.9±0.8) (Fig. 7.2c).

Figure 7.2a-c. Effect of raising season, nodal position and root promoter (auxins) on number of roots produced in stem cuttings of *Osyris lanceolata*

Figure 7.2a. Seasonal effect

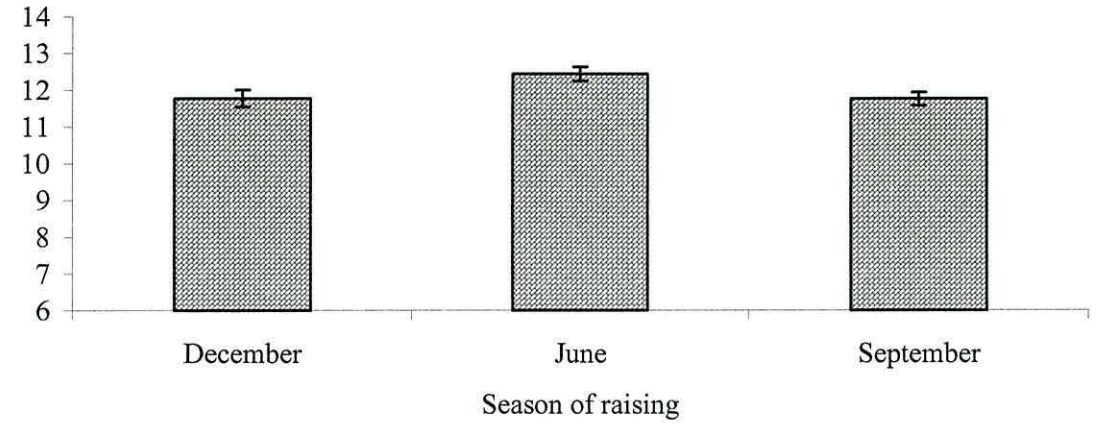


Figure 7.2b. Effect of Auxin type and seasonal of raising

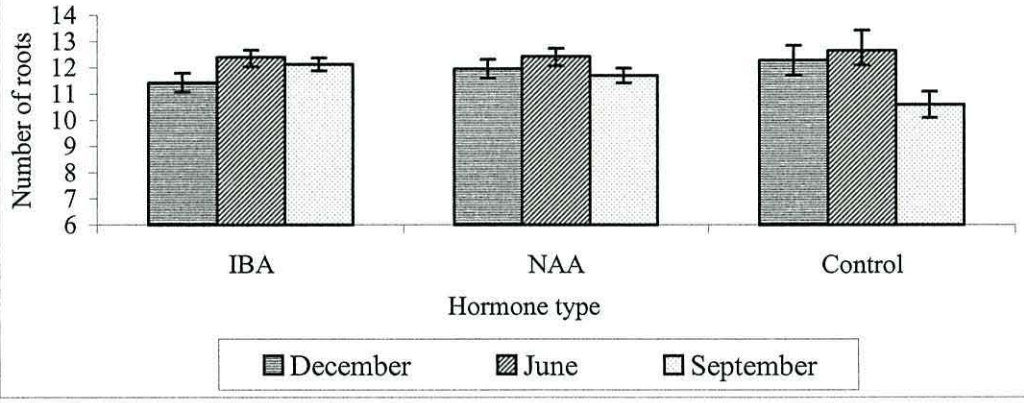
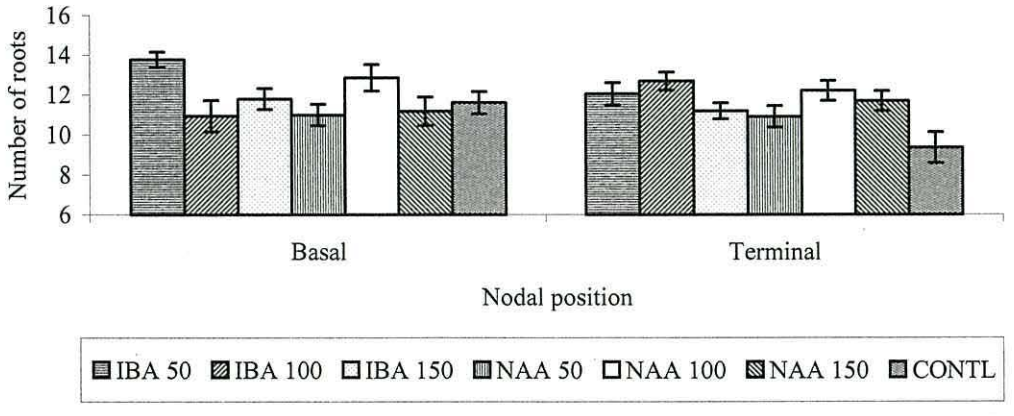


Figure 7.2c. Effect of varried concentrations of auxins and nodal position in September raised cuttings



7.3.1.3. Total root length produced per rooted cutting

The total and mean length of roots produced per cuttings is presented in figure 7.3a-c. Significant difference ($P < 0.01$) in the total root length produced existed among seasons with September cuttings producing longer roots than December. Nodal position had no effect when considered alone, but its interaction with season influenced the total length of roots produced ($P = 0.04$). Basal cuttings of September had longer roots (154.2 ± 3.6 cm) than cuttings of both basal and terminal positions of December and terminal cuttings of June. Shortest roots were produced in cuttings of basal origin raised in December (104.1± 3.6 cm) (Figure 7.3a).

The type of auxins did not have significant effect ($P = 0.08$), but their varied concentrations had influence on the total root length attained ($P < 0.01$). There also existed an interactive effect between the varied concentrations of auxins and seasons on root length ($P < 0.01$). Stem cuttings treated with 50 ppm IBA, collected and raised in September produced longer roots (181.5 ± 5.7 cm). Shorter roots were in stem cuttings treated with 150 ppm IBA during December (105.4 ± 3.5 cm) (Figure 7.3b).

Within each season, nodal position had a significant influence only in cuttings raised in December ($P = 0.01$) and September ($P = 0.05$). In December, total root length was high in terminal cuttings (114.5 ± 2.8 cm) while basal cuttings had only a mean total root length of 104 ± 3.6 cm. In September, longer roots were realized in basal cutting with a total root length of 154.2 ± 3.6 cm while terminal cuttings attained 147 ± 3.8 cm (Figure 7.3a).

The type of auxins influenced the total length of roots produced in cuttings raised in September ($P = 0.001$), but had no significant influence on cuttings raised in December and June. However the varied concentrations of the auxins had an influence on June ($P = 0.001$) and September ($P = 0.001$) cuttings (Figure 7.3b).

Figure 7.3a-c. Effect of raising seasons, position of stem cutting and root promoter (auxins) on roots elongation in stem cuttings of *Osyris lanceolata*

Figure 7.3a. Effect of position of stem and season of raising

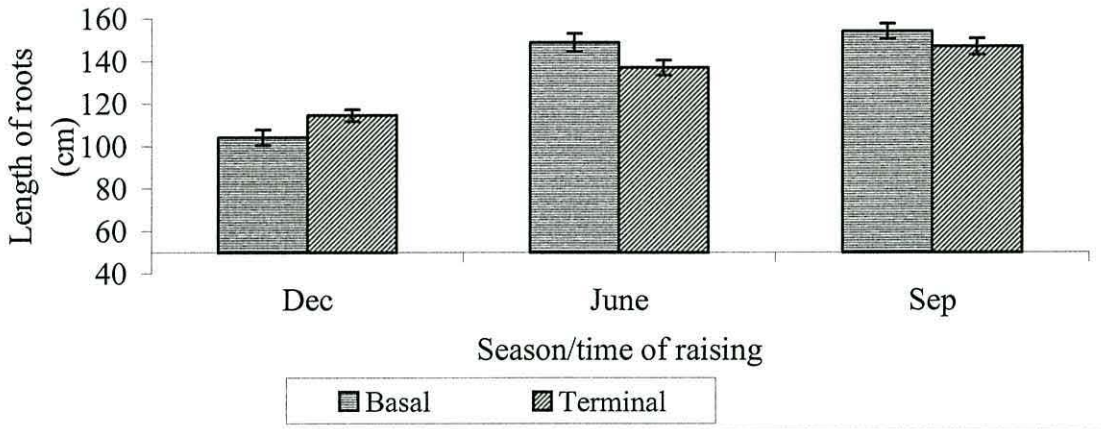


Figure 7.3b. Effect of varried concentrations of auxins and season

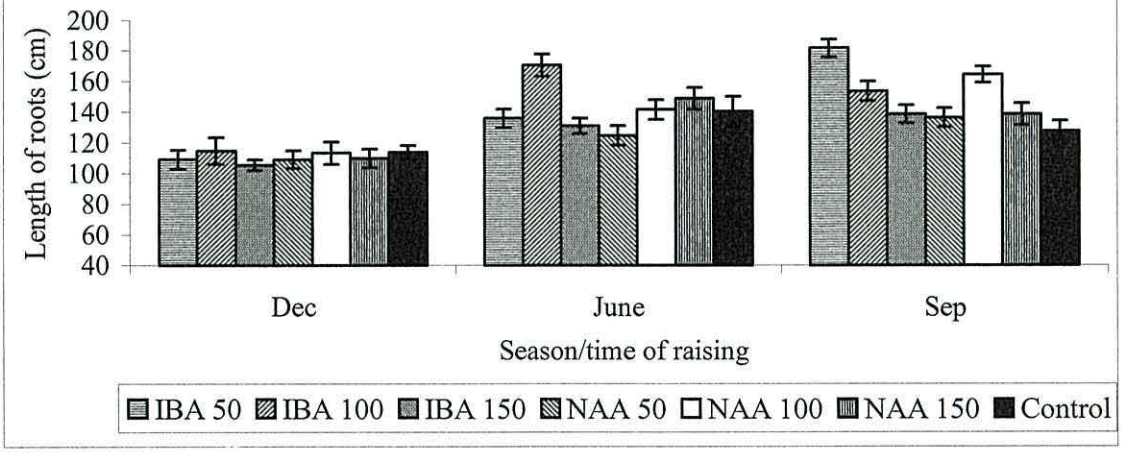
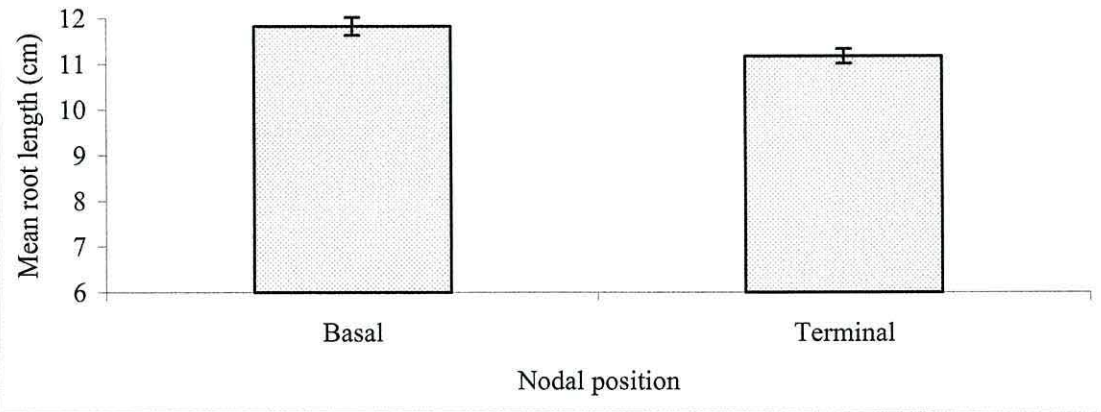


Figure 7.3c. Effect of nodal position on mean root length per cutting in June raised cuttings



In June, stem cuttings treated with 100 ppm IBA had longer roots (170.3 ±7.2 cm) while shorter roots were in stem cuttings treated with 50 ppm NAA (124 ± 6.5 cm). In September, auxins concentrations had an interactive effect with the nodal position in influencing the total length of roots produced ($P = 0.001$). Treating basal cuttings with 50 ppm IBA produced significantly the longest roots (195.6 ± 6.7 cm). Shortest roots were observed in terminals cuttings treated with the control that had a total root length of 113.7 ± 9.5 cm.

