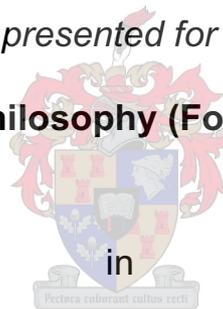


**EFFECT OF POPULATION SIZE ON VIABLE SEED OUTPUT, SEED
RAIN AND NATURAL REGENERATION PATTERN OF A TROPICAL
CONIFER *WIDDRINGTONIA WHYTEI* - RENDLE IN MALAWI**

by

Tembo Faera Chanyenga

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Doctor of Philosophy (Forest Science)



Department of Forest and Wood Science
Faculty of AgriSciences
**Stellenbosch University,
Stellenbosch, South Africa**

Supervisor: Prof. Coert Johannes Geldenhuys
Co-Supervisor: Dr. Moctar Sacande

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DECLARATION

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ABSTRACT

Widdringtonia whytei is a tropical endemic, fire-adapted pioneer coniferous tree species within natural fire-fragmented Afromontane forest patches in a confined area on Mulanje Mountain in Malawi. Natural and anthropogenic fires within the surrounding fire-prone landscape, insect attacks, and uncontrolled harvesting of mature trees for timber threaten the survival of *W. whytei*. This study investigated the effects of population fragmentation on the reproductive biology of *W. whytei*, through four specific studies: effects of population size, tree size and crown position on viable seed output; seed rain variation among population sizes; effects of temperature and light on viability and germination of *W. whytei* seeds; and the influence of population size on natural regeneration patterns.

The study was conducted during 2008 and 2009 on three sites using three *W. whytei* population sizes: small (≤ 10 reproductively mature cone-bearing *W. whytei* trees), medium (11-20 cone-bearing trees) and large (> 20 cone-bearing trees). Data were collected using field and laboratory experiments and a review of secondary information. The effect of fragment size and crown position on viable seed output and seed rain pattern was tested with a nested linear mixed model. The influence of stem diameter (dbh) on viable seed output was tested using a separate data set. The variation in seed rain was explored with a generalised linear model (GLM) with a negative binomial link function. Variations in seed germination was analysed with a Weibull regression model. The influence of fragment size on seedling regeneration was tested with a GLM (binary logistic regression).

Viable seed output from *W. whytei* cones was very low (23%) and was not affected by population size, tree diameter or crown position. *W. whytei* population size influenced seed rain with large fragments having higher seed rain densities. Number of dispersed seeds was generally very low and poorly dispersed. *W. whytei* cones are semi-serotinous and depends on moderate to severe fires for heavy seedfall and wider dispersal. Such fires did not occur during the study period. Temperature is a critical factor for seed germination. Seeds germinate between 15 and 25°C with the optimum at ~ 20°C either under light or dark conditions. Regeneration density followed the seed rain pattern indicating that seed availability is a critical factor for regeneration. Regular cool fires outside the forest patches and along the edges during the hot dry season, and the thick litter layer and shady conditions inside forest patches, caused most of the seedling mortality.

This study highlighted the difficulties *W. whytei* experiences with low output of viable seed, limited seed dispersal and low seedling regeneration in and around forest patches, i.e. factors important for conservation management of this species. It is recommended that gaps with diameter equal to canopy height and occasional spot fires would promote seed dispersal onto exposed mineral soil for rapid seedling establishment and subsequent population growth. Such natural fire disturbance events may show the true seed rain and seedling establishment patterns in this species.

OPSOMMING

Widdringtonia whytei is 'n tropiese endemiese, vuuraangepaste pionier naaldhoutagtige boomsoort binne die natuurlike vuurgefragmenteerde Afrotropiese woudkolle binne 'n beperkte area op Mulanje berg in Malawi. Natuurlike en menslike vure binne die omringende vuurgeneigde landskap, insekaanvalle, en onbeheerde kap van volwasse bome vir hout bedreig die voortbestaan van *W. whytei*. Hierdie studie het die effek van populasiefragmentasie op die voortplantingsbiologie van *W. whytei* ondersoek, deur vier spesifieke studies: die effek van populasiegrootte, boomgrootte en kroonposisie op produksie van lewenskragtige saad; saadreënvariasie tussen populasiegroottes; die effek van temperatuur en lig op lewenskragtigheid en ontkieming van *W. whytei* saad; en die invloed van populasiegrootte op natuurlike verjongingspatrone.

Die studie is gedurende 2008 en 2009 uitgevoer op drie groeiplekke met drie *W. whytei* populasiegroottes: klein (≤ 10 keëldraende (volwasse) *W. whytei* bome), medium (11-20 keëldraende bome) en groot (> 20 keëldraende bome). Data is versamel in veld- en laboratorium eksperimente asook 'n oorsig van sekondêre inligting. Die invloed van populasie fragmentgrootte en kroonposisie van bome op saadproduksie en die saadreënpatroon is getoets met 'n geneste liniêre gemengde model. Die invloed van boomstamdeursnee op die produksie van kiemkragtige saad is met 'n aparte datastel getoets. Die variasie in saadreënpatroon is met 'n algemene liniêre model (GLM) met 'n negatiewe binomiese skakelfunksie getoets. Variasies in ontkiemingspersentasie van sade is met 'n Weibull regressiemodel ontleed. Die invloed van fragmentgrootte op saailingverjonging is met 'n algemene liniêre model (binêre logistiese regressie) getoets.

Produksie van lewenskragtige saad in *W. whytei* keëls was baie laag (23%) en was nie deur populasiegrootte, boomstamdeursnee of kroonposisie beïnvloed nie. *W. whytei* populasiegrootte het saadreën beïnvloed en groot fragmente het groter saadreëndigthede gehad. Die aantal verspreide sade was in die algemeen baie laag en swak versprei. *W. whytei* keëls is semi-saadhoudend (serotinous) en is afhanklik van matige tot intense vure vir massiewe saadvrystelling en wyer saadverspreiding. Sulke vure het nie gedurende die studieperiode voorgekom nie. Temperatuur is 'n kritiese faktor vir saadontkieming. Sade ontkiem goed tussen 15 en 25°C met die optimum rondom $\sim 20^\circ\text{C}$ onder beide lig en donker toestande. Fragmentgrootte het nie die saailingverjonging in *W. whytei* beïnvloed nie. Verjongingsdigtheid het die saadreënpatroon gevolg, wat daarop dui dat saadbeskikbaarheid 'n kritiese faktor is vir saailingverjonging. Gereelde koel vure buite die woudkolle en langs die woudrand gedurende die droë warm seisoen, en die dik lae blaarval (litter) en skadutoestande binne 'n woud, het meeste van die saailingmortaliteit veroorsaak.

Hierdie studie het die probleme beklemtoon wat *W. whytei* ondervind met die lae produksie van kiemkragtige saad, saadverspreiding en saailingverjonging binne en rondom die woudkolle, m.a.w. faktore wat belangrik is vir bewaringsbestuur van hierdie boomsoort. Die aanbeveling word gemaak dat kroonopeninge met 'n deursnee gelyk

aan minstens die kroonhoogte, en sporadiese kolbrande binne die woudkollie kan saadverspreiding op blootgestelde minerale grond vir vinnige saailingvestiging en gevolglike populasiegroei bevorder. Sodanige vuurversteuringsgeleenthede mag die werklike patrone in saadreën en saailingvestiging in hierdie spesies toon.

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DEDICATION

This thesis is dedicated to the following: my wife Margret and my six children Dominic, Vincent, Francis, Ruth, Manuel and Kelvin for enduring with me throughout the study period. They have always been wondering as to when I would stop being a student. The thesis is also dedicated to all those who endure hard conditions.

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LIST OF ACRONYMS

CP	Crown Position
DBH	Diameter at Breast Height
FAO	Food and Agriculture Organisation
FD	Forestry Department
FRIM	Forestry Research Institute of Malawi
GLM	General Linear Model
ISTA	International Seed Testing Association
LMAFDP	Lake Malawi Artisan Fisheries development Project
MNTSC	Malawi National Tree Seed Centre
MMCT	Mulanje Mountain Conservation Trust
MMFR	Mulanje Mountain Forest Reserve
MSBP	Millennium Seed Bank Project - Kew
NSO	National Statistics Office

CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND

How would one deal with an endemic tree species which has a fragmented distribution on top of an isolated mountain massif but which has shown economic potential as timber tree? What are the ecological characteristics of this species that enabled it to survive on this isolated habitat and how could this be used to implement a management system that would ensure survival of this species on the mountain but enable development of its economic potential? The species in question here is *Widdringtonia whytei* (Mulanje cedar) on Mulanje Mountain in south-eastern Malawi.

Malawi is a long, narrow country in southern Africa, lying between latitudes 09°22' and 17°08'South and longitudes 33°40' and 35°55'East. It covers an area of 11.8 million hectares, of which Lake Malawi, Lake Chilwa and Lake Malombe occupy 2.4 million hectares (Chirwa 2002).

The country supports one of the highest population densities in Africa, its inhabitants numbering nearly 14 million (NSO 2008). About 90% of this population lives in rural areas and depends on subsistence agriculture and forest resources for livelihood. In the early 1900s the Malawi Government set aside some areas for permanent forest cover

such as forest reserves, national parks and wildlife reserves for water, soil and wildlife conservation. The forest resources, however, have attained a retrogressive trend over the years due to deforestation. At present, forests cover only about 2.6 million hectares, or 27.2% of the total land area of Malawi (FAO 2010).

The study area, Mulanje Mountain, is located in the south-eastern corner of southern Malawi, between latitudes 15°50' and 16°03'South, and longitudes 35°30' and 35°47'East (Figure 1.1). Mulanje Mountain rises spectacularly above the plains and is the highest mountain in tropical southern Africa, rising to >3000 m above sea level. It covers an area of c 650 km² (Chapman et al. 1991). Dixey (1927) described the physiography of Mulanje Mountain in great detail. It is an example of an inselberg – a formation composed of erosion-resistant rock formed some 130 million years ago (Crow 1981). The soils generally consist of humic ferrisols, containing high levels of silt fraction, and are acidic with a pH ranging between 4.2 - 4.9 (Chapman and White 1970). In 1924 the Nyasaland (Malawi) Geological Survey discovered the existence of superficial deposits of bauxite on the Lichenya Plateau of the Mountain (Dixey 1925).

The mountain exhibits a 'dry region' predominantly on the west and north side, and a wet region on the east and south sides. The wetter south-east side is due to the prevailing weather fronts from the Mozambique coast and mountains. On average 2859 mm of rain is recorded from the Lichenya Plateau, which is on the wetter south-east (Chapman 1995).