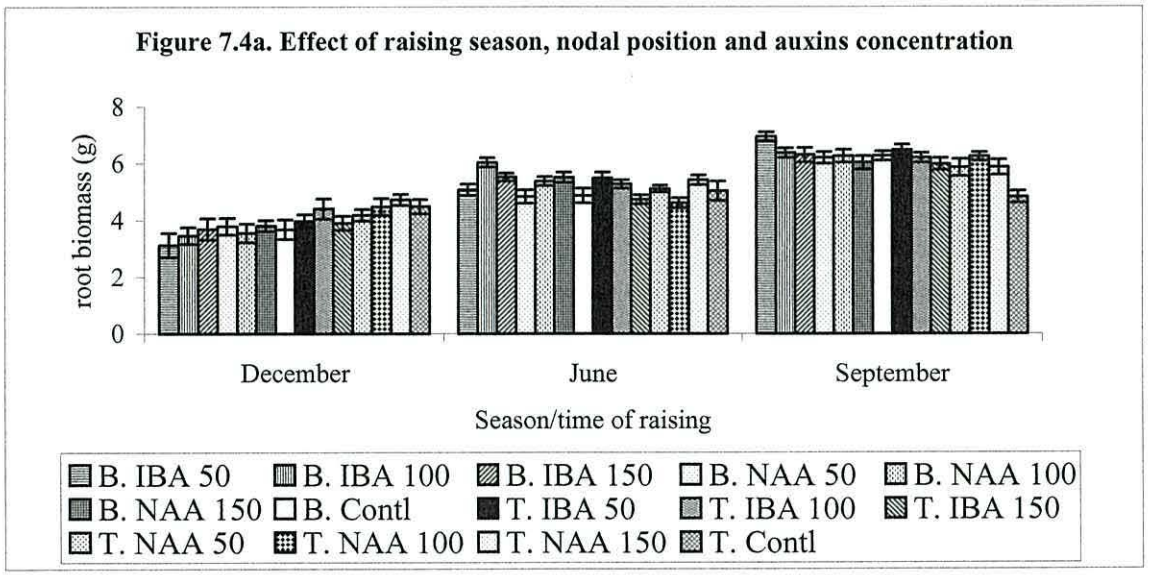
The mean root length followed a similar trend to the total root length produced when considered in general in most aspects with an exceptional of a few. Nodal position continued to have a significant effect when in interaction with seasons of raising ($P < 0.01$), giving the highest mean of 12.8 ± 0.2 cm for basal cuttings raised in September and a minimum of 9.0 ± 0.1 cm for basal portions raised in December. The effect of auxin type that had no effect on total root length became apparent in the mean root length ($P < 0.01$) with IBA having longer roots that either NAA or control. The interactive effect of auxin type and raising seasons observed in the total root length ($P = 0.027$) was not important in the mean root length produced per cutting ($P = 0.29$). However, the varied concentrations of auxins continued to behave as in total root length as well as its interaction with season of raising. The mean root length attained in cuttings raised in September and treated with IBA 50 ppm was 14.0 ± 0.1 cm. The shortest roots were in cuttings of December and treated with NAA 150 ppm with a mean root length per cutting of 9.2 ± 0.2 cm.

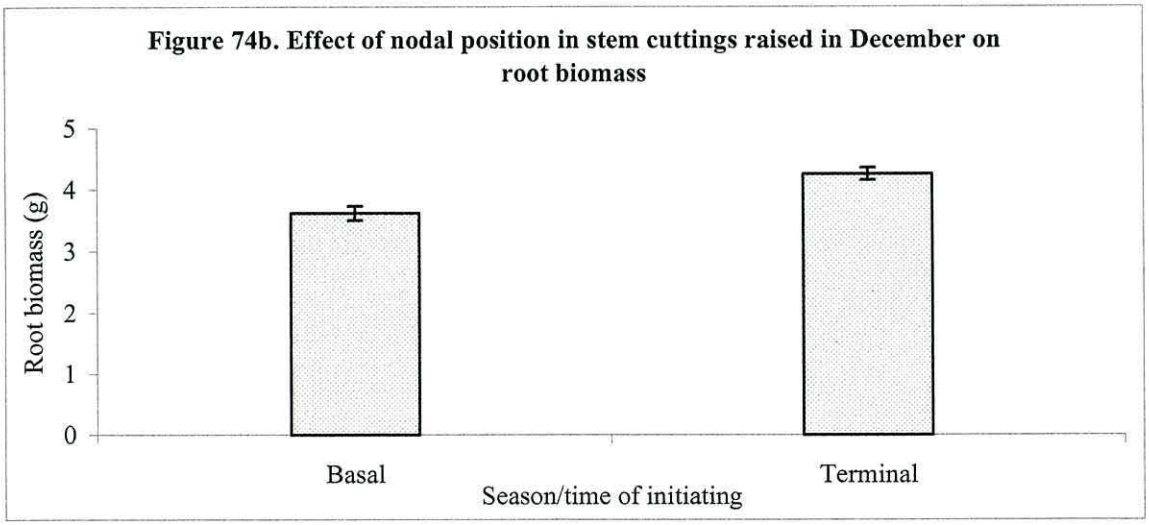
Within each season, the mean root length differed with the total root length. Position of the cuttings that had an effect on total root length of September cuttings ($P = 0.048$) had no significant influence on the mean root length ($P = 0.661$). However, nodal position influenced the mean root length of June cuttings ($P = 0.044$) with basal portion giving a mean root length of 11.8 ± 0.2 cm against terminal portion which had 11.2 ± 0.2 cm (Figure 7.3c). Hormone concentrations had a similar effect to what was observed in total root length.

7.3.1.4. Total root biomass produced per rooted cutting

Figure 7.4a-b presents the outcome of the effect of season, nodal position and auxins application on root biomass production. Season of cutting collection influenced the total root biomass produced per cutting ($P < 0.01$). Similarly, type of auxins and their varied concentrations had an influence on root biomass ($P = 0.04$ and $P = 0.04$, respectively). A significant interactive effect was also observed between the type of auxin and season ($P < 0.01$). Furthermore, auxin concentration interacted with season to give a significant difference in root biomass produced ($P < 0.01$).

Figure 7.4a-b. Effect of raising seasons, position of stem cutting and root promoter (auxins) on root biomass produced in stem cuttings of *O. lanceolata*





Nodal position seemed to have no significant influence when considered alone (P= 0.56), but had an interactive effect with season ($P < 0.01$). The three factors, i.e. season, nodal position and auxin concentration had an interactive effect on root biomass ($P = 0.01$). Root biomass was the highest in basal cuttings that were raised in September and treated with 50 ppm IBA (6.95 ± 0.15 g). The least biomass was in basal cuttings of December treated with 50 ppm IBA (3.13 ± 0.43 g) (Figure 7.4a).

Within each season, nodal position had an influence in all seasons and it was the only factor that influenced root biomass produced per cutting in December ($P < 0.01$). Terminal cuttings of December had significantly more root biomass (4.25 ± 0.10 g) compared with basal cuttings that had a mean biomass of 3.62 ± 0.11 g (Figure 7.4b). Nodal positions differed in the weight of roots produced for June ($P = 0.04$) and September ($P = 0.001$) cuttings. An interactive effect also existed between nodal position and auxin concentration June ($P < 0.02$) and September ($P = 0.03$). In June more biomass was attained in basal cuttings treated with 100 ppm IBA while the least biomass (4.62 ± 0.18 g) was in terminal cuttings treated with 100 ppm NAA. In September, more biomass was produced when basal cuttings were treated 50 ppm IBA (6.95 ± 0.15g) while terminals of the control had the least biomass of 4.83 ± 0.21 g (Figure 7.4a).

7.3.2 Vegetative propagation by marcotting

7.3.2.1. Rooting, shooting and survival of the marcotts in the field and nursery

Plate 7.3 shows the rooted marcotts, eighth week after field initiation i.e. during transplanting while plate 7.4 shows successful established marcots, two months from transplanting. Season at which marcotts were initiated influenced rooting success ($P < 0.01$) with marcotts initiated in September having better rooting success (72.7 ± 2.1%) compared to those initiated at any other time except June. The least rooting was in February that had a mean rooting success of 29.6 ± 2.1% (Figure 7.5a). Auxin type differed significantly in their ability to promote rooting ($P = 0.001$), as well as their varied concentrations ($P < 0.01$). Marcotts treated with 100 ppm IBA had better rooting (66.9 ± 3.7%) while the least rooting was in the control in which rooting

success was 50 ± 1.6% (Figure 7.5b). There was no significant interactive effect between auxin concentration and season (P = 0.198), although marcotts raised in September and treated with 50 ppm IBA gave high rooting percentage (85 ± 6.4%).

Plate 7.3. Rooted marcotts, eight weeks from date of initiation

Plate 7.4 Successful established marcots, two months from transplanting.

Figure 7.5a-d. Effect of seasons of raising and root promoters (auxins) on rooting and shooting success in marcotts of *Osyris lanceolata*

Figure 7.5a. Effect of season of raising

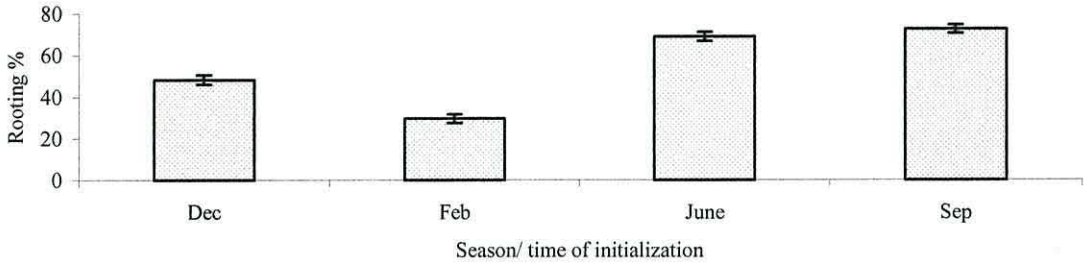


Figure 7.5b. Effect hormone concentrations

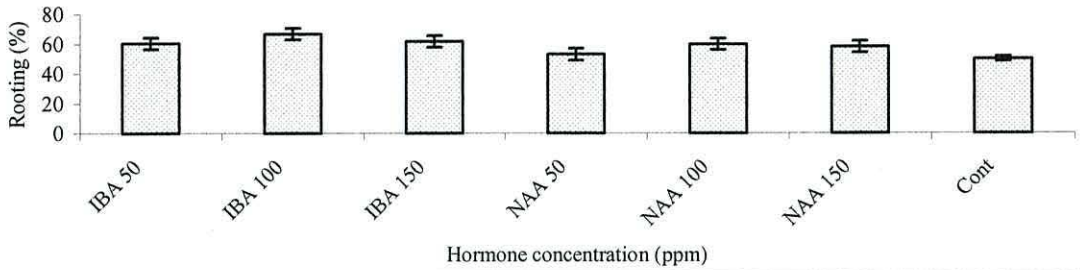


Figure 7.5c. Effect of varied concentrations of auxins at different seasons.

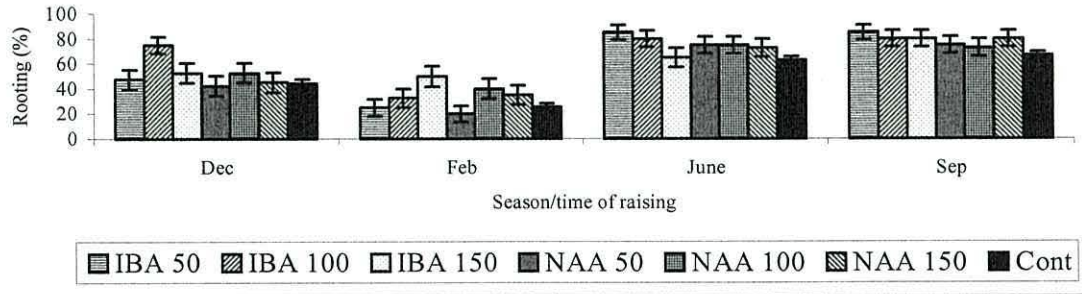
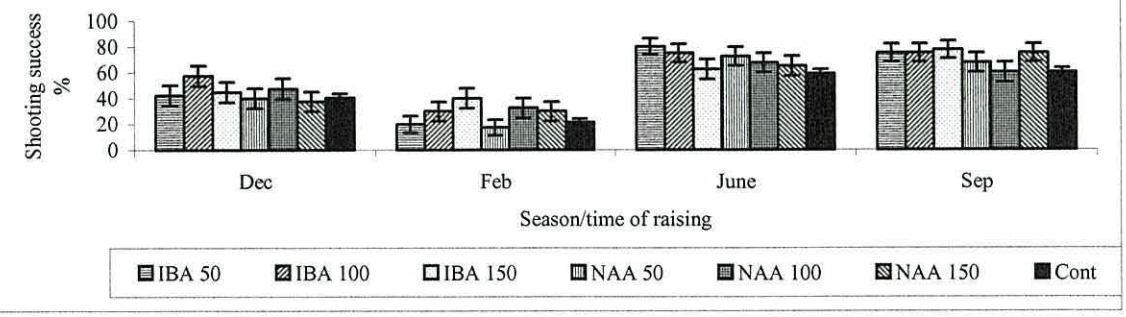


Figure 7.5 d. Shooting success as a proportion of total marcotts raised at different seasons as influenced by the auxins concentration



Within each season, variation in rooting was observed (Figure 7.5c). In December, type of auxin had a significant influence ($P = 0.04$) as well as their varied concentrations ($P = 0.03$) on rooting of marcotts. 100 ppm IBA resulted in better rooting ($75 \pm 6.9\%$) compared to the control that had the least rooting of $44.2 \pm 3\%$. In February, type of auxin did not show any significant difference in promoting rooting ($P = 0.11$). However, auxin concentrations varied in their effect ($P = 0.02$). Marcotts treated with 150 ppm IBA had better rooting ($50 \pm 8.0\%$) than those treated with 50 ppm NAA ($20 \pm 6.4\%$) and the control ($25.4 \pm 2.9\%$). The amount of rooting attained in June and September cuttings were also influenced by the type of auxin applied ($P = 0.01$ and $P = 0.01$, respectively). In both seasons, IBA had better rooting than the control but similar to NAA. Though the amount of rooting due to varied concentrations of auxins differed significantly for marcotts raised in June ($P = 0.04$), the best result was obtained with 50 ppm IBA with a mean rooting of $85 \pm 5.7\%$ and the control had the least rooting ($62.9 \pm 3.1\%$). In September, the rooting success achieved with IBA and the control was $81.67 \pm 2.7\%$ and $66.7 \pm 2.9\%$, respectively.

Shooting and survival of the marcotts in the nursery following transplanting depended on what was achieved in the field. Most marcotts that rooted survived and produced new shoots as a sign of their successful establishment. Neither season nor auxins influenced shooting and overall survival of the marcotts in the nursery. However, considering shooting as a proportion of the total marcotts that were initiated, concentrations of auxin had an interactive effect with season. Marcotts raised in June and treated with 50 ppm IBA had the highest shooting success ($80 \pm 6.4\%$) while the least shooting was in February marcotts treated with 50 ppm NAA with mean shooting of $17.5 \pm 6.1\%$ (Figure 7.5d).

7.3.2.2 Number of roots produced per rooted marcott

The number of roots produced per marcott following the treatments is presented in Figure 7.6a-c. Season at which marcotts were initiated influenced the number of roots produced per marcott ($P < 0.01$), with marcotts of June producing more roots than other seasons. The type of auxin as well as auxin concentration had significant effects on the number of roots produced ($P < 0.01$). Both IBA and NAA produced more roots compared with the control, and the difference between the two auxins not significant.

Figure 7.6a-c. Effect of seasons of raising and root promoters (auxins) on the number of roots produced per marcot in *Osyris lanceolata*

Figure 7.6a. Effect of varried concentrations of auxin and raising season

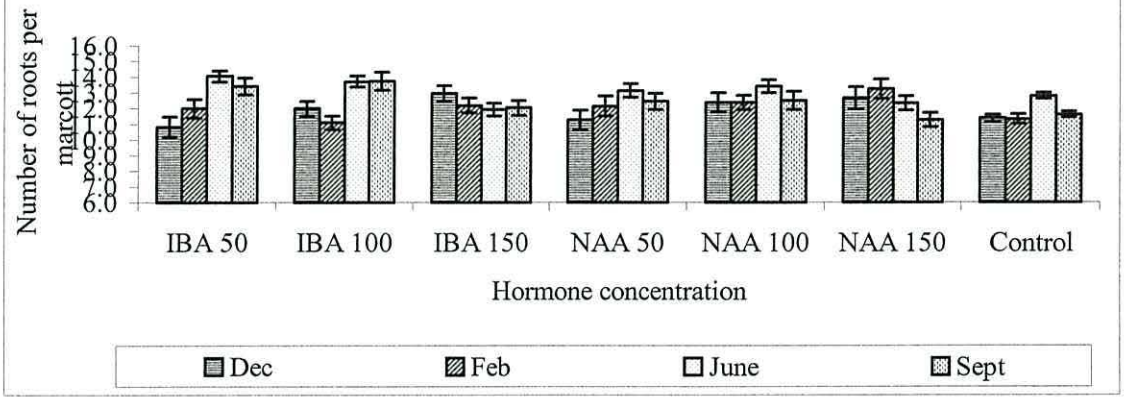


Figure 7.6b. Effect of auxin concentration at different seasons on the number of roots produced in marcotts

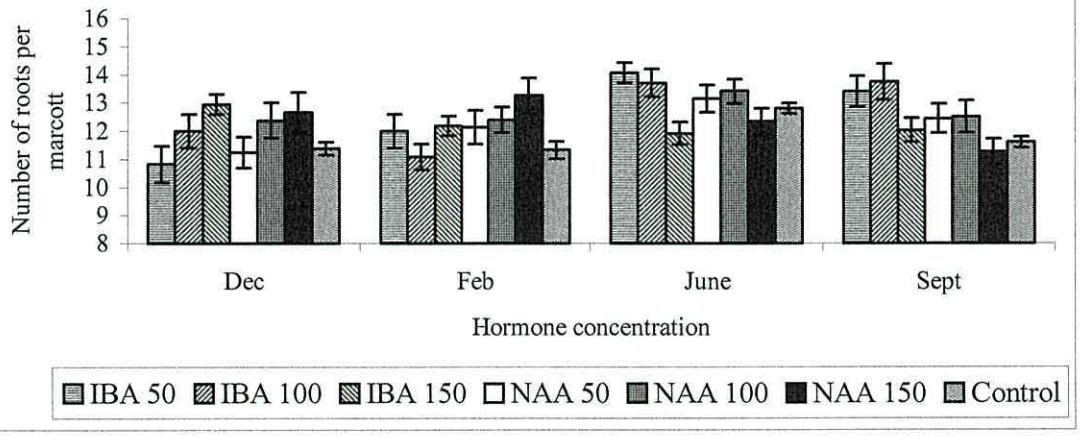
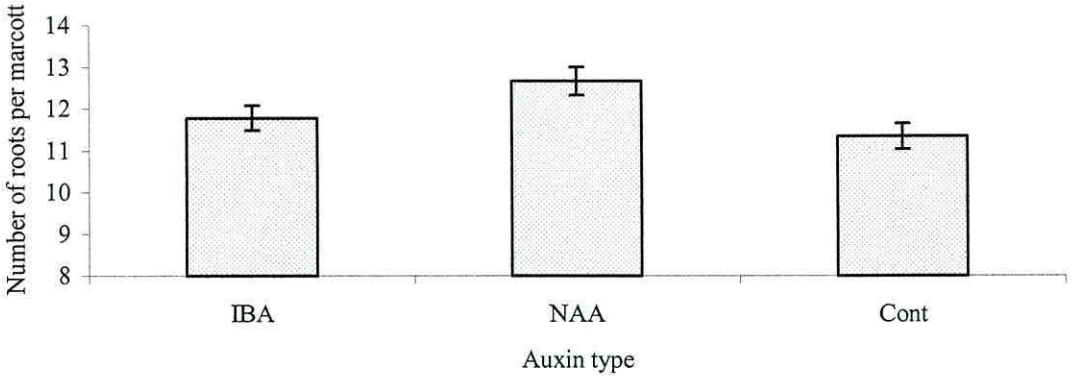


Figure 7.6c. Effect of type of Auxin on the number of roots produced in nmarcotts raised in February



Of the auxin concentrations, 100 ppm IBA produced significantly more roots than the control but similar with the other concentrations. A significant difference was observed in the interactive effect between concentration of auxin and season ($P < 0.01$). Marcotts raised in June and treated with 50 ppm IBA had significantly the highest number of roots (14.1 ± 0.4). The least number of roots (10.8 ± 0.6) was in marcotts of December treated with 50 ppm IBA (Figure 7.6a).

Within each season, auxin types had a variable effect on the number of roots produced per marcott. Auxin type had no significant effect on marcotts raised in December ($P = 0.19$). However, auxin concentration had different outcome ($P = 0.04$). More roots (12.9 ± 0.5) were observed in marcotts treated with 150 ppm IBA though Tukey's pair wise comparison revealed no significant difference with the marcotts treated with 50 ppm IBA ($P = 0.11$) that had the least number of roots (10.8 ± 0.6). Auxin type had a significant effect on the number of roots produced in February raised marcotts ($P = 0.01$) but concentration of auxins had no significant effect ($P = 0.054$). NAA produced more roots (12.7 ± 0.3) than the control (11.3 ± 0.3) but similar to IBA (Figure 7.6c). Though the type of auxin had no significant effect ($P = 0.208$) for marcotts raised in June, auxin concentrations had ($P = 0.001$). Marcotts treated with 50 ppm IBA produced more roots (14.0 ± 0.4) compared with those treated with either IBA or NAA at 150 ppm and the control. Least roots were found in marcotts treated with 150 ppm IBA (11.9 ± 0.4) (Figure 7.6b).

In September, the type of auxin as well as their varied concentrations differed significantly in their ability to induce the number of roots in marcotts (all $P < 0.01$). Application of IBA produced more roots compared with NAA and the control. Furthermore, marcotts treated with 100 ppm IBA had more roots (13.7 ± 0.6) than those treated with 150 ppm NAA and the control. Least number of roots (11.3 ± 0.5) was attained with 150 ppm NAA (Figure 7.6b).

7.3.2.4 Total and mean root length per rooted marcott

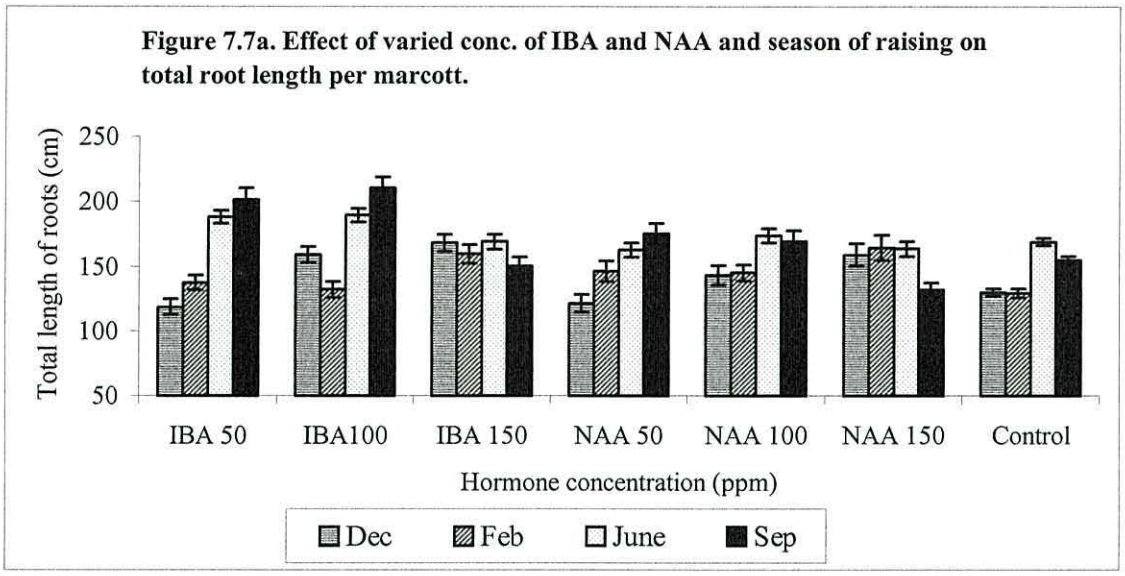
Total root length produced per marcott differed between seasons ($P < 0.01$) with June marcotts having significantly the highest total root length (172.2 ± 1.7 cm). Both the

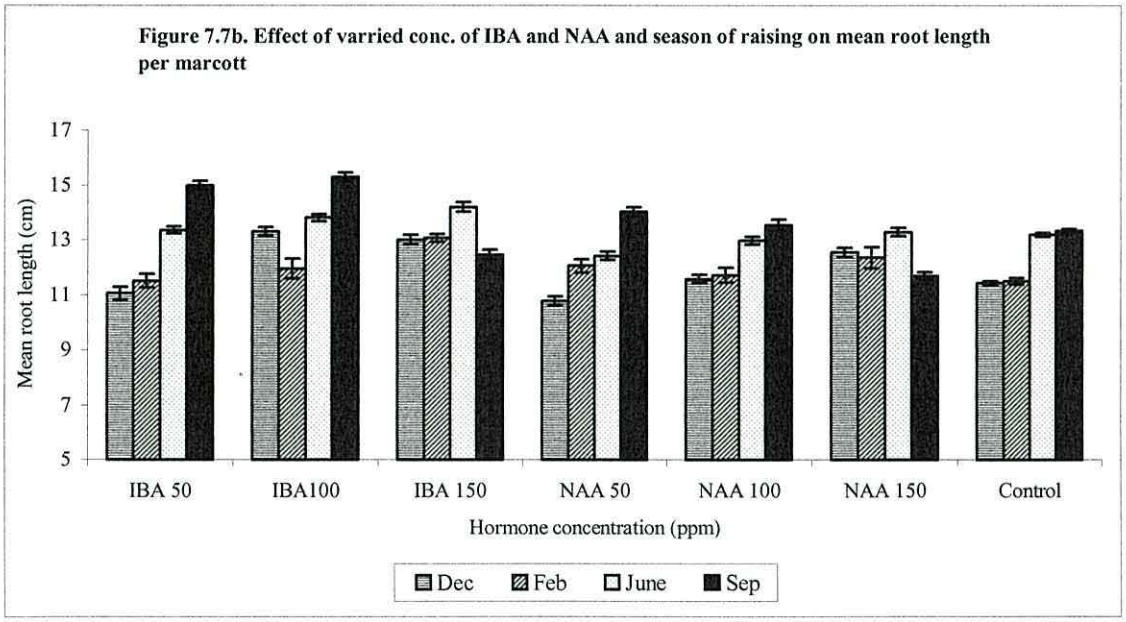
type and concentrations of auxins had significant influences on total root length (both $P < 0.01$) with IBA in general producing longer roots than either NAA or the control. Concentrations of auxins had also an interactive effect with season ($P < 0.01$). Marcotts initiated in September and treated with 50 ppm IBA had the longest total root length (210.1 ± 8.9 cm). Shorter total root length was observed in marcotts raised in December and treated with 50 ppm IBA (118.6 ± 6.2 cm) (Figure 7.7a). The mean root length (Figure 7.7b) was highest in marcotts raised in September and treated with IBA at 100 ppm (15 ± 0.1 cm) than all other interactions except in marcotts raised in September and treated with IBA 50 ppm. The lowest mean was in marcotts of December and treated with NAA 50 ppm that had 10.9±0.2 cm.