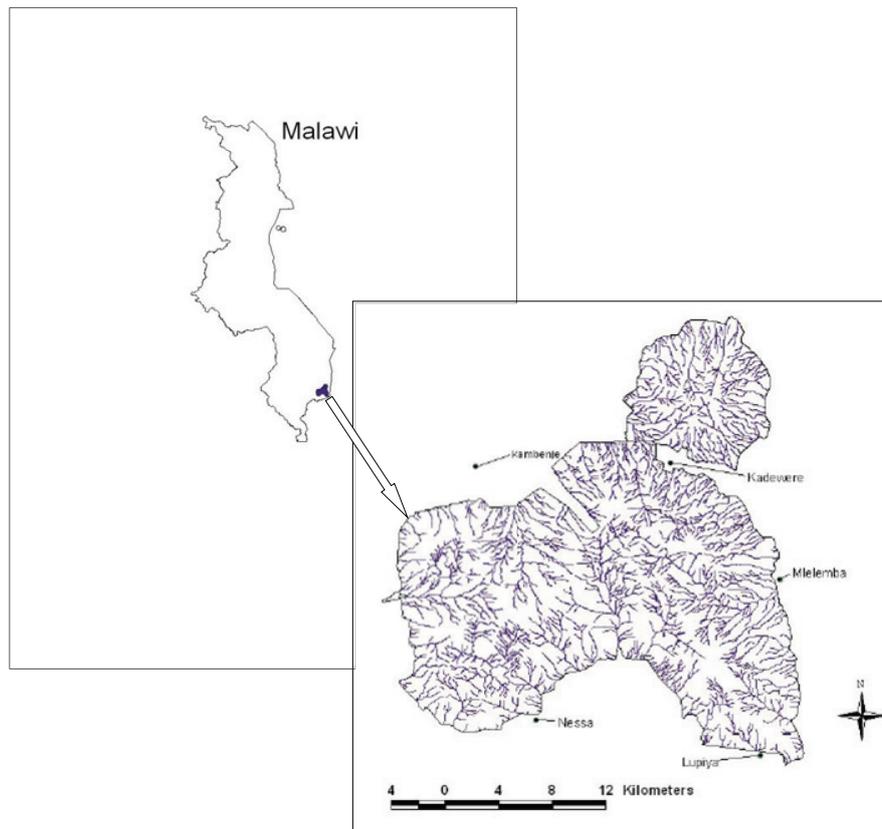


Figure 1.1: Location of Mulanje Mountain in Malawi (Bayliss et al. 2007)

At first glance, the Mulanje Mountain looks like a massive barren rock but when hiking the mountain one is bound to come across three types of forests (White 1970).

1. *Lowland forests:* They comprise Miombo woodland and lowland rainforest that exist at 600-950 m above mean sea level (asl) as relics around the foothills in the south and southeast of Mulanje Mountain. Miombo woodland is the principal woody community of the drier lower northern, eastern and western piedmont slopes. These open to closed woodlands belong to the Zambezian Centre of Endemism (White 1983). Miombo woodland contains a number of species of *Brachystegia* and common related genera such as *Julbernardia*. The lowland

rainforest on the other hand, occurs as remnants along the streams on the lower slopes and on tea estates at the foot of the mountain. The most dominant species of these forests are *Newtonia buchananii* and *Khaya anthotheca*. In Mulanje, lowland forests are extremely limited and being of lower altitude, have suffered greatly from timber felling, simple cultivation and estate clearances. For instance, the low elevation forests at Chisongole and the Ruo Gorge (400 ha) have been completely cleared (Johnston-Stewart 1984). Paradoxically, it is only on the estates where any of this woodland type still exists unexploited, and it is possible that the protection against fire on the estates may facilitate the woodlands to develop towards rainforest.

2. *Mid-altitude forests*: These forests, found between 900 m and 1500 m asl, are more strongly transitional and considered part of the submontane floristic region (Dowsett-Lemaire 1989). Structurally, mid-altitude forests have richer species composition and a considerably lower proportion (~40%) of endemic woody species than the Afromontane forests. This forest type has a low canopy and is hung with many lichens, mosses and epiphytes (Dowsett-Lemaire 1989). *N. buchananii* dominates these forests with *Albizia adianthifolia*, *Funtumia africana*, *Chrysophyllum gorungosanum* and *Trilepisium madagascariense* as main associate tree species. Isolated old *W. whytei* tree stumps are also found in some gorges of the mid-altitude forests, i.e. an indication of the presence of the species previously (pers. obs.). Considering that *W. whytei* is a pioneer light-demanding species, its presence on such sites suggests that the sites were more

open before as a result of occasional extreme fires. To date, however, most of the mid-altitude forest area is under cultivation (pers. obs). The largest remaining block of the mid-altitude forest is found at Chisongole, on the south east of the mountain.

3. *Afromontane forests*: These forests on the plateaux (1850-2300 m asl) are found in gullies, gorges and ravines where they are sheltered from strong winds and fires (Chapman 1995). Much of the Afromontane forest is fragmented in widely scattered patches. These forests are taller than the mid-altitude forests and *Olea capensis* and *W. whytei* are the most common emergent trees. Where *W. whytei* is present in forest patches it always occurs in more abundance at the forest edges as young trees and sporadically as large emergent trees in the forest core (Chapman 1995). Other important canopy trees present include *Drypetes gerrardii*, *Ekebergia capensis*, *Olea capensis*, *Cassipourea malosana* and *Podocarpus latifolius* (Dowsett-Lemaire 1989). Although the adult trees are almost always found in the forest patches, *W. whytei* does not regenerate under closed canopy (Edwards 1982). Presumably the seedlings are light demanding. Slow-growing gymnosperm seedlings would, however, not stand a chance in the competitive environment of a tree-fall gap, so *W. whytei* has no opportunity at all for becoming established in the mature forests which consists largely of angiosperms and its strategy is that of a forest pioneer (Edwards 1982). Lianas are not common but there is great abundance of ferns (particularly epiphytic species), lichens, mosses and bryophytes, especially in wetter sites.

Much of the botanical studies on Mulanje Mountain tended to focus on *W. whytei* principally because of its uniqueness and commercial value and most of the interest over the last 80-90 years was related to its exploitation (Chapman 1995). The first check-list of plants of Mulanje Mountain was produced as early as 1897 (Wild 1964). However, following an initial paper on *W. whytei* in 1961, a comprehensive account of the vegetation of the mountain was published (Chapman 1962). A large number of endemic plant species were identified such as *W. whytei* and *Helichrysum whyteanum* (Chapman 1962,1990, Wild 1964, Strugnell 2002).

1.1.1 *Widdringtonia whytei* and its economic importance

1.1.1.1 *Taxonomy and biological characteristics*

The genus *Widdringtonia* is named after captain *Widdringtonia* who published a book on European pines in 1843 (Chapman 1995). The genus *Widdringtonia* contains several species from southern Africa. Three species grow in very small and disjunct populations: *W. cedarbergensis* (Clanwilliam Cedar) is confined to the Cedarberg Mountains, 250 km north of Cape Town in the Western Cape Province of South Africa (Marsh 1966); *W. schwarzii* (Willowmore Cedar) is confined to the Baviaanskloof Mountains in the Eastern Cape Province of South Africa (Luckhoff 1963, Marsh 1966) and *W. whytei* (Mulanje Cedar) is confined to Mulanje Mountain in south-eastern Malawi (Figure 1.2). *Widdringtonia cupressoides* (syn. *W. nodiflora*) has a much wider range from the Cape Peninsula through the mountains of the Western Cape to the Drakensberg between KwaZulu-Natal and Lesotho, and Limpopo Province, in South

Africa, Eastern Border Mountains of Zimbabwe and Mozambique to Mulanje Mountain in southern Malawi (Figure 1.2). *Widdringtonia cedarbergensis* is easily killed by fire, even by quick, light veld fires, regenerates from seed only, and regeneration after fire is very good (Manders et al. 1990). *Widdringtonia schwarzii* does not coppice once killed by fire and regenerates from seed only (Palgrave 1977). Similarly, *W. whytei* is unable to coppice after being burnt, and is restricted to fire protected valleys, where it is a forest pioneer which persists as a single-stemmed canopy emergent (Pauw and Linder 1997). *Widdringtonia cupressoides*, on the other hand, has the ability to survive a fire by coppicing from a ligneous base, and typically grows as a multi-stemmed bush in fire prone shrublands (Pauw and Linder 1997). On Mulanje Mountain, as earlier stated, both the *W. cupressoides* and *W. whytei* exist (Bayliss et al. 2007) and are ecologically distinct (Pauw and Linder 1997).

Widdringtonia whytei produces two kinds of leaves. Juvenile leaves are cultrate (the shape of a knife blade), spirally arranged, fresh green, up to 2 cm long (Chapman 1995). Adult leaves that are scale-like, closely appressed to the stem, decussate, about 2 mm long, dark green, begin to appear when *W. whytei* trees are 1.5-2 m tall. Male and female cones are borne on the same tree (Chapman 1995) and sometimes on the same branch (pers. obs.). Male cones are terminal and yellow-brown in colour while female cones are white-blue in colour (Figure 1.3 left). Mature female cones are globose, 1.5-2 cm in diameter, dark brown with four scales and take 2 years to develop (Figure 1.3 right).