Variation in total length of roots produced existed within each season. In all seasons, both the type and concentration of auxins had significant influences on total root length (both $P < 0.01$). IBA was observed to be more effective than the control and sometimes NAA in influencing total root length. In December, 150 ppm IBA produced longer total root length (167 ±6.5) while marcotts with shortest total root length were those treated with 50 ppm IBA that totaled to 118.6 ± 6.2 cm (Figure 7.7a). In the same season, the mean root length was high in marcotts treated with IBA 100 ppm that attained with a mean root length of 13.3 ± 0.1 cm. Marcotts with lowest mean root length were those treated with NAA 50 ppm, with a mean root length of 10.8 ± 0.2 cm (Figure 7.7b).

In February, total root length was highest in marcotts treated with 150 ppm NAA (164.3 ± 9.7 cm) while the lowest was in those treated with the control that had a total length of 129.4 ± 3.6 cm (Figure 7.7a). The highest mean root length per cuttings in this season was 13.1 ± 0.2 cm observed in marcotts treated with IBA at 150 ppm while those treated with IBA at 50 ppm attained the lowest mean of 11.5 ± 0.2 cm (Figure 7.7b). In June, 100 ppm IBA produced longest total root length (189.3 ± 5.3 cm) while 50 ppm NAA had the shortest (162.9 ± 5.4 cm) as observed in Figure 7.7c. The highest mean root length was 14.2 ±0.2 attained in marcotts treated with IBA at 150 ppm while the lowest was 12.4 ± 0.1 cm in marcotts treated with NAA at 50 ppm (Figure 7.7b).

Figure 7.7a-b. Effect of seasons of initialization and root promoters (auxins) on length of roots produced per marcott in *Osyris lanceolata*





In September the highest total root length was obtained in marcotts treated with 50 ppm IBA (210.1 ± 8.9 cm) while marcotts with shortest total roots length were observed in those treated with 150 ppm NAA that had a total length of 131.8±5.6 cm (Fig 7.7a). The mean root length was high in marcotts treated with IBA 100 ppm (15 ± 0.1 cm) while the shortest were in marcotts treated with NAA at 150 ppm that gave 11.7 ± 0.1 cm (Fig 7.7b).

7.3.2.4 Total root biomass production per marcott

Root biomass differed depending on season and the type and concentration of auxins applied (all $P < 0.01$). Marcotts raised in September had significantly more biomass (6.98 ± 0.08 g) than those raised at other seasons. Both auxins i.e. IBA and NAA promoted higher root biomass than the control but no significant difference existed between the two auxins. Applications of 100 ppm IBA had significantly higher root biomass (6.72 ± 0.8 g) than other concentrations. A significant interactive effect existed between concentrations of auxins and season ($P = 0.01$) as observed in Figure 7.8a.

Figure 7.8a-b. Effect of raising season and application of root promoters (auxins) on root biomass produced per cutting

Figure 7.8a. Seasonal variation in root biomass as influenced by auxin concentration

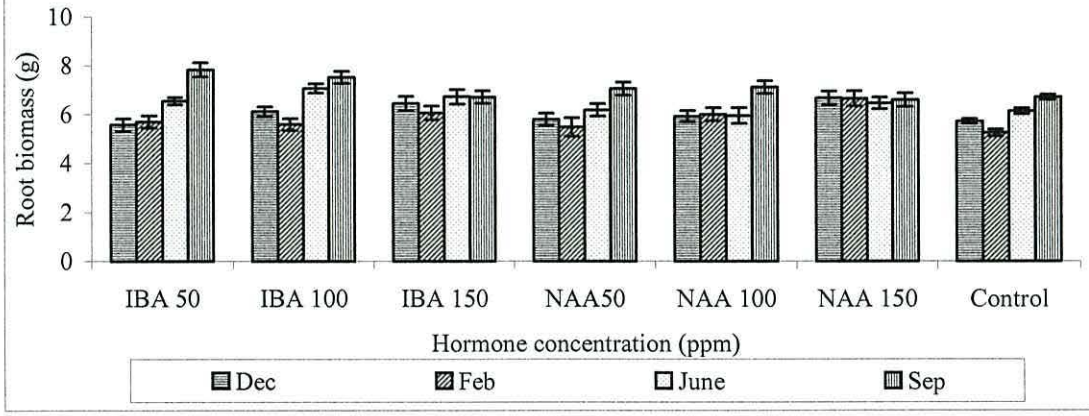
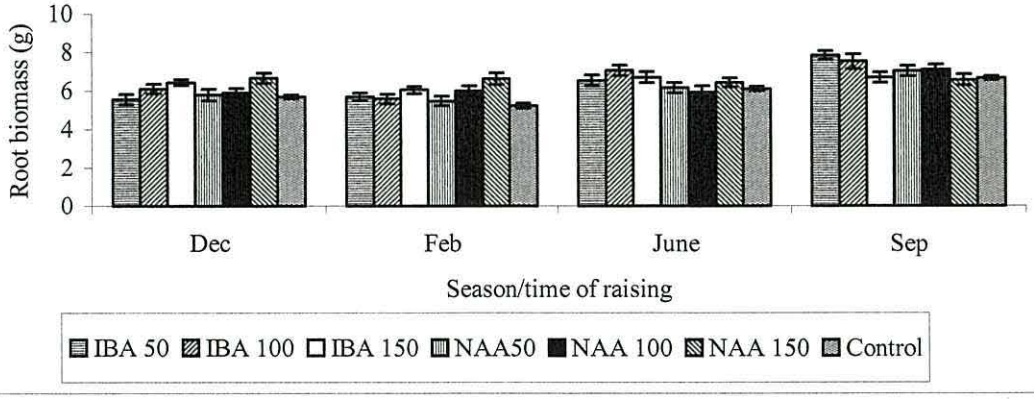


Figure 7.8b. Effect of varied concentrations of auxins at different seasons of raising



Marcotts initiated in September and treated with 50 ppm IBA had the highest root biomass (7.84 ± 0.30 g). The least biomass was observed in the control initiated in February with a mean biomass of 5.23 ± 0.14 g (Figure 7.8a). Variation was also observed within each season. Type of auxins influenced root biomass produced in December, February, June and September (P = 0.04, < 0.01, <0.01 and <0.01 respectively). Auxins concentrations also produced different root biomass in all seasons (all P = 0.01). In December and February, 150 ppm NAA gave significantly more root biomass per marcott (6.66 ± 0.27 g and 6.63 ± 0.31 g, respectively). The least biomass for the two seasons were obtained with 50 ppm IBA (5.6 ± 0.26) and the control (5.23 ± 0.14 g), respectively. For marcotts raised in June, 100 ppm IBA produced significantly higher biomass (7.07 ± 0.19 g) compared with the control (6.11 ± 0.12 g) and those treated with100 ppm NAA (5.94 ± 0.32 g). In September, marcotts treated with 50 ppm IBA gave the highest biomass (7.84 ± 0.13 g). Least biomass was attained with 150 ppm NAA (6.58 ± 0.28 g) (Figure 7.8b). High concentrations of either IBA or NAA were generally as worse as the control (Figure 7.8b).

7.4.5. Discussion

5.1 Effect of season on the performance of stem cuttings and marcotts

In both stem cuttings and marcotting experiments, a good performance was achieved by raising/initiating during the months of June and/or September. While no stem cutting survived among those that were raised in February, some success was achieved with marcotts initiated during the same month. The season during which cuttings or marcotts are made had been observed to have an influence on the rooting of many species especially in those that are hard to root (Nanda and Anand, 1970; Leakey, 1985; Joshi *et al.*, 1992; Kantari, 1993a). The difference in the performance of cuttings and marcotts between seasons is mainly attributed to the amount of food reserve present in a plant and its allocation to various parts of the plant which tend to vary with seasons. Also levels of flower stimulus antagonistic to rooting and levels of endogenous regulator substances necessary for root initiation have been reported to be high in some periods of the year than others (Nand and Anand, 1970; Leakey, 1985).

The above facts may explain the success of marcots and stem cuttings initiated in September and June in the present experiment. While September falls within the dry season during which most plants including *O. lanceolata* are dormant, June is the period which marks the end of the rainy season, soon before the plant enters its dormant stage after being photosynthetically active throughout the rainy season that ends in around April-May at the present study site. During these periods, most parts of plants including branches are already rich in nutrition status having been accumulated during the active growing seasons. This food reserve provides the cutting with enough energy required to initiate and grow roots (Priston *et al.*, 1953; Nand and Anand, 1970; Hartmann and Kester, 1997; Kijkar 1992b).

Also the absence of plant developmental activities such as bud initiation and flowering, makes little competition, leaving stem cuttings and marcotts with enough food materials that can be directed to rooting (Haissig, 1984; Kantari, 1993a; Joshi *et al.,* 1992). Similar observations have been reported in most European species where, the ability of the cuttings to root decreases during summer and increases considerably during winter. The decreased rooting ability in summer is associated with high levels of irradiance, water stresses and the incidence of flowering all of which disfavour rooting (Klahr and Still, 1979; Lux 1982). The fact that levels of endogenous auxins and rooting co-factors are high in some periods of the year than others may have also played a role in the successful rooting of marcotts/stem cuttings initiated in June and September. However, there was no evidence to prove that high levels of these compounds existed in June and September since they were not monitored. In general, cuttings harvested when these compounds are high tend to root easily than those collected at other times as reported by Nand and Anand (1970), Haissig (1984), Hartmann and Kester (1997), Kantari (1993a) and Forestry Commission (1995).

The poor performance of cuttings and marcotts raised in December and February can be related to the presence of active growth and closeness to the flowering period. In December *O. lanceolata* is actively producing new shoots and leaves. This phenomenon may have a divergent effect on resources in plant body. Most resources tend to be allocated to bud initiation and leafing, thus leaving little in other parts of the body. Thus

cuttings/marcotts collected/initiated in December are likely to have suffered from nutrition problems especially carbohydrate leading to their poor rooting. Cuttings/ marcots initiated in February might have suffered from similar situation where by *O. lanceolata* tends to allocate most of its resource to flowering, as this period is just a few weeks before active flowering takes place in the area. Thus low levels of nutrition status of the marcotts/cuttings coupled with high levels of flower stimulus antagonistic to rooting and competition for food materials necessary for both rooting and flower formation might have resulted in no rooting in stem cuttings and very little in marcotts (Johnson, 1970; Kantari, 1993a; Joshi *et al.,* 1992).

7.4.2 Effect of auxins on the performance of stem cuttings and marcotts

Auxin application proved to be advantageous in promoting the rooting ability of both marcotts and stem cuttings in the present experiment. It enhanced the number of marcotts that rooted, the number of roots formed per stem cutting as well as length and biomass of roots. Many woody plants such as *Tilia platyphyllos* (Howard and Harrison-Muray, 1985), *Triplochiton scleroxylon* (Leakey *et al.*, 1982), *Cordia alliodora, Albizia guachapele* and *Vochysia hondurensis* (Leakey *et al.*, 1990); *Acacia* hybrid (*A. mangium x A. auriclriformis*) (Kijkar, 1992a), and *Parkia biglobosa* (Teklehaimanot *et al.,* 1996) have been shown to respond similarly to external hormone application. Auxins influence adventitious root formation by hastening cell division and thus increasing the ability of cuttings to root (Hartmann and Kester, 1997; Chauhan *et al.,* 1994).