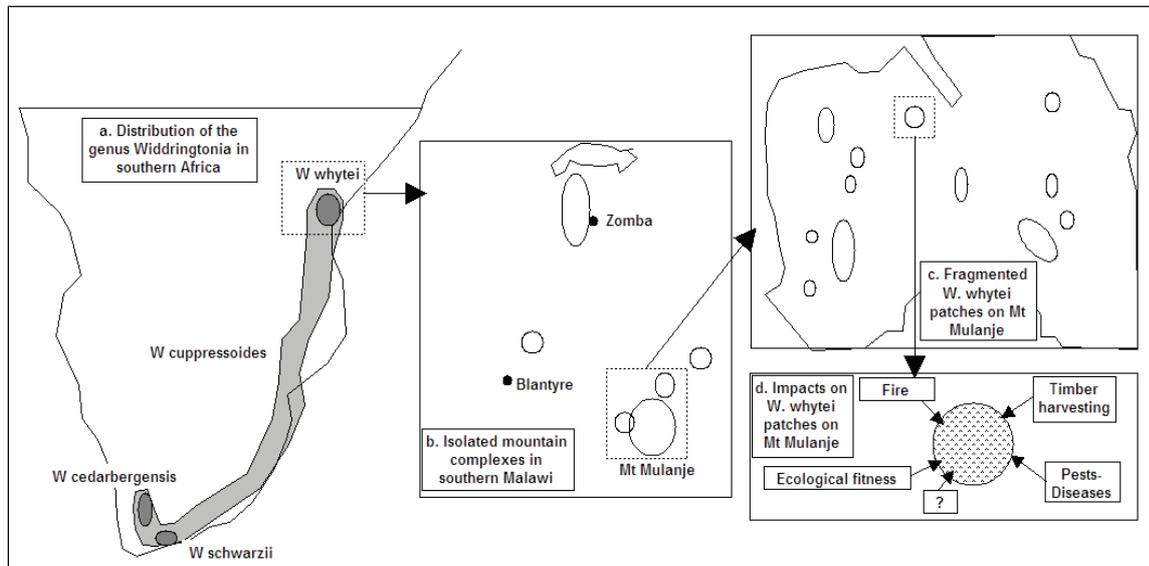


Figure 1.2: Distribution of the genus *Widdringtonia* in southern Africa

The bark of *W. whytei* is thick and spongy (Chapman 1995) and the thickness increases with age (Pauw and Linder 1997). For instance, the bark of a 3 m tall *W. whytei* sapling may be only 1 mm thick, smooth and uncracked but the thickness increases very rapidly with increasing diameter. They observed that trees with 10 cm diameter at breast height (dbh) had bark thicknesses ranging from 5 to 10 mm and split along longitudinal cracks. Trees greater than 20 cm in diameter had bark more than 10 mm thick (Pauw and Linder 1997). According to Chapman (1995), the presence of a thick bark on *W. whytei* is a characteristic to adapt to destructive fires as one way of ensuring survival of sufficient seed trees.



Figure 1.3: *Widdringtonia whytei* female (white-blue) and male (yellow-brown) cones (left) and mature female cones (right).

1.1.1.2 Distribution and population status of *W. whytei* on Mulanje Mountain

Widdringtonia whytei is endemic to Mulanje Mountain (Chapman 1962). Through the earlier part of its life, the appearance is typically coniferous. Older trees frequently develop flattened crowns and sparse foliage draped with lichens in most cases (Figure 1.4 left). It grows up to 40 m in height and ± 1 m in stem diameter at breast height (dbh) (Figure 1.4 right). However, the growth rate of *W. whytei* is slow (Sakai 1989). For instance, a mature tree 82 years old growing at Zomba Mountain produced 100 growth rings (Sakai 1989). This indicates that the growth rings on *W. whytei* are not necessarily annual rings.

Chapman (1995) described *W. whytei* as a pioneer tree species that is unable to regenerate under a closed canopy. Where there is fire protection, *W. whytei* seedlings and saplings are found sporadically at the edge of the forest where the litter layer is

minimal (Topham 1936). *Widdringtonia whytei* grows in association with evergreen tree species such as *Erica benguelensis*, *Hypericum revolutum* and *Myrica humilis* (Chapman 1995). Once the fire has destroyed this vegetation, seed from a few *W. whytei* mature trees in the stand germinates on the exposed mineral soil (Topham 1936). *W. whytei* will normally be the first species to re-establish itself after fires followed by the evergreen tree species which in most cases regenerate through vegetative (coppice) regrowth. It is a paradox that despite its extreme flammability, without the intervention of fire, *W. whytei* would be unable to compete with this evergreen forest vegetation (Chapman 1995). However, the dominance of *W. whytei* in such stands depends upon the amount of viable seeds produced and dispersed and number of seedlings regenerating and establishing.



Figure 1.4: *W. whytei* lichen-draped foliage (left) and mature stem (right) at Sombani

Several assessments of *W. whytei* have been undertaken on Mulanje Mountain (Edwards 1982, Sakai 1989, Lawrence et al. 1994, Makungwa 2004). All the studies noted a low natural regeneration of *W. whytei* and relatively high mortality rates of standing trees (currently 32.6% of standing cedar is dead). High mortality rate of

standing cedar trees could affect seed output, seed dispersal and natural regeneration patterns in *W. whytei*. Using similar methods, Sakai (1989) calculated an area of *W. whytei* equivalent to 1462 hectares whereas Makungwa (2004) calculated an area of 845.3 hectares, representing a decrease of 616.7 hectares in 15 years (a 2.8% loss per year). In the 845.3 hectares, overall mean stem density of *W. whytei* ranged from 36 stems per hectare at Chambe to 99 stems per hectare at Sombani and most of the cedars were in >55 cm dbh class (Table 1.1), signifying maturity of the remaining stands. Decreasing population size and ageing tree populations are known to affect viable seed output, seed dispersal as well as natural regeneration in some plant species (Rajora et al. 2002, Laurance et al. 2006, Tomita et al. 2008, Wang et al. 2010).

Table 1.1: Mean density (stems ha⁻¹) of live and dead (in parentheses) cedar by stem diameter class (DBH) for the six regions of Mulanje Mountain as of July 2004 (after Makungwa 2004)

Region name	Density by stem diameter class (DBH in cm)						Overall
	5-14.9	15-24.9	25-34.9	35-44.9	45-54.9	>55	
Chambe	13 (3)	15 (2)	1 (0)	0 (0)	3 (0)	4 (0)	36 (5)
Chinzama	19 (0)	7 (0)	10 (11)	16 (3)	15 (7)	10 (33)	77 (54)
Lichenya	1 (1)	7 (7)	10 (11)	16 (9)	7 (5)	15 (2)	56 (35)
Madzeka	0 (0)	0 (0)	0 (10)	2 (8)	2 (4)	45 (17)	49 (39)
Sombani	15 (0)	14 (3)	28 (3)	13 (16)	13 (5)	16 (0)	99 (27)
Thuchila	7 (1)	3 (8)	7 (12)	6 (6)	10 (4)	24 (3)	57 (34)

1.1.1.3 Conservation status of *W. whytei* on Mulanje Mountain

Widdringtonia whytei is a protected endemic tree species within the Mulanje Mountain Forest Reserve (MMFR) and licenses are only available from the Forestry Department

(FD) for the exploitation of dead trees. The Mulanje Mountain Conservation Trust (MMCT) was set up to provide long-term support for the research and conservation of biological diversity (including *W. whytei*) in the reserve and the sustainable utilization of its natural resources. The Trust is working with the Forestry Department in bringing in community participation to manage and protect *W. whytei* from fires. MMCT and FD focus on fire prevention measures only, which include total fire prevention in the *W. whytei* forest patches, fire break maintenance around the forest patches and early burning in the fire zones.

Widdringtonia whytei naturally has a scattered (patchy) distribution on Mulanje Mountain (Figures 1.5 and 1.6) from about 1500-2200 m above sea level, with the majority of the population between 1800 m and 2100 m. However, there are several threats to the *W. whytei* fragments (patches) on the mountain. The most serious of these are fire damage, illegal logging, lack of natural regeneration, colonisation by invasive species and conifer aphid attack (Bayliss et al. 2007). Two types of fires occur on the mountain, namely natural fires and human made fires (Chapman 1995). Natural fires are less frequent and are mostly caused by lightning during the rainy season. They cause less damage since they cause individual tree deaths. Human made fires on the other hand, are the most frequent especially during the hot dry months of the year (Chapman 1995). They include prescribed burns, hunting fires and crop residual-burning fires (Bayliss et al. 2007). The application of prescribed, controlled early burns is the major means of managing grass vegetation on Mulanje Mountain (Bayliss et al. 2007). Prescribed burns are applied in climatic conditions likely to result in less-intense fires and lower cedar

mortality. The intention is to pre-empt the occurrence of very intense wildfires that in most cases result in high cedar mortality. Occasionally, these fires go out of hand and cause damage to cedar trees (pers. obs.). Hunting fires on the other hand, are widespread and burn with high intensities although technically no hunting is allowed inside the forest reserve boundary. These fires have devastated parts of many forest patches on the mountain (pers. obs.). Crop residual-burning fires are also a common occurrence and often spread out of control onto the mountain from cultivated fields at the foot of the mountain. This is common on the north-eastern side of the mountain where the reserve borders with the communities. Such fires mostly occur during the months of August and September when most people are preparing their gardens (pers. obs.). These fires cause considerable damage on *W. whytei* patches located on the north-eastern slopes especially the regenerating seedlings that mostly occur on the forest margins (Chapman 1995).

Illegal logging of Mulanje cedar is also significant in areas not normally visited by FD and MMCT officials. Most of the illegal harvesting targets live and large trees. Removal of live and large trees reduces the number of potential seed trees that can have a detrimental long-term impact on the species regeneration.

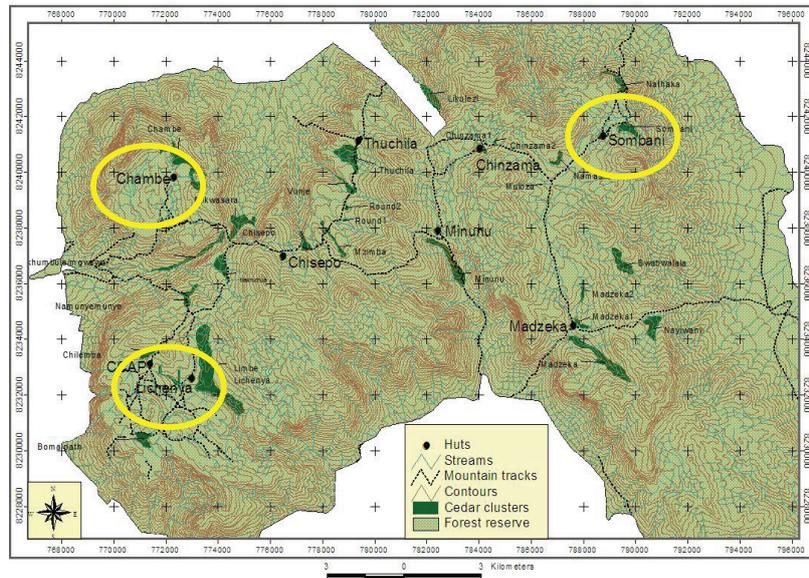


Figure 1.5: *W. whytei* fragments on Mulanje Mountain as at 2004 (from Makungwa 2004) and location of study sites

On the other hand, removal of live potential parent trees creates large gaps that may be beneficial to the regeneration of this pioneer tree species.

The spread of invasive species on the mountain is well documented (Chapman 1995, Bayliss et al. 2007). The most serious is the Mexican pine (*Pinus patula*), originally planted as a nurse crop for Mulanje cedar. *P. patula* and *W. whytei* are in direct competition as both are pioneer tree species depending on fire for their natural spread and are light demanding for their seed germination and establishment. *W. whytei* cannot establish under *P. patula*. Another threat is the aphid (*Cinara cupressi*), originally found on the Mexican cypress *Cupressus lusitanica* planted c 80 years ago on the mountain for timber (Chapman et al. 1991). The aphid attacked the *W. whytei* and was the cause of death of many trees (Chilima 1989). As shown in Table 1.1, about 32.6% of *W.*

whytei standing trees are dead. In some plant species, mature trees release large amounts of seed when attacked, with good regeneration, if there are suitable conditions for germination (Botha 1990).



Figure 1.6: A *Widdringtonia whytei* forest fragment near Chinzama Hut, Mulanje Mountain, Malawi.

The Mulanje Mountain Forest Reserve (MMFR) was gazetted in 1927. Most of the community surrounding the mountain is rural and poor. Forests surrounded by developing poor rural communities are often degraded by traditional subsistence practices to satisfy the livelihood needs for building material, fuelwood, food, medicine, and other household goods (Geldenhuys 2004). High poverty levels coupled with high population density and national demand for wood products has resulted in the over-exploitation of *W. whytei* population on Mulanje Mountain. For instance, the Forestry Department in the Ministry of Mines and Energy, opened a *W. whytei* sawing season for the year 2007. The reason for opening was, among other reasons, to supply plank material to the Lake Malawi Artisan Fisheries Development Project (LMAFDP) for the

construction of up to 400 plank boats. Pit-sawyers are normally awarded licenses to saw during a specified period. Licenses were given to 45 sawyers however records showed that 11 sawyers did not buy any cedar tree suggesting that some form of fraud took place during the whole exercise despite every sawyer sawing during that season (Makungwa and Chanyenga 2007). Furthermore, the policy of the government on *W. whytei* tree sawing is to saw dead trees only. But during the 2007 sawing season, 31.8% of the total trees sawn were live trees (Table 1.2). Removal of live trees reduces the number of potential seed-producing trees in fragments that may also affect seed output and natural regeneration.

Table 1.2: Number of *Widdringtonia whytei* trees harvested by region in 2007 on Mount Mulanje

Region name	Trees harvested	Proportions of dead and live trees	
		Dead (%)	Live (%)
Chambe	50	27 (54.0)	23 (46.0)
Chinzama	235	182 (77.6)	53 (22.4)
Lichenya	184	148 (80.5)	36 (19.5)
Madzeka	301	164 (54.5)	137 (45.5)
Sombani	163	123 (75.8)	40 (24.2)
Thuchila	300	197 (65.7)	103 (34.3)
Total	1233	841 (68.2)	392 (31.8)

Source: Makungwa and Chanyenga (2007)

1.1.1.4 Economic importance

Widdringtonia whytei is an excellent timber that has been used for construction, furniture, panelling and wood crafts for over 100 years. Due to its economic importance, the European settlers made great efforts during the early 1900s to plant the species in plantation stands on Zomba Mountain and Viphya Plateau in order to create a supply of

readily available timber and to alleviate pressure on the natural populations on Mulanje Mountain. Unfortunately, it was only on Zomba Mountain where the species has shown the potential to grow well (Chapman 1995). *Widdringtonia whytei* is a high altitude tree that grows between 1500-2200 m asl. But the area available for growing *W. whytei* on Zomba Mountain is very limited (2445 ha which is also planted with other plantation species such as pines) and restricted to the basin of the plateau (1200-1800 m asl). Failure of *W. whytei* to grow successfully outside its natural environment resulted in conservation efforts to concentrate on Mulanje Mountain only as a management strategy. Although the species was declared a Malawi national tree in 1984, its extraction levels on Mulanje Mountain are unsustainable. This has been demonstrated by the volume (m³) of wood harvested during the 2007 sawing season on the mountain (Table 3).

Whyte (1891) described Mulanje cedar wood as equal to the finest yellow pine. The wood is light to moderately heavy, durable (strongly resistant to termites, wood borers and fungal attacks) and fragrant (Chapman 1995). Figure 1.7 shows pit-sawn planks in one of the clusters on the mountain. Its basic density ranges from 385 - 430 kg/m³ (Chapola 1989). Exposed to the atmosphere as with shingles, for which it is very suitable, the wood weathers to silver-grey. The wood is well suited for boat building. The wood has a very high economic value. For example, during January 1992, one large tree cut from natural stands on Mulanje Mountain fetched a price of about £1000 (Chapman 1995). This is a great advantage to a poor country like Malawi if the species is well conserved.

Table 1.3: Volume (m³) of live and dead *Widdringtonia whytei* trees harvested by region during the 2007 sawing season on Mulanje Mountain.

Region name	Total (m ³)	Dead (m ³)	Live (m ³)
Chambe	180.57	114.30	66.27
Chinzama	459.21	356.35	102.86
Lichenya	778.79	626.93	151.87
Madzeka	1389.65	757.36	632.29
Sombani	305.96	231.92	74.04
Thuchila	816.40	536.37	280.03
Total	3930.59	2623.23	1307.36

Source: Makungwa and Chanyenga (2007)

Carved *W. whytei* kists, wood-carvings and crafts such as walking sticks are popular among tourists. The wood produces oil locally known as “Mulanje Tar”. Formerly, the oil was distilled from sawdust and waste wood as a preservative against termites. In 1906, 40 gallons were produced, chiefly for the then Nyasaland Public Works Department (Forestry Department 1906). Subsequent analysis by the Tropical Research and Development Institute suggested that the oil might find a use locally as in scented soap. To date, however, nothing has come out of this. While these qualities have fuelled the exploitation of the tree species, they could also help save it through a programme of sustainable resource utilization. Despite the high economic value of *W. whytei* wood, field observations have shown that a large proportion of the tree volume of the cut cedar trees is left on the site as wastage after harvesting on the mountain. For example, after 2007 *W. whytei* sawing season an overall mean of 27.8% (representing 1091.23 m³) was left as wastage (Figure 1.8) across sites on Mulanje Mountain (Makungwa and Chanyenga 2007). The wood carvers and craftsmen are not taking advantage of such an opportunity and that is a clear indication of inefficient use of the resource. Inefficient use of tree resources may accelerate the cutting down of *W. whytei* trees that may

reduce the number of seed producing trees and affect seed production and natural regeneration.



Figure 1.7: Pit sawn *Widdringtonia whytei* planks at Sombani on Mulanje Mountain



Figure 1.8: A *Widdringtonia whytei* tree log left on a logging site as wastage at Madzeka on Mulanje Mountain during the 2007 sawing season.

1.2 FOREST/POPULATION FRAGMENTATION

Forest or population fragmentation has been defined as the splitting of large areas of contiguous native forest or species populations into smaller and more isolated remnant forest patches or species populations. Forests may be fragmented by a number of activities or events, such as road construction, logging, conversion to agriculture, or wildfire (Granger 1984, Geldenhuys 1994). Ultimately, the cause of fragmentation is either anthropogenic or natural in origin.

Forest fragmentation causes many physical and biological changes as a result of habitat loss and insularization (Lovejoy et al. 1986). Population size and/or spatial distribution of some forest species are reduced when forest landscapes become increasingly fragmented (Tabarelli et al. 1999). A reduction in forest species population size affects demographic processes such as seed production, seed dispersal and natural regeneration in plant species but the effects vary from species to species (Cunningham 2000). For example, seed from smaller populations of *Pinus strobus*, a light demanding species during the establishment phase, had a lower ratio of filled seed than seed from large populations, demonstrating that a reduction in the number of individuals in a population negatively affects viable seed output in *P. strobus* (Rajora et al. 2002). In contrast, a reduction in population size of *Picea jezoensis*, another light demanding conifer tree species during its early life-history stages, increased total viable seed per cone (Tomita et al. 2008). Large population size acted as a barrier to distant pollen sources and was responsible for low viable seed output in *P. jezoensis* (Tomita et

al. 2008). However, no simple generalizations can be made; the effect of forest fragmentation on viable seed output is highly variable among plant species.

Forest fragmentation and isolation modifies several post-pollination processes such as seed rain (Jules and Rathcke 1999, Cunningham 2000). Seed rain can be described as the total amount of seeds received by any habitat during a certain period of time, and its magnitude and composition is driven by seeds that just drop beneath seed trees as well as by seeds actively dispersed (Du et al. 2007). The quantity and composition of seed rain into fragments could be altered by changes in population size (Santos and Telleria 1994). For instance, seed rain densities in *Nothofagus nitida* differed significantly between large and small population fragments; with large fragments receiving as high as 60 seeds/m²/day and small fragments receiving only 20 seeds/m²/day (Armesto et al. 2001). However, they concluded that the effect of forest fragmentation on seed rain densities varies amongst species. Although forest fragmentation is a well-known problem, few studies have investigated tree species seed rain patterns in forest fragments.

Increasing fragmentation of forested landscapes has drawn attention towards regeneration dynamics in fragmented forest tree communities (Laurance et al. 2006). Fragmentation affects seedling regeneration, establishment and abundance (Janzen 1983, 1988, Lovejoy et al. 1986, Saunders et al. 1991, Esseen 1994, Millis 1995, Murcia 1995). Regeneration of forest plants may be affected by fragmentation, but conclusions

are not straightforward. Some studies have demonstrated reduced seedling regeneration in small fragments compared to large fragments (Benitez-Malvido 1998) whereas other studies have reported increased seedling regeneration in small fragments compared to large fragments (Laurance et al. 1998). Other studies have reported increased seedling regeneration in forest fragment interiors compared to edges while others have detected no difference in seedling regeneration between the edge and interior of fragments (Lawes et al. 2005). For instance, tree seedling densities of pioneer tree species (*Cecropia sciadophylla*, *Vismia guianensis* and *V. amazonica*) were higher on the forest fragment edges than in fragment interiors (Laurance et al. 2006). Fire and light penetrations were higher on the edges of forest fragments that promoted growth of these pioneer tree species (Laurance and Bierregaard 1997). In contrast, the shade tolerant *Cryptocarya alba* occurred with lower seedling densities on the forest fragment edges compared to forest interiors in Chile (Guerrero and Bustamante 2009). These observations clearly demonstrate that fragmentation modifies forest conditions such as light, moisture and temperature that also affect seed germination and seedling survival in many plant species but the effects are highly variable among species (Ferner and Thompson 2005, Arrieta and Saurez 2006, Keeley et al. 2000).