Of the two auxins applied, IBA gave a better result in most of the parameters assessed compared to NAA. While there is a general acceptance that, IBA is the most effective of all the root promoters in many species tried (Leakey, 1985; Leakey *at a*l., 1990), its effectiveness in *Osyris lanceolata* over NAA cannot be overemphasized. Only some concentrations of IBA were effective rather than IBA as a whole. Similarly, few concentrations of NAA gave almost comparable results to what was attained with the best concentrations of IBA. These cannot be neglected. However, most concentrations of NAA were inferior. In some plants such as *Triplochiton scleroxylon,* a mixture of IBA and NAA was found to be most useful than either of the two alone (Leakey *et al.*, 1982).

The effectiveness of auxins and the amount applied varied with seasons. A strong interaction existed between auxin concentrations and season. The variation in the effectiveness according to season may be due to the amount of endogenous hormone and associated root co-promoters in plants, which vary with season (Nand and Anand, 1970; Cheffins and Howard, 1982a; Hartmann and Kester, 1997; Kantari, 1993a; Joshi *et al.,* 1992). These may influence the amount of auxin to be applied as more is needed when the concentration of these compounds is low compared with the time when they are high. While, 50 ppm of IBA was sufficient enough to enhance rooting in cuttings/marcotts initiated in September, over 150 ppm seemed to be required in marcotts/cuttings raised in February. Cuttings raised in June and December rooted better when 50 – 100 ppm auxin was applied. The order in the amount of IBA needed to stimulate rooting in both cuttings and marcotts was September ≤ June ≤ December ≤ February.

September and June are associated with high content of food reserves in plants and thus cuttings are likely to respond more readily with little aid of auxin. February is associated with the peak of active flowering during which most food reserves are directed toward this activity leaving little for rooting. In addition, levels of flower stimulus antagonistic to rooting are likely to be high during this time (Cheffins and Howard, 1982; Kantari, 1993a; Joshi *et al.,* 1992; Nand and Anand, 1970). Thus, trials to initiate roots in marcotts/cuttings during this time are likely to succeed with difficulty. It may be necessary to apply higher concentrations of auxin in February to increase endogenous auxin activity or increase the involvement of auxins co-factors or other processes necessary to induce rooting (Howard and Harrison-Murray, 1985).

7.4.3 Effect of nodal position on the performance of stem cuttings

Stem cuttings of *Osyris lanceolata* originating from basal portion seem to perform better than those of terminals. Many researchers have reported similar finding in other species such as *Triplochiton scleroxylon* (Leakey, 1983), *Populus ciliata* (Srivastava and Mangil, 1981) and in some Dipterocarps (Singh *et al.,* 1984). This difference in the performance between the two nodal positions could be the result of uneven distribution of inhibiting substances in different parts of the shoots. Most of these are

produced in the apical parts and they have been proved to have an inhibitory effect on root initiation (Viart 1979; Orourke (1940) in Hartmann and Kester, 1997). Stem cuttings originating close to terminals are thus expected to have more of these inhibitors compared to those of basal origin. This could have contributed to their poor performance. Also the numbers of pre-formed root initials, which are important to initiate adventitious roots, are reported to decrease from the base to the tip of the shoot. Consequently, cuttings from basal portions perform better compared to the terminals (Hartmann and Kester, 1997).

The difference in performance could also be due to the differences in the nutritional status (Singh *et al.*, 1984, Haissig, 1986). A considerable body of evidence suggests that, cuttings with higher C/N ratios root better than those with lower ratios. This is because higher ratios are associated with higher food reserves especially carbohydrates which is important for root initiation (Leakey, 1985). The ratios generally decrease from the base to the top of any single shoot from which cuttings are made. Thus cuttings from the basal portion are better in nutrition than terminal cuttings and thus they are expected to perform better. However, this reason does not need to be over emphasized since very little amount of carbohydrate have been shown to be really needed during root regeneration. It is argued that, high levels of C/N promote rooting mainly due to low level of nitrogen that is positively correlated with rooting and not the high food content theory (Hambrick *et al.*, 1985). Also cuttings with high nitrogen level tend to be tender and prone to desiccation before rooting takes place (Kantari, 1993).

A good performance of terminal cuttings reported by Kijkar (1992) and Kumar *et al.* (1993) as a result of juvenility did not seem to hold for this species. Possibly the concentration of root inhibitors produced in the terminals and low nutritional status superseded the juvenility effect. Although maintenance of some leaves on terminal cuttings had been proved to be useful in some species (Longman, 1993), this did not seem to be the case in the present experiment. It is likely that the two pair of leaves (~

20 cm^2) left on the terminal cuttings of the present experiment encouraged excessive transpiration before roots were initiated. This could have caused early desiccation to the cuttings and hence their poor performance (Longman, 1993; Aminah, 1991).

CHAPER VIII

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General discussion

Osyris lanceolata Hochst. & Steud. which is an aromatic tree species indigenous to Tanzania, is now being used as a substitute/supplement of Indian sandalwood, *Santalum album* (Hill, 1937; Metcalfe, 1950; Schery, 1954; Walker, 1966, Heywood, 1978). The value and utilization of *O. lanceolata* in perfumery seems to have been known for sometime (Hill, 1935; Dale and Greenway, 1961; Walker, 1966), although its full commercial recognition and suggestion for improvement in Tanzania are rather recent developments (Mbuya *et al.*, 1994; Mwang'ingo and Mwihomeke, 1997). Due to unregulated and destructive method of harvesting that have been taking place for almost five decades in Tanzania, it was speculated that *O. lanceolata* may be under threat (Mwang'ingo and Mwihomeke, 1997). As a first step towards management and conservation of the species, the Government of Tanzania banned further harvesting of the species in 1990s as little was known on the resource status of the species. However, some illegal smuggling has been reported to still take place that may have put further threat to the species (S.T Mwihomeke, personal communication). In order to arrest further degradation of the species the Government of Tanzania has been encouraging all sectors of the community to domesticate the species.

Although the will to domesticate *O. lanceolata* has been there, its implementation has been a problem due to lack of information on the silviculture and ecological requirement of the species. Information on the species is fragmented and quite superficial for effective use. The only information that was found of value was that of Rao (1942a, b), Kuijt (1969), Joshi (1960) and Herrera (1984a, 1988a, b). Yet, these are based on the Mediterranean range of the species that could hardly be extended to the tropical environment. This is not surprising, as most research work in the tropics have always focused more on few species of commercial importance,

particularly timber yielding species, with little or no effort placed on species producing non-timber forest products. However, recent realization of the fact that some non-timber forest products have for many years been contributing to overall economy of many rural communities with some of them having a value exceeding by far even that of timber have made the recognition of such species as *O. lanceolata* to become more apparent (Simons, 1996b). This being the case in Tanzania, the present study was therefore carried out to investigate the current population status, biology, oil chemistry and ecology of *O. lanceolata* in Tanzania and develop methods for its domestication that could guide future strategies on the management and conservation of the species.

Six research activities were undertaken in the present study. The first activity involved collating existing knowledge with regard to distribution, population status, ecology, biology and oil chemistry of *O. lanceolata* from literature. There was a significant dearth of information in its East African range and apparently nothing on Tanzania, the only African country where the species has been exploited by the sandalwood oil industry. Thus, the rest of the research activities in the present study were aimed at providing the basic information which are required in order to develop strategies and programs for effective management and conservation of the species and sustainable utilization of the resource. Knowledge on population structure of *O. lanceolata* provides a picture on the future prospects of the resource and how possibly it can be managed in a sustainable way (Leek, 1965; Bawa and Krugman, 1991). It is also an important process that can assist in the identification of potential sources for genetic improvement and *in-situ* conservation of the species (Kemp and Palberg-Lerche, 1994). Successful management of *Osyris lanceolata* in the wild also depends on a thorough knowledge on the reproductive biology of the species (Janick *et al.*, 1982). The most desirable means by which the sustainability of *Osyris lanceolata* can be ensured is through domestication. Hence, there is need to develop appropriate methods of seed storage and pre-treatment techniques and methods of propagating the species through vegetative means in order to facilitate its domestication.

The results of the resource assessment study carried out under the present study revealed that the populations in Tanzania, despite many years of unregulated exploitation, were still stable. However, the populations differed in tree and site characteristics between the north and south of Tanzania. In the populations of the northern ecozone the density of trees was higher and the trees were smaller in stature than in the populations in the Southern ecozone. This was attributed to soil and climatic differences between the two ecozones. The Northern ecozone is more arid, stony and rocky which are characteristic features of sites reported in the literature as the natural habitat for the species (Herrera, 1984a, 1988a). The significant negative correlation found between tree diameter and soil pH indicates that *O. lanceolata* favours soils with low pH. The significant positive correlations between nitrogen content and almost all morphological parameters also indicate that nitrogen can enhance the growth of *O. lanceolata*. Soil nutrient status and acidity have been reported in the literature as being responsible for influencing the distribution and morphological variations of species within and among populations of several species of trees (Bell, 1982; Crawley, 1986).

O. lanceolata, being a dioecious species, the distribution of male and female plants within a population and the difference in morphological characteristics between them were also investigated in the present study. The results suggest that both male and female plants were randomly distributed within each population and there was no difference in morphological characteristics between them. According to Bawa (1976; 1980b), random sex distribution as opposed sex clustering in a population is ideal for effective reproduction. According to the results of the reproductive process study flower formation times differed in male and female plants. This could be a strategy by the species to ensure successive fertilization (Bawa, 1980b; Bullock and Bawa 1981). However, the results of the reproductive success study suggest that pollen availability is limiting in *O. lanceolata* as it was observed that many flowers were aborted. According to Wiebes (1979), Handerson (1986) and Simons (1996a) poor pollination may be a consequence of insufficient pollen production or failure of the pollen to move across trees. The fact that female flowers of *O lanceolata* offer little reward in terms of nectar and are inconspicuous to pollinators and hence rarely

visited (Herrera *et al.*, 1984), may also contribute to the problem of pollen availability. Although the distance between individual female and male plants was found to be far in the populations studied (23 m), this may not be the only factor responsible for the observed increased abortion rate. Therefore, further work is required to ascertain the exact causes of flower abortion in *O lanceolata*.

Difference between populations of the northern and the southern ecozones was also observed in terms of oil quantity and quality in the wood of *Osyris lanceolata*. According to the results of the present study the oil content of *Osyris lanceolata* was in general higher in the Northern ecozone than in the Southern ecozone. In particular the population at Bereko in the Northern ecozone yielded 7.25% oil, which was almost twice as high than the oil content reported in *Santalum album* (4.2%). However, the santalol content of the oil produced per unit kg of wood was 36% less than what is reported for *Santalum album*. Yet, this is significant in terms of oil production from the point of view of the perfumery industry under the present condition of scarcity of the resource. The dry climate and low soil fertility conditions prevalent in the Northern ecozone may be some of the factors responsible for the higher oil content and quality of the Northern populations than the south. Santalol has been reported to be an outcome of heat and water stress (Iyenga, 1968; Srinivasan *et al.*, 1992). Along with santalol, other compounds of commercial importance were also identified in the oil of *Osyris lanceolata* in the present study. These include lanceol, bisabolene, bisabolol and nuciferol. These compounds are likely to widen the utilization and market potential of *Osyris lanceolata* in the future.

O. lanceolata, being a hemi-parasite species, host plant species were also identified in the present study. *O. lanceolata* was found to be non-host specific as reported by Rao (1942a). However, the number of host plant species utilised by *O. lanceolata* varied between populations. *O. lanceolata* utilised a wide range of plant species as hosts in populations which received less rainfall and had low fertile soils. Similar observations have been reported in most parasitic plants growing in poor soils (Lamont, 1982). Under these conditions many host species are needed to enable *O.*

lanceolata to meet its water and nutrient requirements. The most frequent host species identified in the present study were *Brachystegia spiciformis* and *Brachystegia utilis*, *Tecomaria capense ssp nyasae, Mayetenus senegalensis*, *Rhus natalensis*, *Aphloia theiformis* and *Rhus natalensis*. Nursery studies conducted to investigate the influence of some of the most frequent host plants on growth of *Osyris lanceolata* showed that *Brachysstegia spiciformis, Rhus natalensis* and *Casuarina equisetifolia* had positive effects on growth of *Osyris lanceolata*. This suggests that selection of host species is important at nursery stage if *Osyris lanceolata* has to be raised in plantations.