1.3 PROBLEM STATEMENT

Widdringtonia whytei has become a prestige wood with high scarcity value. However, its population on Mulanje Mountain naturally has a very fragmented distribution pattern.

The fragments have further been impacted on by human resource use, wildfire damage, the spread of invasive species into *W. whytei* patches and increased tree mortality due to aphid attack. Very low natural regeneration has been reported in recent surveys. *W. whytei* regenerates through seed only and the reduction in natural regeneration observed for the species could be due to inadequate viable seed output and variation in seed rain (dispersal) pattern. Understanding *W. whytei* regeneration pattern requires an insight of its seed availability. However, data to indicate whether or not regeneration in this species is sensitive to fluctuation in viable seed output is limited. Furthermore, phenological pattern of seed rain (dispersal) of *W. whytei* has hitherto received no attention and is little known. Seed rain forms the template for later regeneration and plant recruitment. However, little is known about how seed rain influences seedling regeneration and abundances in different *W. whytei* patches or fragment sizes. Temperature and light are important ecological factors that regulate seed germination of many plant species. Temperature and light requirements for germination are unexplored in *W. whytei*. Determining the relationship between viable seed output, seed rain, seed germination and seedling regeneration pattern under different conditions may therefore be pivotal in understanding the recruitment dynamics of this very important species. The absence of this knowledge deprives forest managers, ecologists and conservation biologists from the Department of Forestry (FD) and Mulanje Mountain Conservation Trust (MMCT) of a key tool towards achieving sustainable management of *W. whytei* forest patches on Mulanje Mountain.

1.4. STUDY OBJECTIVES AND CONCEPTUAL FRAMEWORK

1.4.1 General objective

The overall objective of this study is to assess the effects of population fragmentation and isolation on viable seed output, seed rain and natural regeneration patterns in *W. whytei* for the formulation of *in-* and *ex-situ* conservation strategies of the species. The conceptual framework for the study of *W. whytei* on Mulanje Mountain is shown in Figure 1.9.

1.4.2 Specific objectives

1. To determine the effects of *W. whytei* population size, tree diameter and crown position in forest canopy on viable seed output per cone.
2. To understand the phenological pattern of seed rain in *W. whytei* and how it is affected by population size.
3. To understand seed germination requirements of *W. whytei*.
4. To establish the influence of population size on seedling regeneration patterns in *W. whytei* and how it relates to seed rain pattern.

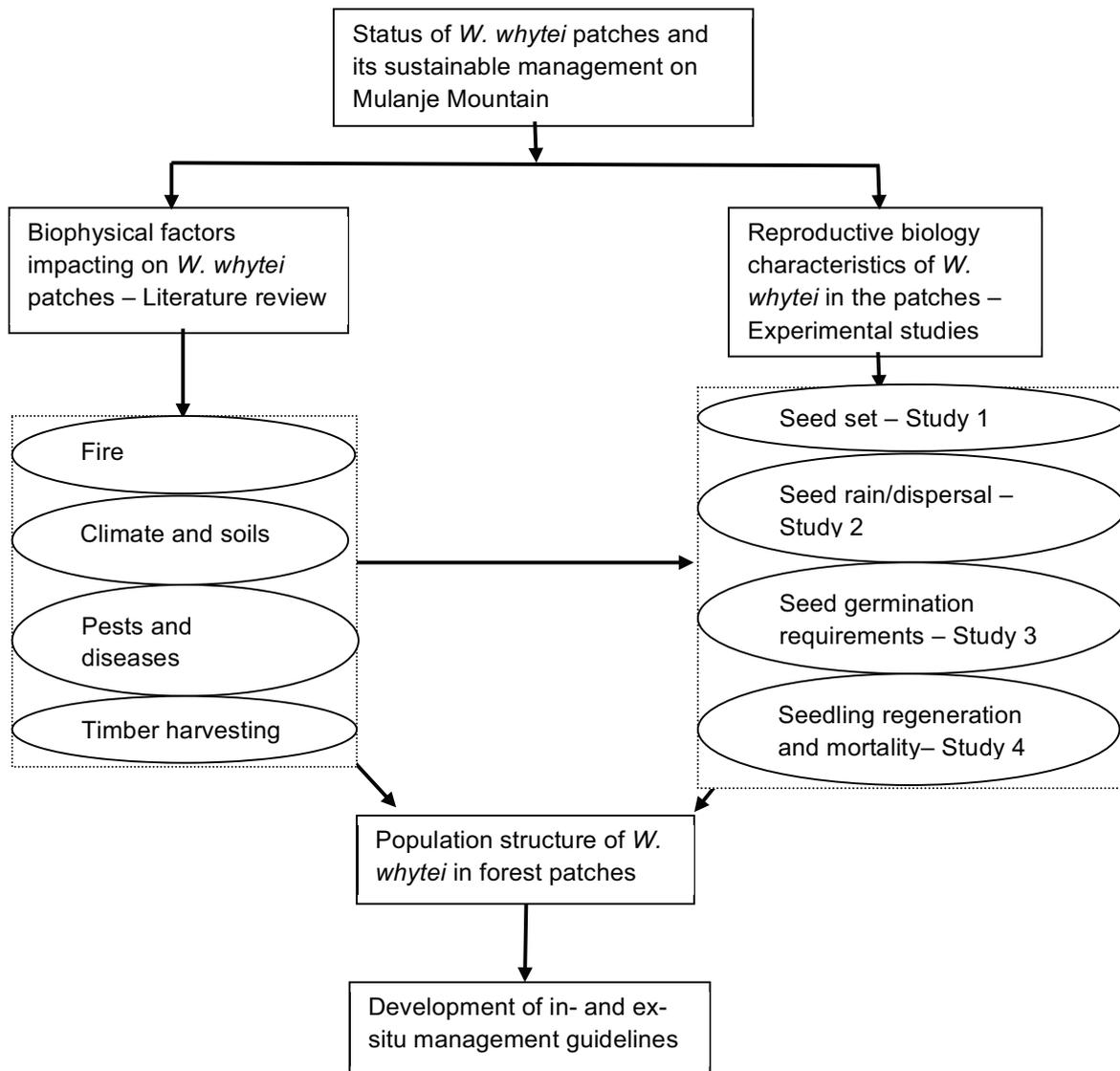


Figure 1.9: A conceptual framework for the study of *Widdringtonia whytei* on Mulanje Mountain.

1.4.3 Specific objectives and general sampling study designs

Objective 1: To examine the effects of *W. whytei* population size, tree diameter and crown position on viable seed output per cone.

Research questions:

1.1. Does population size of *W. whytei* within a forest patch influence viable seed output per cone?

1.2. Does tree size and crown position in the forest canopy influence viable seed output per cone?

Hypotheses:

The hypotheses being tested are that:

1.1. Large population sizes of *W. whytei* within a forest patch or fragment produce more viable seeds per cone than small population sizes.

1.2. Cones from large trees produce more viable *W. whytei* seeds than cones from small trees.

1.3. Trees with well-lit crowns produce more viable seeds per cone than trees with suppressed crowns.

Study design

In this study, a stratified random sampling design will be used to select fragment sizes based on number of cone-producing *W. whytei* trees. Three fragment sizes will be selected on three sites. Four *W. whytei* seed trees will be selected from each fragment for cone collection. One isolated tree (>500 m from the nearest *W. whytei* fragment) will also be selected on each site for cone collection. Selection of seed trees will be based on the accessibility, distance between trees (25-30 m apart), presence of cones, tree health, crown position in the forest canopy, and stem diameter (≥ 30 cm). Germination

followed by tetrazolium and cutting tests will be conducted to determine the seed viability following the International Seed Testing Association (ISTA) rules (2012).

Objective 2: To determine the effect of *W. whytei* population size within a site on seed rain density and its dispersal pattern.

Research questions:

2.1. Does seed rain density vary amongst *W. whytei* population sizes both inside and outside forest patches and sites?

2.2. When does most of the seed rain occur?

Hypothesis:

The hypotheses being tested are that:

2.1. Large *W. whytei* fragments produce higher seed rain densities both inside and outside the forest patches than small fragments and they vary between sites on the mountain.

2.2. Seed rain occurs during a specific time of the year.

Study design

Funnel seed traps will be used to collect seed falling from *W. whytei* trees in all study population forest fragments. Seeds will be collected from the traps on a monthly basis for a minimum period of 24 months.

Objective 3: To determine the temperature and light requirements for seed germination of *W. whytei*.

Research question:

3.1. To what extent does temperature and light influence seed germination in this pioneer tree species of *W. whytei*?

Hypothesis:

3.1. Seed germination in *W. whytei* is influenced by temperature and light.

Study design

Seeds of *W. whytei* will be germinated at different temperature and light conditions in the laboratory.

Objective 4: To examine whether *W. whytei* population size influences seedling regeneration, survival and establishment and when and where such regeneration and mortality occur.

Research questions:

4.1. Does *W. whytei* forest fragment size influence seedling regeneration and mortality?

4.2. When and where does seedling regeneration and mortality occur?

Hypothesis:

The hypotheses being tested are that:

4.1. Small *W. whytei* forest fragments have lower seedling regeneration densities and higher seedling mortality both inside and outside the forest patches than large fragments on all sites on the mountain.

4.2. Seedling regeneration and mortality occur at specific times of the year.

Study design

Plots will be used to evaluate the impact of *W. whytei* forest fragment size on *W. whytei* seedling regeneration and mortality both inside and outside forest patches. Fixed regeneration plots measuring 3 x 15 m will be placed adjacent to the seed traps at every 10 m both outwards up to 30 m and inwards up to the centre of the fragment. Seedling counts will be done on a monthly basis for a minimum period of 24 months. Seedlings will be marked for easy identification in the subsequent assessments.

1.5 GENERAL DESCRIPTION OF THE STUDY SITES

This study will be conducted on Mulanje Mountain at three sites namely Sombani, Chambe and Lichenya (Figure 1.5). General sites conditions are presented in Table 1.4. Temperature and rainfall details on the Mountain are presented in Figure 1.10. Today, these sites are characterised by *W. whytei* forest fragments of different sizes ranging from 0.8 - 177.9 ha with most of them confined to the ravines and hollows on the plateaux and below cliffs and the gorges where the terrain affords some protection from fires.