From the results of the present study it was shown that *Osyris lanceolata* regenerated naturally from both seed and coppice/rootstock origin, with 61% being from coppice or rootstock origin. The low level of regeneration from seed may be due to mechanical dormancy, which has been reported to hinder spontaneous germination in seeds of *Osyris lanceolata* (Msanga, 1998). In addition, the recalcitrant character of the seeds of *Osyris lanceolata* (Mbuya *et al.,* 1994) associated with rapid loss of viability may also contribute to reduced level of regeneration. Experiments carried out in the present study to alleviate these problems indicated that germination of seeds of *Osyris lanceolata* can be enhanced by applying seed pre-treatments techniques including complete removal of the seed coat and soaking seeds in hot water before seed sowing. These techniques significantly increased the cumulative germination percentage, germination energy and germination value in *O. lanceolata.* These pre-treatment techniques have been reported to be successfully in many species with hard seed coats (Msanga and Kalaghe, 1992; Sadhu and Kaul, 1989; Maghembe and Msanga, 1988; Onywekwelu, 1990; Asenga and Otysina 1996; Redhead and Hall, 1992; Adjers and Srivastava, 1993). The problem of viability due to the recalcitrant character of the seeds has also been shown in the present study to be alleviated by applying appropriate seed storage methods. The results of the present study showed that seeds of *O. lanceolata* could be stored at 3-5 °C with moisture content of 20% for at least 36 weeks without losing their viability. This means *O. lanceolata* can be propagated from seed despite the cost involved in the pre-treatment and storage of seeds.

In order to supplement propagation of *O. lanceolata* from seed as well as to reduce the additional cost of pre-sowing treatment and storage of seeds, further research was carried out in the present study to investigate the possibility of propagating *O. lanceolata* from stem cutting and air layering/marcotting. The results indicated that *O. lanceolata* could easily be propagated from both stem cuttings and air layers. Seasons when cutting or marcots are made, application of auxins and nodal position of cuttings influenced rooting success. Best rooting success in both stem cuttings and marcots was achieved during the months of June and September. The effect of season has been observed on the rooting of many species especially in those that are hard to root (Nanda and Anand, 1970; Leakey, 1985; Joshi *et al*, 1992; Kantari, 1993a). Auxin application proved to be advantageous in promoting the rooting ability of both marcotts and stem cuttings of *O. lanceolata* in the present study. It enhanced the number of marcotts that rooted, the number of roots formed per stem cutting as well as length and biomass of roots. Similar results have been reported for many woody plants (Howard and Harrison-Muray, 1985; Leakey *et al.*, 1982; Teklehaimanot *et al.,* 1996). Stem cuttings originating from basal portion also performed better than those of terminals as in many other woody species (Leakey, 1983; Srivastava and Mangil, 1981; Singh *et al.,* 1984).

8.2 General conclusions and recommendations

The following conclusions and recommendations have been drawn from the present study:

From the results of the study on the status of populations of *Osyris lanceolata,* it is concluded that populations of *Osyris lanceolata* in Tanzania are stable. The species occurs in a variety of climatic conditions and soil characteristics in Tanzania although areas with arid and semi-arid climate associated with rocky characteristics are the most favorable habitats for the species. Tree size is influenced both by climate and soil fertility status. Trees tend to be reasonably big where rainfall is high and fertility of the soil is good. High density of trees occurs in semi-arid to arid climate. *Osyris lanceolata* seems to have little ability to compete with other species

as it was found to show suppressed growth in stands with very high tree stocking density where shading effect is high. The regeneration of the species relies heavily on rootstock source although the importance of seed source cannot be overlooked. Thus, the harvesting method currently employed, which involves removal of the whole root system has to be discouraged in order to encourage natural regeneration from root suckers. The present study also showed that *Osyris lanceolata* can be raised artificially from seeds by applying pre-sowing treatments to seeds if establishing commercial plantations are desired. Sex distribution within a population is generally random with little evidence of sex segregation. The time taken from flower initiation until when ripe fruits are formed is about 100 days. Of the flower initiated, 45% are expected to produce viable seeds. This low level of seed formation may be due to lack of sufficient pollen resulting into increased abortion rate. It is recommended that harvesting of trees should take into consideration the amount and spatial distribution of the tree sexes as over harvesting of one sex is likely to impair the whole reproductive process. Assisted pollination has also been shown to have a potential for increasing pollination rate and hence the amount of fruits/seeds that can be produced in a population.

The results of the study on quantity and quality assessment of essential oil in *Osyris lanceolata* revealed that sandalwood populations vary a lot in their ability to produce quantity and quality of oil. This confirms the speculation that good quality oil may be the reason why exploitation of the species by the perfumery industry has been concentrated in the northern region of Tanzania. Sandalwood from the Northern ecozone yielded more quantity and better quality of oil compared to the Southern ecozone. There was, however, no solid evidence to suggest that root system was superior to the shoot system in quantity and quality of oil produced. The oil content was found concentrated close to the ground level both below and above ground and decreased as one moved towards the root or shoot tips. Thus, it is concluded that the uprooting of the tree as a method of harvesting is unjustified and must be stopped. *Osyris lanceolata* also contains many other chemical compounds, which have not yet been exploited apart from santalol. The variety of chemical compounds observed in the species is likely to widen the utilization of *Osyris lanceolata*. This could

perhaps widen the market potential of the species to strongly justify its domestication. Exploitation of the possible use of the other compounds is thus worth investigating. There were also a number of unidentified chemical compounds, which need further investigations. Finally, it is recommended that more care is needed in harvesting of *Osyris lanceolata*, as populations with good quality sandalwood seem to be few. Uprooting the species needs to be discouraged as this is the best means through which the species regenerates naturally. The variation in oil content and quality and morphological attributes of trees need to be taken into consideration if improvement of the species is desired. A combination of tree characteristics including big size of trees and high amount and better quality of oil may be given a priority in selection processes for improvement of the species. There is a need to incorporate more populations of sandalwood in quality assessment including small populations as they may prove to be more productive. Cross border study is also highly recommended particularly towards Kenya and Uganda where the species is also reported to occur in order to broaden the scope of identifying and capturing the genepool of *Osyris lanceolata* with better quality oil.

Based on the results of the study on identification of host plants of *Osyris lanceolata* and their interactions, it is concluded that the host species range of *Osyris lanceolata* is wide. Yet some tendency of preference for some species is shown. The study on growth of *Osyris lanceolata* in the nursery showed that tree species with light crowns are the best hosts. These include *Brachystegia spiciformis*, *Rhus natalensis* and *Casuarina equisetifolia*. Moreover, species that combine light crown with the ability to fix nitrogen also proved more advantageous as revealed by the best growth performance of *Osyris lanceolata* observed under *Casuarina equisetifolia*. It is, therefore, recommended that the best host species observed in the present study (*Casuarina equisetifolia, Brachystegia spiciformis and Rhus natalensis*) be utilised in the early stage of *Osyris lanceolata* plantation establishment. However, it is unknown whether these species could remain the best host at later stages of *Osyris lanceolata* growth. Thus, further research is required to assess their potential for longer period. It has been observed in some parasitic plants that, some host plants that proved to be good in an early stage became inferior at a later stage as some

parasitic plants tend to prefer different hosts as their growth progresses. In *Santalum album*, host plants are classified into three groups depending on the stage at which they were found suitable. These categories include initial hosts (suitable at nursery stage), intermediate hosts (suitable within few years after field planting) and long-term hosts that support the parasite to maturity stage. Therefore, the three species identified in the present study may only fall under the category of initial hosts.

From the results of the study on storage and pre-treatment of seeds of *Osyris lanceolata*, it is concluded that the longevity of *O. lanceolata* seeds can be prolonged by slowly dehydrating the seeds to moisture content of about 20% and storing at temperatures around 3-5 °C. Moisture content of 15% or below seems to be lethal especially when storage had to exceed 12 weeks due to excessive dehydration. High moisture content (25% or more) is also lethal for long storage period due to excessive respiration and likely overheating effect. Low temperatures (possibly below 3 °C) have a chilling effect while high temperatures (possibly above 13 °C) encourage excessive respiration and hence rapid loss of seed viability. However, the precise temperature of storage depends on the period at which seeds are intended to be stored. For short-term storage (within 8-12 weeks), dehydrating seeds to about 20% moisture content and storing at 13-15 °C (almost room temperature) could suffice. Beyond 12 weeks, the recommended moisture content of 20% and temperatures of around 3-5 °C should be adapted. Seeds of *Osyris lanceolata* can be stored only for short-term supply even under best laboratory conditions. In general drying seeds under shade to moisture content of about 20% and storing at 3-5 °C can maintain reasonable viability of seeds for some time. About 60% of seeds are expected to be viable after storing for 36 week. Temperatures below 1 °C are to be avoided due to chilling effect, which ultimately kills seeds.

From the pre-sowing treatment trials, it is concluded that the thin seed coat covering the embryo of *O. lanceolata* plays a significant role in limiting germination by restricting the entry of gases and water, which are essential for germination. The thin coat also plays part in providing mechanical barriers to the embryo growth leading to the overall poor germination. Therefore, it is recommended that complete removal of

the seed coat and soaking seeds in hot water are required in order to overcome the physical and mechanical dormancies observed in *O. lanceolata.* These treatments are also effective in shortening the dormancy periods and promoting early growth of the seedlings and thus utilising the nursery space more effectively. Thus, propagation of *O. lanceolata* can be done through the use of seeds. For optimal germination, seeds should be pre-treated by soaking in hot water, or their seed coat should be completely removed before sowing. However, owing to the difficulties, time consumption and possible destruction of seeds, complete removal of the seed coat may be avoided and hot water soaking adopted which also gives satisfactory results. Even with the best treatment (complete removal of the seed coat), germination attained is still not very high (66.5%) indicating that seeds of *O. lanceolata* may still have other types of dormancies not yet identified in the present study. It is therefore recommended that further work is needed to identify other types of dormancies such as chemical dormancy and see if further improvements in germination rates can be achieved.

From the results of the study on vegetative propagation of *Osyris lanceolata,* it is concluded that stem cuttings and air layering have a big potential to serve as alternative propagation techniques for *Osyris lanceolata.* Marcotting gives better result compared to cuttings although wide applicability of the technique could be limited compared to cuttings as it involves two stages of work: an initial extended period in the field for rooting of marcotts and acclimatisation of rooted marcotts in the nursery. The best season for harvesting of stem cuttings is September while marcotts can be initiated at any time between June and September. Of the two nodal positions of stem cuttings, basal cuttings are better than terminals and should be adopted whenever raising the species through the use of stem cuttings is considered. Application of Indole-3-Butyric Acid (IBA) has been shown to be useful in promoting the propagation of *Osyris lanceolata* by stem cuttings and marcots. The concentration to be used should be 50 ppm for those raised in September. However if cuttings or marcotts have to be initiated in June, then a concentration of 100 ppm may be appropriate.

8.3. Specific recommendations for further investigation

The following are recommended for further investigation

1. Cross border assessment and inclusion of more populations to cover national and regional scales are recommended. As observed in the present study, there exists a big difference in sandalwood quality among populations within and between ecozones. The northern ecozone have shown superiority in oil quality to the southern ezozone. However, morphologically, the southern ecozone has bigger trees compared to the northern ecozone. More sampling is thus recommended to further confirm these. What factors exactly control the quality of wood need also to be determined. This is an essential procedure if breeding programme for improvement of the species for quality wood has to be successful. Breeding programmes should aim at capturing both good quality oil and growth rate of the species.
2. While the amount of santalol in *Osyris lanceolata* has been used as a determinant of wood quality, the existence of other useful compounds in lager quantities as in the case lanceol (57%) in comparison with santalol, which was only 36% may serve as additional criteria for quality assessment if the use and market potentials of these compounds are properly studied. Other useful compounds could also be identified if similar study is extended to other populations within Tanzania and elsewhere.
3. Trials to plant *Osyris lanceolata* and observe its performance in the field is recommended as next step in further studies. This should be coupled with investigation on the suitability of various hosts at different stages of growth of *Osyris lanceolata*. As reported in *Santalum album*, suitability of hosts changes at different stages as the plant grows to maturity. Host species with commercial value such as timber, fuelwood and fruits might also be worth investigating so as to derive multiple benefits from the same piece of land.
4. More trials to enhance the regeneration of *Osyris lanceolata* in its wilderness need to be established to hasten its recovery especially in over-exploited stands. Techniques such root wounding and trenching might prove worth trying. The

importance of regeneration from seed should not also be overlooked, as its contribution in overall regeneration is not insignificant and there may be possibilities to enhance this. For effective pollination and seeding, determination of critical distance between males and females need to be assessed as pollen has been shown to be somehow limiting in *Osyris lanceolata* . This critical distance will assit in determining how trees should be harvested in a stand without interfering much in the reproductive processes.

5. Further work is still needed to improve the seed storage method in order to increase the viability of seeds of *Osyris lanceolata* over longer period. The most important aspect to consider is perhaps maintenance of moisture content seeds that have proved useful. Dehydration or excessive absorption of moisture in the stored seeds might change the initial moisture content, thus lowering the viability due to collapse of cell walls or excessive respiration. Incorporation of seed storage gases in the containers might help to reduce respiration and prolong the life span of seeds.

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APPENDICES

Appendix 1. Composition of species encountered in *Osyris lanceolata* supporting populations at six sites, their stocking/ hectare and basal area (cm^2) contribution/per hectare

Species	Family	Nundu-Njombe		Sao Hill-Mufindi		Image-Iringa		Bereko-Babati		Mgwashi Lushoto		Gubali-Kondoa	
		Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area
Acacia gerrardii	Mimosaceae	-	-	-	-	-	-	1.7	82	0.4	21	-	-
Acacia hockii	Mimosaceae	-	-	-	-	16.4	585	84.2	4894	46.8	468	42.2	1081
Acacia kirkii	Mimosaceae	-	-	-	-	0.5	19	-	-	-	-	-	-
Acacia nilotica	Mimosaceae	-	-	-	-	0.8	135	26.9	2646	-	-	0.8	58
Acacia robusta	Mimosaceae	-	-	-	-	0.3	98	-	-	-	-	-	-
Acacia senegal	Mimosaceae	-	-	-	-	-	-	-	-	-	-	0.6	10
Acacia sieberiana	Mimosaceae	-	-	0.7	302	3.3	854	-	-	-	-	-	-
Acokanthera schimperi	Apocynaceae	-	-	-	-	1.0	11	-	-	-	-	-	-
Aeschynomene abyssinica	Papilionaceae	-	-	-	-	6.7	71	-	-	-	-	-	-
Agauria salicifolia	Ericaceae	31.0	5179	-	-	-	-	-	-	-	-	-	-
Albizia glaberrima	Mimosaceae	-	-	-	-	17.7	1265	1.7	253	-	-	-	-
Albizia gummifera	Mimosaceae	12.8	1091	8.1	375	-	-	-	-	-	-	-	-
Albizia harveyi	Mimosaceae	-	-	-	-	1.5	214	3.4	329	-	-	0.3	4
Albizia petersiana	Mimosaceae	-	-	-	-	2.8	120	-	-	-	-	5.6	564
Albizia tanganyicensis	Mimosaceae	-	-	-	-	-	-	-	-	-	-	0.6	115
Albizia zimmermanni	Mimosaceae	-	-	-	-	-	-	-	-	-	-	1.9	163
Allophyllus africanus	Sapindaceae	-	-	-	-	1.5	46	-	-	-	-	-	-

Species	Family	Nundu-Njombe		Sao Hill-Mufindi		Image-Iringa		Bereko-Babati		Mgwashi Lushoto		Gubali-Kondoa	
		Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area
Allophyllus congolanus	Sapindaceae	-	-	-	-	0.3	3	-	-	-	-	-	-
Aphloia theiformis	Flacourtiaceae	466.9	15773	-	-	-	-	-	-	-	-	-	-
Apodytes dimidiata	Icacinaceae	14.5	595	32.6	1088	1.3	36	-	-	0.4	3	-	-
Azanza garckeana	Malvaceae	-	-	-	-	-	-	4.3	141	-	-	-	-
Bersama abyssinica	Melianthaceae	22.1	390	9.5	118	-	-	-	-	-	-	-	-
Boscia salicifolia	Capparidaceae	-	-	-	-	-	-	-	-	-	-	0.3	39
Brachystegia glaberrima	Caesalpiniaceae	-	-	-	-	140.3	16373	-	-	-	-	-	-
Brachystegia microphylla	Caesalpiniaceae	-	-	-	-	-	-	-	-	-	-	3.6	115
Brachystegia spiciformis	Caesalpiniaceae	-	-	-	-	74.1	6639	165.0	8537	-	-	2.5	108
Brachystegia utilis	Caesalpiniaceae	-	-	-	-	152.8	11287	222.2	8317	-	-	1.1	118
Bridelia micrantha	Euphorbiaceae	6.9	174	0.5	62	2.3	49	-	-	-	-	-	-
Buddleia salviifolia	Loganiaceae	2.1	71	4.4	133	-	-	-	-	-	-	-	-
Byrsocarpum orientalis	Connaraceae	-	-	17.4	211	0.5	12	-	-	-	-	-	-
Cadaba farinose	Capparidaceae	-	-	-	-	-	-	0.4	42	-	-	-	-
Caloncoba welwitschii	Flacourtiaceae	0.3	13	-	-	-	-	-	-	-	-	-	-
Canthium burttii	Rubiaceae	6.6	209	-	-	1.0	11	3.4	142	-	-	5.8	90
Canthium mombazense	Rubiaceae	-	-	-	-	-	-	-	-	0.4	3	-	-
Carissa edulis	Apocynaceae	-	-	0.2	3	2.3	52	-	-	12.1	107	-	-
Catha edulis	Celastraceae	65.9	3548	9.5	329	0.3	2	-	-	14.6	133	-	-
Canthium oligocarpum	Rubiaceae	-	-	-	-	0.3	3	-	-	-	-	-	-
Catunaregam spinosa	Rubiaceae	-	-	-	-	6.2	108	1.3	22	-	-	2.5	97
Chassalia parvifolia	Rubiaceae	0.3	3	-	-	-	-	-	-	-	-	-	-
Cissus milnei	Vitaceae	-	-	-	-	-	-	0.4	7	-	-	-	-
Clausena anisata	Rutaceae	3.4	31	1.6	18	-	-	-	-	-	-	-	-

Species	Family	Nundu-Njombe		Sao Hill-Mufindi		Image-Iringa		Bereko-Babati		Mgwashi Lushoto		Gubali-Kondoa	
		Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area
Clerodendron myricoides	Verbenaceae	-	-	0.5	4	15.6	192	5.1	154	0.4	4	1.9	19
Combretum molle	Combretaceae	-	-	-	-	-	-	21.8	1395	15.4	157	59.7	1857
Combretum zeyheri	Combretaceae	-	-	-	-	28.5	1851	0.4	42	-	-	17.5	600
Commiphora africana	Burseraceae	-	-	-	-	-	-	1.3	47	-	-	0.3	81
Commiphora eminiii	Burseraceae	-	-	-	-	1.0	24	-	-	-	-	-	-
Commiphora mossambicensis	Burseraceae	-	-	-	-	1.3	33	0.9	52	-	-	1.7	130.1
Cordia monoica	Boraginaceae	-	-	-	-	-	-	-	-	-	-	0.3	4
Crotolaria laburnifolia	Papilionaceae	-	-	-	-	0.5	4	-	-	-	-	-	-
Croton macrostachys	Euphorbiaceae	-	-	0.5	21	1.0	22	-	-	-	-	-	-
Croton polytrichus	Euphorbiaceae	-	-	-	-	-	-	-	-	-	-	0.6	7
Cussonia spicata	Araliaceae	6.9	352	2.3	313	-	-	-	-	-	-	-	-
Dais cotinifolia	Thymeleaccae	31.0	1189	3.0	81	-	-	-	-	-	-	-	-
Dalbergia nitidula	Papilionaceae	-	-	-	-	22.6	728	2.1	107	-	-	49.7	1245
Dichrostachys cinerea	Mimosaceae	-	-	-	-	19.5	294	0.9	16	9.6	102	0.3	4
Diospyros whiteana	Ebenaceae	39.7	411	-	-	-	-	-	-	-	-	-	-
Diospyros zombensis	Ebenaceae	-	-	-	-	12.8	292	-	-	-	-	-	-
Dissotis bussei	Melastomataceae	5.5	295	-	-	0.5	18	-	-	-	-	-	-
Dodonaea viscose	Sapindaceae	6.9	105	23.3	861	15.9	190	5.6	102	17.1	151	4.2	41
Dombeya burgessiae	Sterculiaceae	0.7	49	-	-	-	-	-	-	-	-	-	-
Dombeya rotundifolia	Sterculiaceae	-	-	15.1	491	-	-	-	-	-	-	-	-
Dombeya shupangae	Sterculiaceae	-	-	0.5	175	8.7	236	-	-	-	-	-	-
Dracaena deremensis	Agavaceae	0.7	11	-	-	-	-	-	-	-	-	-	-
Ekebergia benguellensis	Meliaceae	-	-	-	-	-	-	-	-	-	-	2.8	130
Erica arborea	Ericaceae	-	-	0.7	17	-	-	-	-	-	-	-	-

Species	Family	Nundu-Njombe		Sao Hill-Mufindi		Image-Iringa		Bereko-Babati		Mgwashi Lushoto		Gubali-Kondoa	
		Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area
Erythrina abyssinica	Papilionaceae	-	-	2.6	383	1.3	86	-	-	-	-	-	-
Erythroxylum fischeri	Erythroxylaceae	35.2	3691	-	-	2.6	88	-	-	-	-	-	-
Euclea divinorum	Ebenaceae	12.8	415	-	-	-	-	-	-	-	-	-	-
Euclea natalansis	Ebenaceae	-	-	0.2	14	20.0	711	8.5	375	6.4	64	25.8	964
Euclea racemosa	Ebenaceae	-	-	-	-	-	-	2.1	54	-	-	-	-
Euphorbia candelabrum	Euphorbiaceae	-	-	-	-	2.6	130	26.5	3951	2.1	135	23.9	1726
Euphorbia cuneata	Euphorbiaceae	-	-	-	-	-	-	0.9	98	-	-	5.0	131
Euphorbia grantii	Euphorbiaceae	-	-	-	-	-	-	5.1	164	-	-	12.2	175
Euphobia usambarica	Euphobiaceae	0.3	3	-	-	-	-	-	-	-	-	-	-
Euphorbia nyikae	Euphobiaceae	-	-	-	-	-	-	-	-	76.8	4263	-	-
Faurea rochetiana	Proteacea	-	-	-	-	3.8	163	-	-	-	-	-	-
Faurea saligna	Proteacea	-	-	-	-	23.1	1577	-	-	-	-	-	-
Ficus natalensis	Moraceae	-	-	-	-	-	-	-	-	-	-	0.6	14
Ficus scassellatii	Moraceae	-	-	-	-	3.1	342	-	-	-	-	-	-
Ficus thonningii	Moraceae	-	-	-	-	0.5	7	-	-	-	-	-	-
Flacourtia indica	Flacourtiaceae	0.3	3	3.0	425	10.0	172	0.4	71	-	-	-	-
Flueggea virosa	Euphorbiacea	-	-	-	-	0.3	2	-	-	-	-	0.6	5
Garcinia huillensis	Guttiferae	-	-	-	-	1.8	27	-	-	-	-	-	-
Gardenia ternifolia	Rubiaceae	-	-	-	-	2.3	100	-	-	0.4	3	-	-
Gnidia sp	Thymelaceae	-	-	0.2	2	-	-	-	-	-	-	-	-
Grewia bicolor	Tiliaceae	-	-	-	-	-	-	2.6	89	-	-	-	-
Grewia holstii	Tiliaceae	-	-	-	-	-	-	-	-	-	-	3.6	85
Grewia similes	Tiliaceae	0.7	7	-	-	-	-	-	-	-	-	-	-
Grewia tembensis	Tiliaceae	-	-	-	-	-	-	0.4	14	-	-	-	-