Table 1.4: General site conditions at the selected three study sites on Mulanje Mountain, Malawi (source: Lawrence et al. 1994)

Site variable	Sombani	Chambe	Lichenya
Latitude (South)	15°52'41"	15°62'40"	15°62'33"
Longitude (East)	35°40'89"	35°30'72"	35°30'73"
Altitude, m a.s.l.	1660 – 2265	1820 – 1900	1790 – 1960
Steepness, ° slope	9 – 46	7 – 20	9 – 45
Terrain	Easy to extremely difficult access; sheltered to exposed, with some rocky outcrops	Medium to difficult access; rocky outcrops; deep humus layer; lush	Easy to very difficult access; exposed; very damp; lush

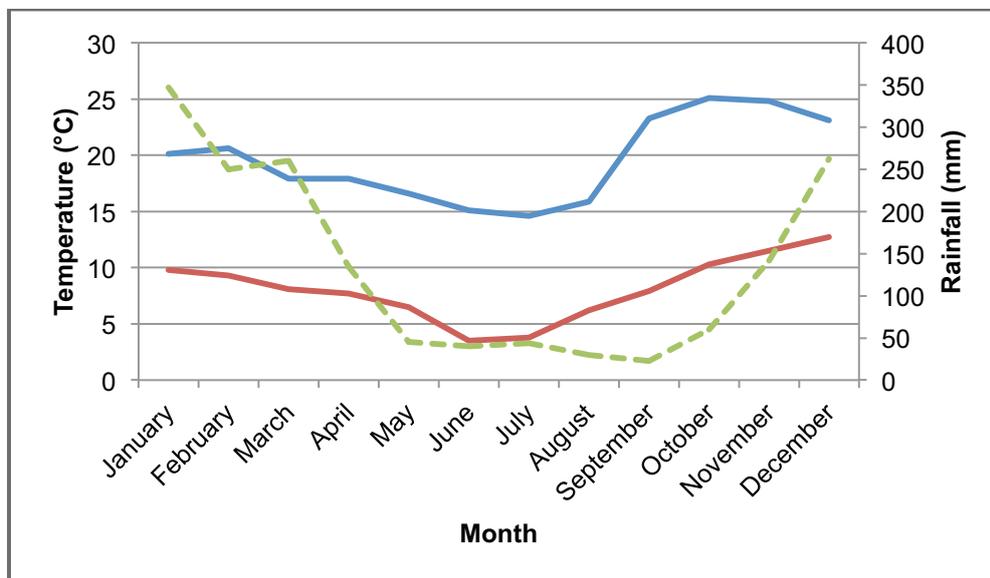


Figure 1.10: Mean monthly minimum and maximum temperatures (solid lines) and rainfall (dotted line) for Lichenya Hut on Mulanje Mountain.

1.6 IMPORTANCE OF THE STUDY

The study is designed to provide a basis for a dual management approach, i.e. for a conservation management strategy to ensure the survival of this endemic tropical

conifer tree species on Mulanje Mountain, and for the production of plants for the establishment and silvicultural management of planted stands of this species away from Mulanje Mountain to provide in the socio-economic needs for this species. It will assist to understand the reproductive ecology of *W. whytei*, such as factors that influence viable seed output, seed rain, seed germination and phenological dispersal pattern. The results would be of great value for improving the controlled burning programme on Mulanje Mountain, the harvesting management of the species, from both natural stands on the mountain and in planted stands elsewhere, and to assist the Malawi National Tree Seed Centre (MNTSC) to develop a seed collection calendar for the species which will subsequently reduce the seed collection costs. Information on seedling regeneration pattern is critical for the implementation of appropriate silvicultural treatments on the residual *W. whytei* stands. The above information will also assist with the formulation of both in- and ex-situ conservation and management strategies of the species.

1.7 STRUCTURE OF THE THESIS

The study is divided into six chapters. Chapter 1 (this chapter) presents the background of the study, the problem statement followed by the general and specific objectives, related research questions and hypotheses, importance of the study and the structure of the thesis. Chapter 2 shows how population size, tree diameter and crown position (illumination class) in forest canopy affects viable seed output in this tropical montane conifer. Chapter 3 looks at the seed rain pattern and seed limitation in different *W. whytei* fragment sizes on different sites in all seasons of the year. Chapter 4 presents

seed germination requirements of this species. Chapter 5 shows how natural regeneration is guided by population sizes and season. Chapter 6 is a general discussion on effects of fragmentation on *W. whytei* in terms of viable seed output, seed rain, seed germination requirements and natural regeneration patterns. It ends with conclusions and recommendations in terms of this thesis.

The chapters have been prepared as papers to be submitted for publication, for this reason, the formats may differ from chapter to chapter and may include duplication because of that.

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CHAPTER 2

EFFECT OF POPULATION SIZE, TREE DIAMETER AND CROWN POSITION ON VIABLE SEED OUTPUT IN *WIDDRINGTONIA WHYTEI*¹

2.1 INTRODUCTION

Forest landscapes and species populations continue to be fragmented by natural events such as fires, insects and diseases and by human activities such as agriculture, urbanization, and forest harvesting (Bowman and Prior 2004). Populations of some tree species have become more isolated with decreasing population spatial distribution, numbers of sub-populations, stem densities within fragments and increasing distances between fragmented sub-populations (O'Connell et al. 2006). Fragmentation, decrease in stem densities within fragments and isolation of plant populations are thought to affect demographic processes as a result of reduced pollen receipt and increased pollen limitation (Ashman et al. 2004, Pauw 2007, Wang et al. 2010).

Studies on the effects of population fragmentation on viable seed output of particular plant species reported contrasting results depending on the biological and ecological characteristics of the species concerned. Seed from smaller populations of *Pinus strobus*, a wind-pollinated tree species, had a lower ratio of filled seed than from larger populations in Canada (Rajora et al. 2002). Similarly, the percentage of viable seeds per cone significantly decreased with decrease in patch size in *Pinus tabulaeformis* in China (Wang et al. 2010). In contrast, population fragmentation in the conifer tree *Picea*

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jezoensis increased the total number of viable seeds per cone as a result of enhanced pollen movement (Tomita et al. 2008). These findings demonstrate that population fragmentation effect on viable seed production varies from species to species.

Several factors contribute to variation in viable seed output in forest trees. In many studies variation in seed set has been attributed to climatic as well as biological factors (Kainer et al. 2007). Climate as a major contributor to crop size variation, leads to a resource matching hypothesis that annual viable seed output patterns match annual rainfall distribution and some key seasonal temperature variables (Kainer et al. 2007). On the other hand, most woody plants biologically adhere to alternating supra-annual schedules of low and high viable seed output years (Herrera et al. 1998, Kelly and Sork 2002). This provides strong evidence that rainfall and temperature, which are more normally distributed and much less variable (Koenig and Knops 2000), are not the only determinants of viable seed output variation. Total tree height (Layne and Abrahamson 2004) and tree stem diameter (Ares and Brauer 2004) have all been reported to affect seed viability output. For example, Snook et al. (2005) investigated the influence of tree stem diameter on viable seed production in the mahogany tree *Swietenia macrophylla*. They observed that viable seed output increased with increasing tree stem diameter. Most tree species produce higher viable seed output in their middle ages, which may last from decades to centuries, followed by decline and senescence (Harper and White 1974).

Canopy structure is one of the most important factors influencing ecological processes in forest trees (Iwaizumi et al. 2008). Crown position in the forest canopy determines light interception and photosynthesis and reproduction (Okubo and Levin 1989, Nathan et al. 2002, Falster and Westoby 2003, Petit and Hampe 2006, Poorter et al. 2006, Seki 2008). Viable seed output in plant species is not necessarily uniform and often varies within the crown of the trees and among crowns of different trees depending upon the position of the tree crowns within the forest canopy (Iwaizumi et al. 2008). For example, some studies reported greater seed set in canopy than understory trees (Karlsson 2000, Debain et al. 2003). These variations are generally considered to be associated with resource related factors such as light intensity (Despland and Houle 1997). Therefore, a better knowledge of viable seed output in relation to crown position in forest canopies is indispensable for managing forests appropriately.

Widdringtonia whytei naturally has a fragmented distribution pattern on Mulanje Mountain but the fragments have further been impacted on by wildfires and human resource use (Chapman 1995). In these fragments *W. whytei* stands comprise scattered trees of both smaller and larger stem diameters. Both suppressed and emergent tree canopies exist in the stands (pers. obs.). However, very low natural regeneration has been reported in recent surveys (Makungwa 2004 Bayliss et al. 2007). Given the importance of viable seed availability in plant species regeneration, this study was carried out to examine the impact of *W. whytei* population fragment size on viable seed output per cone. The study also assessed whether tree stem diameter and crown position of trees in relation to the forest canopy affected viable seed output per cone.

The first hypothesis was that viable seed output per cone in *W. whytei* trees decreased with decreasing population size. The second hypothesis was that tree stem diameter and crown position in the forest canopy positively influenced viable seed output per cone.

2.2 MATERIALS AND METHODS

2.2.1 Study sites

This study was conducted on Mulanje Mountain in Malawi at three sites, namely Sombani (15°52'41"S; 35°40'8"E), Chambe (15°62'40"S; 35°30'72"E) and Lichenya (15°62'33"S; 35°30'73"E). The altitude of these sites ranges from 1660-2265 m asl (Lawrence et al. 1994). These sites are characterised by *W. whytei* forest fragments of different sizes ranging from 0.8 - 177.9 ha with most of them confined to the ravines and hollows on the plateaux and below cliffs and gorges where the terrain affords some protection from fires.

2.2.2 Study species

Widdringtonia whytei is a wind-pollinated coniferous tree endemic to Malawi and valued for its fine timber with attractive fragrance (Bayliss et al. 2007). Its commercial exploitation began in 1898 and continued until 1955, with large areas of forest cleared

(Chapman 1995). It grows up to 40 m in height and c. 1 m in stem diameter at breast height (dbh). Male and female cones are borne on the same tree (Chapman 1995) and sometimes on the same branch (pers. obs.). Cones are globose, 1.5-2 cm in diameter, dark brown with four scales (Chapman 1995). The number of cones per cluster is much less variable, with generally two cones per cluster. Female cones seldom remain closed for more than two years (Pauw and Linder 1997). A cone generally contains 4-8 seeds (Pauw and Linder 1997). *Widdringtonia whytei* seed is 25 mm long and 12 mm wide on average with a wing (Pauw and Linder 1997) and is wind-dispersed (Chapman 1995).

2.2.3 Population selection

Two sets of data were collected. In the first study, a stratified random sampling method was followed to select trees to assess the effect of *W. whytei* fragment size on viable seed output per cone. At each selected study site on the Mountain, all *W. whytei* population sizes were recorded and grouped into four categories (isolated trees, small, medium and large). Small fragments consisted of groups of ≤ 10 cone-bearing (reproductively mature) trees in an area either surrounded by other tree species or grassland vegetation. Medium-sized fragments consisted of groups of 11-20 cone-bearing individuals while large forest fragments comprised groups of >20 cone-bearing trees. Only trees in the small and medium fragments were physically counted. A tree was considered isolated if it was separated by more than 500 m from the nearest individual or group of *W. whytei* trees either surrounded by grassland or other vegetation types.

Other factors were considered during forest fragment selection, such as accessibility, distance between forest fragments (1 km was considered the minimum distance between selected forest fragments) and comparable stand characteristics such as orientation, elevation and slope despite most fragments being confined to the ravines (slopes) and hollows (plateaux). In each size group (small, medium and large) on each site, after excluding potentially unsuitable fragments, one fragment was randomly selected, and one tree from the isolated trees. A total of nine fragments and three isolated trees were selected for the study.

In each selected fragment, four cone-bearing trees were randomly selected, based on accessibility, distance between trees (25-30 m apart), presence of cones and tree health. Cones were also collected from one isolated tree on each study site. Each selected tree was recorded by location, fragment number and tree number, for easy identification.

Mature cones which were about to open (Figure 2.1a), were collected in 2008 and 2009 following a pre-determined sampling plan. Sample size (number of cones) was determined using Cochran (1963) formula: $n_o = Z^2 pq / e^2$ where n_o = sample size; Z^2 = standard deviation of normally distributed population (1.96); p = maximum variation (0.5); q = confidence interval (0.5) and e^2 = standard error (5%). Based on this formula, 385 cones from 39 trees (approximately 10 cones per tree) were supposed to be evaluated in a year. In this study, however, 20 mature cones were collected and

evaluated from each tree giving a total of 780 cones per year. Sample size was deliberately doubled to capture maximum variation in viable seed output per cone.

A tree climber collected the cones using an arm pruner. The crown of each sample tree was divided into north, east, south and west portions. From each portion, the crown was also subdivided into top, middle and lower. Where possible, five cones per branch and one cone per cluster were collected from one or two randomly chosen middle branches of each portion. Cones collected from each tree were bulked.

A separate study was conducted to investigate the effect of stem diameter and crown position on the viable seed output per cone in *W. whytei*. Initially, a subsample of trees at each site was selected to determine the diameter distribution of the population and crown position of each tree to guide the selection of trees to ensure sampling of a broad range of sizes. This study focused only on the Sombani site because the Chambe site had inadequate numbers of accessible small trees and Lichenya lacked large trees. A sample of 86 trees was selected from which 20 mature cones were collected per tree. The stem diameter at breast height (dbh) of sampled trees ranged from 13 to 61cm. The crown position (CP) of each selected tree within the canopy was scored as: (1) understorey or suppressed (no direct light) (CP1); (2) intermediate (some overhead or side light) (CP2); (3) Co-dominant (full overhead light) (CP3) and (4) dominant or emergent (full overhead and side light) (CP4) (Snook et al. 2005).

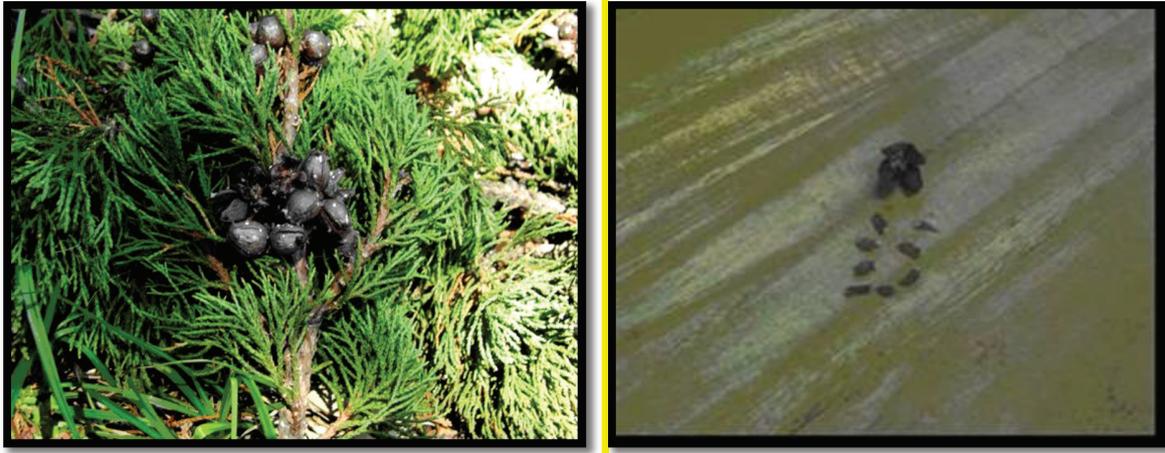


Figure 2.1: Cones and seeds of *W. whytei*: (a) Left: mature cones which are about to open; (b) Right: dry open cone with some seeds.

2.2.4 Seed viability determination

Each cone was placed in the shade and allowed to open separately in order to determine total number of seeds per cone. Seed viability was determined by conducting germination tests followed by tretrazolium and cutting tests (ISTA 2012). All seeds from each cone (Figure 2.1b) were placed in separate plastic petri dishes filled with solid culture media prepared from Agar-agar granules (10g/l) (Figure 2.2) for germination tests. Germination experiments were performed in a germinator having a day and night light cycle of 12 hours at 20°C (Gondwe 2010). Germination counts were made daily for a period of 30 days. At the end of the experiment, the ungerminated seeds were soaked in water for 24 hours and tested in a tretrazolium solution for 8 hours at 25°C (ISTA 2012) to check if the seeds were still viable and this was followed by cutting tests as a confirmation.

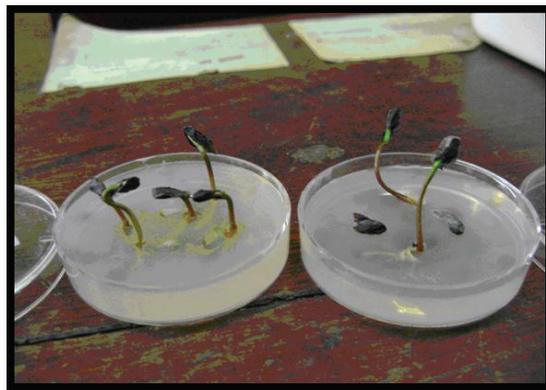


Figure 2.2: Germinated and ungerminated *W. whytei* seed in Agar-agar with each petri dish representing the seed collected per individual cone.

2.2.5 Data analysis

An exploratory analysis of the effect of fragment size on proportion of viable seed (PVS) per cone was conducted by plotting the kernel density and normal probability density functions for each fragment using the SAS system. As the data were found to be non-normal, they were subjected to a generalized linear model (GLM) assuming binary logit where fragment size, site and year were entered in the model as the fixed effect (Sileshi 2012). The statistical power of the test was also calculated. The statistical power of a significance test is the long-term probability (given the population effect size, alpha, and sample size) of rejecting a false null hypothesis. The statistical power of the test for the fixed effects of site and fragment was sufficiently high (>0.99). While the GLM analysis shows the mean effect of population size, it does not reveal how seed viability is distributed in each fragment. Therefore, the probability (P) of obtaining a given seed viability value was estimated for each fragment using categorical models. Then the cumulative probability distribution of seed viability was plotted to allow examination of the stochastic dominance of one fragment over the other (Sheather and Jones 1991). In

order to evaluate the effect of tree stem diameter and crown position on proportional seed viability, data were subjected to polynomial (parabolic) regression.

2.3 RESULTS

2.3.1 Relationship between seeds produced and viable seeds

In total, 1560 cones were sampled during the 2-year study period, which produced 10,214 seeds or 6.5 seeds per cone. Only 2350 seeds were viable, representing 1.5 viable seeds per cone, or 23% viable seeds per cone. The frequency distribution of total and viable seeds per cone is shown in Figure 2.3. Cones producing 6 seeds had the highest frequency (261 cones) followed by cones producing 7 seeds (233 cones). Cones producing one viable seed had the highest frequency (415 cones) followed by cones producing zero (398 cones) or two viable seeds (302). Only one cone produced 15 viable seeds and was the highest (Figure 2.3).

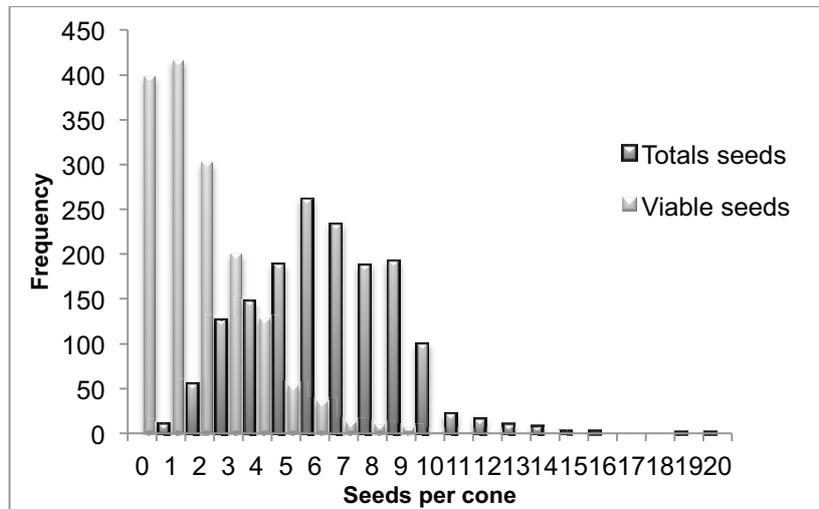


Figure 2.3: Frequency of the total and viable number of *Widdringtonia whytei* seeds per cone as assessed from 1560 cones collected from 39 sampled trees on Mulanje Mountain, Malawi.

2.3.2 Effect of fragment size on viable seed output

Large population sizes produced the highest proportion of viable seeds per cone (29%), while medium-sized populations produced the lowest proportion of viable seeds per cone (18%) (Table 2.1; Figure 2.4). Cones at Sombani produced a significantly higher proportion (29%) of viable seeds per cone compared with those at Chambe and Lichenya (22% and 20% respectively) (Table 2.1).