Species	Family	Nundu-Njombe		Sao Hill-Mufindi		Image-Iringa		Bereko-Babati		Mgwashi Lushoto		Gubali-Kondoa	
		Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area
Halleria lucida	Scrophulariaceae	0.7	19	0.2	4	-	-	-	-	-	-	-	-
Heteromorpha trifoliata	Umbelliferae	29.7	1327	-	-	6.4	108	-	-	-	-	-	-
Hymenodictyon floribundum	Rubiaceae	-	-	-	-	0.3	3	-	-	-	-	0.3	13
Indigofera rhynchocarpa	Papilionaceae	-	-	-	-	7.7	83	9.8	238	-	-	3.1	46
Jasminum odoratissimum	Oleaceae	40.7	684	-	-	-	-	-	-	-	-	-	-
Kigelia africana	Bignoniaceae	-	-	-	-	0.3	98	-	-	-	-	-	-
Kotschya goetzei	Papilionaceae	-	-	2.1	19	-	-	-	-	-	-	-	-
Kotschya aeschynomenoides	Papilionaceae	12.1	316	-	-	-	-	-	-	-	-	-	-
Landolphia buchananii	Apocynaceae	-	-	-	-	0.5	8	-	-	-	-	-	-
Lannea schimperi	Anacardiaceae	-	-	3.5	137	10.3	1055	1.7	394	1.4	27	9.4	600
Lippia kituiensis	Verbenaceae	-	-	-	-	-	-	-	-	0.7	6	-	-
Lonchocarpus bussei	Papilionaceae	-	-	-	-	-	-	2.6	152	-	-	1.1	55
Ludia mauritiana	Flacourtiaceae	-	-	-	-	-	-	-	-	0.7	5	-	-
Maesa lanceolata	Myrsinaceae	10.7	825	0.2	3	-	-	-	-	-	-	-	-
Maprounea africana	Euphorbiaceae	-	-	-	-	7.2	102	-	-	-	-	-	-
Markhamia lutea	Bignoniaceae	-	-	-	-	-	-	-	-	-	-	0.3	6
Markhamia obtusifolia	Bignoniaceae	-	-	-	-	3.1	144	-	-	-	-	0.8	22
Maytenus heterophylla	Celastraceae	2.8	36	129.5	2340	-	-	-	-	15.0	137	-	-
Maytenus mossambicensis	Celastraceae	-	-	-	-	11.5	154	-	-	-	-	-	-
Memecylon greenwayi	Melastomataceae	1.0	8	-	-	-	-	-	-	-	-	-	-
Myrica salicifola	Myricaceae	22.1	2704	22.1	790	-	-	-	-	-	-	-	-
Mundulea sericea	Papilionaceae	-	-	-	-	19.7	488	-	-	-	-	-	-
Myrica africana	Myricaceae	-	-	0.5	4	-	-	-	-	-	-	-	-
Myrsine africana	Myrsinaceae	-	-	1.9	20	-	-	-	-	-	-	-	-

Species	Family	Nundu-Njombe		Sao Hill-Mufindi		Image-Iringa		Bereko-Babati		Mgwashi Lushoto		Gubali-Kondoa	
		Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area
Myrsine melanophloeos	Myrsinaceae	167.9	8507	20.9	515	-	-	-	-	-	-	-	-
Mystroxylon aethiopicum	Celastraceae	-	-	-	-	1.5	51	-	-	-	-	-	-
Nuxia floribunda	Loganiaceae	2.4	721	-	-	-	-	-	-	-	-	-	-
Ochna holstii	Ochnaceae	-	-	0.2	6	14.6	270	0.9	26	-	-	0.3	11
Ochna macrocalyx	Ochnaceae	-	-	-	-	0.3	2	-	-	-	-	-	-
Olea europaea	Oleaceae	-	-	-	-	1.0	209	-	-	1.8	17	-	-
Olinia rochetiana	Oliniaceae	28.3	1360	0.9	60	-	-	-	-	-	-	-	-
Ormocarpum kirkii	Papilionaceae	-	-	-	-	45.6	778	4.3	218	-	-	1.7	39
Ormocarpum trichocarpum	Papilionaceae	-	-	-	-	-	-	-	-	-	-	1.7	35
Osyris lanceolata	Santalaceae	39.9	2209	38.2	1474	44.7	829	75.9	1555	46.2	466	70.0	1282
Ozoroa insignis	Anacardiaceae	-	-	-	-	1.3	172	1.3	201	3.9	48	1.9	139
Pappea capensis	Sapindaceae	-	-	-	-	1.5	156	3.0	405	3.2	36	1.9	134
Pavetta schumanniana	Rubiaceae	-	-	-	-	2.1	22	0.4	12	-	-	1.7	26
Pavetta gardeniifolia	Rubiaceae	-	-	-	-	-	-	11.1	222	-	-	-	-
Peddiea fischeri	Thymelaceae	2.4	43	-	-	-	-	-	-	-	-	0.4	6
Plectranthus barbatus	Labiatae	-	-	-	-	-	-	-	-	3.2	77	1.1	15
Plectranthus igniaris	Labiatae	-	-	-	-	-	-	-	-	3.6	44	-	-
Pleurostylia africana	Celastraceae	-	-	-	-	0.8	29	-	-	-	-	0.6	17
Premna resinosa	Verbenaceae	-	-	-	-	0.3	6	-	-	-	-	0.3	4
Protea madiensis	Proteaceae	-	-	3.5	93	-	-	-	-	52.5	569	-	-
Psorospermum febrifugum	Guttiferae	-	-	6.3	61	-	-	-	-	-	-	-	-
Psychotria cyathicalyx	Rubiaceae	54.8	949	-	-	-	-	-	-	-	-	-	-
Psychotria kirkii	Rubiaceae	-	-	-	-	-	-	-	-	-	-	4.7	62
Psychotria lauracea	Rubiaceae	-	-	4.4	108	-	-	-	-	-	-	-	-

Species	Family	Nundu-Njombe		Sao Hill-Mufindi		Image-Iringa		Bereko-Babati		Mgwashi Lushoto		Gubali-Kondoa	
		Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area
Rhamnus prinoides	Rhamnaceae	30.7	577	-	-	-	-	-	-	0.7	6	-	-
Rhoicissus tridentata	Vitaceae	-	-	-	-	1.3	12	-	-	-	-	-	-
Rhus natalensis	Anacardiaceae	166.6	4266	46.8	1117	51.8	938	97.4	4179	8.2	84	75.0	1272
Rhus vulgaris	Anacardiaceae	-	-	-	-	14.1	377	-	-	18.2	167	-	-
Rothmannia fischeri	Rubiaceae	-	-	-	-	-	-	-	-	-	-	1.1	18
Rutidea orientalis	Rubiaceae	1.0	27	0.5	4	-	-	-	-	-	-	-	-
Rytigynia uhligii	Rubiaceae	-	-	-	-	5.4	58	-	-	0.4	4	0.6	48
Schefflera volkensii	Araliaceae	-	-	2.3	137	0.3	5	0.9	31	-	-	-	-
Schrebera alata	Oleaceae	-	-	4.2	245	22.8	636	-	-	-	-	-	-
Scutia myrtiana	Rhamnaceae	-	-	-	-	-	-	-	-	1.1	10	-	-
Securidaca longepedunculata	Polygalaceae	-	-	-	-	1.5	134	-	-	-	-	-	-
Senna singueana	Caesalpiniaceae	-	-	-	-	19.2	598	3.0	183	1.1	9	6.9	162
Solanesino mannii	Compositae	-	-	-	-	0.3	3	-	-	-	-	-	-
Sterculia quingueloba	Sterculiaceae	-	-	-	-	-	-	-	-	-	-	1.9	272
Steganotaenia araliacea	Umbelliferae	-	-	-	-	0.5	18	0.4	20	-	-	5.8	180
Stoebe kilimanscharica	Compositae	-	-	0.2	5	-	-	-	-	-	-	-	-
Strychnos henningsii	Loganiaceae	-	-	0.7	45	-	-	1.7	40	-	-	-	-
Strychnos spinosa	Loganiaceae	-	-	-	-	2.1	53	-	-	-	-	-	-
Strychnos potatorum	Loganiaceae	-	-	-	-	-	-	-	-	-	-	0.3	8
Syzygium cordatum	Myrtaceae	-	-	1.9	1365	0.3	13	-	-	-	-	-	-
Tapiphyllum cinerascens	Rubiaceae	-	-	-	-	3.8	70	-	-	-	-	0.8	18
Tarenna fisheri	Rubiaceae	-	-	-	-	-	-	-	-	-	-	2.2	8
Tarenna neurophylla	Rubiaceae	-	-	-	-	21.3	766	-	-	-	-	8.1	158
Tecomaria capensis	Bignoniaceae	85.5	1474	67.7	1388	-	-	-	-	-	-	-	-

Species	Family	Nundu-Njombe		Sao Hill-Mufindi		Image-Iringa		Bereko-Babati		Mgwashi Lushoto		Gubali-Kondoa	
		Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area	Stocking	B. area
Tephrosia nyikensis	Papilionaceae	-	-	0.2	2	-	-	-	-	-	-	-	-
Terminalia sericea	Combretaceae	-	-	-	-	-	-	-	-	-	-	3.1	148
Tetradenia riparia	Labiatae	-	-	-	-	-	-	-	-	-	-	1.1	14
Tinnea aethiopica	Labiatae	-	-	-	-	-	-	-	-	0.4	3	-	-
Tricalysia ruandensis	Rubiaceae	-	-	-	-	-	-	-	-	0.4	3	5.0	107
Trimeria grandiflora	Flacourtiaceae	-	-	-	-	-	-	-	-	6.4	75	-	-
Uapaca kirkiana	Euphorbiaceae	-	-	-	-	0.8	34	-	-	-	-	-	-
Uvaria volkensii	Annonaceae	-	-	-	-	-	-	0.4	6	-	-	-	-
Vangueria infausta	Rubiaceae	-	-	7.7	141	1.8	45	9.8	396	3.6	47	2.8	56
Vepris simplicifolia	Rutaceae	-	-	-	-	0.5	6	-	-	-	-	-	-
Vepris stolzii	Rutaceae	0.3	6	-	-	-	-	-	-	-	-	-	-
Vernonia exsertiflora	Compositae	-	-	-	-	1.5	44	0.4	9	-	-	-	-
Vernonia lasiopus	Compositae	-	-	0.5	5	-	-	-	-	-	-	-	-
Vitex payos	Verbenaceae	-	-	-	-	0.8	29	-	-	-	-	-	-
Ximenia caffra	Olacaceae	-	-	-	-	13.6	295	4.7	261	-	-	2.2	45
Zahna africana	Sapindaceae	-	-	-	-	0.3	18	-	-	-	-	-	-
	Total	**1,473**	**59,665**	**503**	**1,5512**	**979**	**5,4203**	**829**	**4,0416**	**375**	**7,453**	**496**	**1,4800**

Appendix 2. Climatic conditions of the study sites for the past 10-30 years

Stand/population	Aspect	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total/mean
Bereko-Babati	Rainfall (mm)	79	90	141	184	69	0	0	0	0	7	56	117	743
	Max. Temp (°C)	26.7	27.3	27.3	25.6	23.6	22.3	21.7	22.8	24.5	26.7	27.3	26.7	25.2
	Min. Temp (°C)	14.7	14.7	16.3	16.3	15.2	13.0	12.5	12.5	13.6	14.1	15.8	15.8	14.5
	Evapotrans (mm)	133	131	141	122	113	105	111	125	139	159	141	133	1550
Gubali-Kondoa	Rainfall (mm)	112	93	112	81	28	2	0	0	0	3	24	103	557
	Max. Temp (°C)	28.3	28.3	27.2	26.7	25.6	25.0	25.0	25.6	27.8	28.9	29.4	29.4	27.3
	Min. Temp (°C)	16.7	16.7	16.7	16.7	15.0	12.8	11.7	12.8	13.9	15.6	17.2	17.2	15.3
	Evapotrans (mm)	120	111	118	108	98	93	103	115	139	157	146	134	1442
Image-Iringa	Rainfall (mm)	131	117	123	52	16	0	0	2	1	6	10	143	600
	Max. Temp (°C)	26.4	25.7	26.0	26.5	26.1	25.6	24.4	25.1	26.7	28.2	29.0	27.7	26.5
	Min. Temp (°C)	15.7	15.2	14.9	14.7	13.7	12.4	12.2	12.0	12.8	13.9	14.7	15.7	14.0
	Evapotrans (mm)	118	103	109	101	102	99	104	116	131	148	141	125	1397
Mgwashi-Lushoto	Rainfall (mm)	80	97	115	232	161	38	39	17	2	91	159	150	1182
	Max. Temp (°C)	22.8	23.9	23.1	22.3	20.2	18.3	18.1	17.2	19.5	20.9	21.3	21.8	20.8
	Min. Temp (°C)	14.1	16.1	16.4	15.1	14.7	12.4	11.4	10.8	11.7	14.3	15.4	13.4	13.8
Nundu-Njombe	Rainfall (mm)	260	219	351	266	33	5	2	2	1	14	109	246	1508
	Max. Temp (°C)	20.2	20.5	20.0	18.2	17.4	17.0	17.0	18.3	19.5	21.5	22.6	20.7	19.4
	Min. Temp (°C)	10.1	10.6	9.6	9.3	7.5	5.2	4.6	5.3	6.2	8.0	9.1	9.3	7.9
	Evapotrans (mm)	92	83	83	70	61	55	66	79	91	111	109	93	993
Sao Hill-Mufindi	Rainfall (mm)	181	156	192	87	16	2	1	1	4	6	52	173	872
	Max. Temp (°C)	21.9	23.8	21.9	21.1	20.2	19.5	18.8	19.9	22.3	23.8	24.2	22.8	21.7
	Min. Temp (°C)	12.9	12.8	12.7	12.2	10.4	8.0	7.4	8.2	9.1	11.2	12.2	12.8	10.8
	Evapotrans (mm)	109	103	107	98	89	80	86	103	130	142	125	117	1289