The effect of year was not significant (Tables 2.2) but some inter-annual variation in proportion of viable seeds per cone was apparent within fragments of the same size (Table 2.1). For example, large populations at Chambe and Lichenya produced higher proportions of viable seeds per cone in 2009 compared with 2008 whereas the large population at Sombani produced higher proportion of viable seeds per cone in 2008

compared with 2009. Isolated trees produced higher proportions of viable seeds per cone in 2009 compared with 2008 at all sites (Table 2.1).

Table 2.1: Mean number of seeds produced and proportion of viable seeds per cone from 20 cones per tree for four selected cone-bearing trees per fragment during 2008 and 2009 in three fragment sizes at three sites on Mulanje Mountain, Malawi.

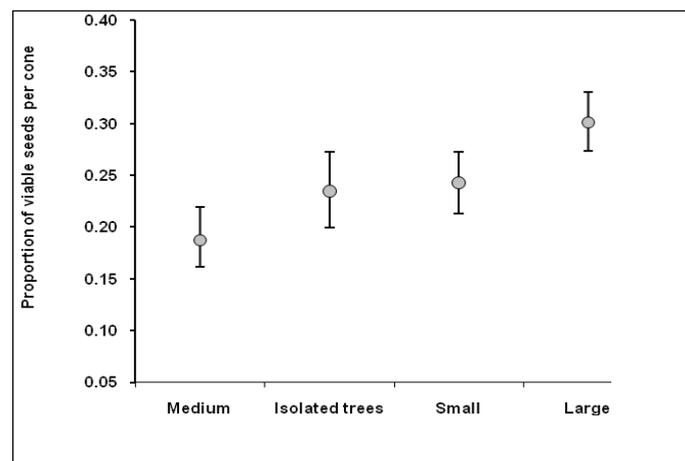
Fragment size	Year	1=Number of seeds produced; 2=Proportion of viable seeds	Sites			Mean per fragment size
			Chambe	Lichenya	Sombani	
Large (> 20 trees)	2008	1	7.01 ± 0.24	5.68 ± 0.24	7.83 ± 0.21	1= 6.58 ±0.23
		2	0.26 ± 0.01	0.17 ± 0.01	0.43 ± 0.02	
	2009	1	6.18 ± 0.22	7.15 ± 0.27	5.66 ± 0.25	2= 0.29 ±0.01
		2	0.31 ± 0.02	0.29 ± 0.02	0.33 ± 0.03	
Medium (11-20 trees)	2008	1	6.05 ± 0.26	6.85 ± 0.29	7.41 ± 0.25	1= 6.27 ±0.25
		2	0.20 ± 0.01	0.27 ± 0.02	0.11 ± 0.01	
	2009	1	4.80 ± 0.26	7.43 ± 0.25	5.12 ± 0.21	2= 0.18 ±0.01
		2	0.18 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	
Small (≤ 10 trees)	2008	1	5.88 ± 0.25	6.06 ± 0.25	8.46 ± 0.33	1= 6.70 ±0.24
		2	0.21 ± 0.01	0.20 ± 0.01	0.37 ± 0.01	
	2009	1	4.86 ± 0.23	7.51 ± 0.17	7.48 ± 0.26	2= 0.23 ±0.01
		2	0.15 ± 0.01	0.22 ± 0.02	0.28 ± 0.01	
Isolated trees	2008	1	7.55 ± 0.53	5.25 ± 0.53	8.55 ± 0.45	1= 6.79 ±0.50
		2	0.19 ± 0.03	0.15 ± 0.04	0.26 ± 0.04	
	2009	1	5.75 ± 0.56	7.50 ± 0.36	6.15 ± 0.61	2= 0.23 ±0.03
		2	0.26 ± 0.03	0.16 ± 0.03	0.36 ± 0.04	
Overall mean: Sites		1	6.01 ± 0.31	6.67 ± 0.29	7.08 ± 0.32	
		2	0.22 ± 0.01	0.20 ± 0.02	0.29 ± 0.02	
Overall mean: Year			2008	2009		
		1	6.88 ± 0.31	6.29 ± 0.30		
		2	0.23 ± 0.01	0.24 ± 0.02		

The proportion of viable seeds per cone was highly variable among fragments (Table 2.2) but the variation was independent of fragment size.

Table 2.2: Significant tests for the effect of site, year, fragment size and crown position on viable seed output in *W. whytei*.

Source	Number Df	Chi-square	Pr > Chi Square
Site	2	28.26	<.0001
Year	1	0.01	0.9105
Fragment size	3	73.53	<.0001
Crown position	3	0.98	0.8066

Exploratory analysis of the distribution of proportion of viable seeds per cone across fragment sizes using kernel density (gray lines), normal probability (black lines) and cumulative plots highlighted some differences between fragment sizes. Medium-sized populations had the highest probability (up to 30%) of cone failure to produce viable seeds (Figures 2.5 and 2.6). Cones from isolated trees produced average viable seeds compared with cones from small and large populations (Figure 2.5). The probability of isolated trees producing empty cones was nil (Figure 2.6).

**Figure 2.4:** Variability in the mean proportion of viable seeds per cone of different *W. whytei* fragment sizes on Mulanje Mountain. Vertical bars indicate 95% confidence intervals.

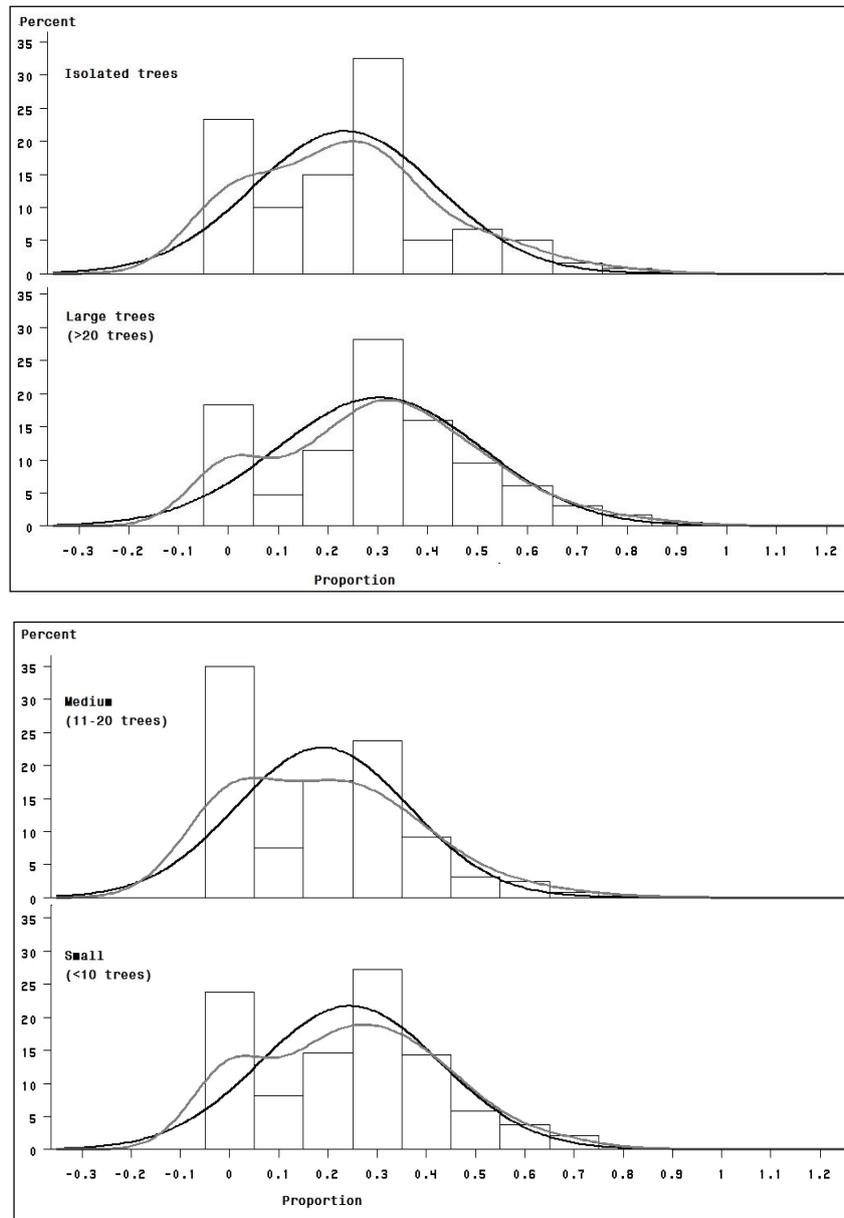


Figure 2.5: Exploratory analysis of the distribution of the proportion of viable seeds across fragment sizes of *W. whytei* using kernel density (gray lines) and normal probability plots. Vertical bars represent the distribution of observed values.

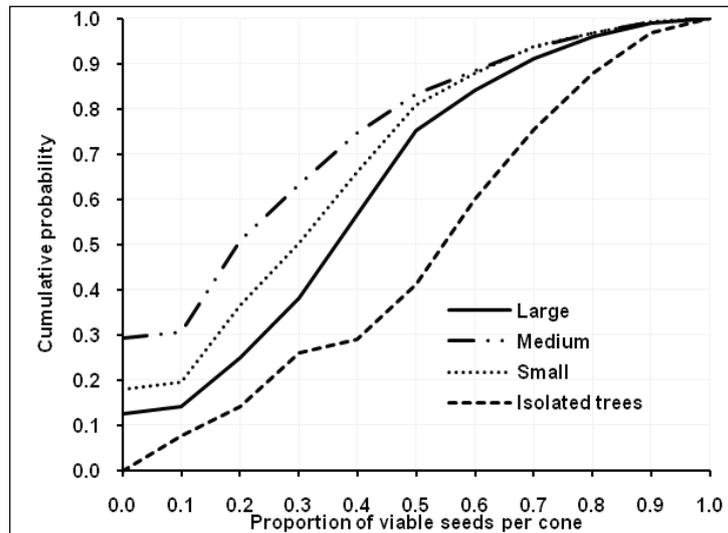


Figure 2.6: Cumulative probability distribution of *W. whytei* viable seed output per cone across fragment sizes.

2.3.3 Effect of crown position on viable seed output

Crown position did not have a significant influence on the proportion of viable seeds per cone (Table 2.2). Cones from the emergent and co-dominant trees produced higher seed viability (60% and 59% respectively), but they were not significantly different from those produced by understorey and intermediate trees (47% and 51% respectively). Cones from understorey trees had the lowest seed viability, while being the most variable (Figure 2.7).

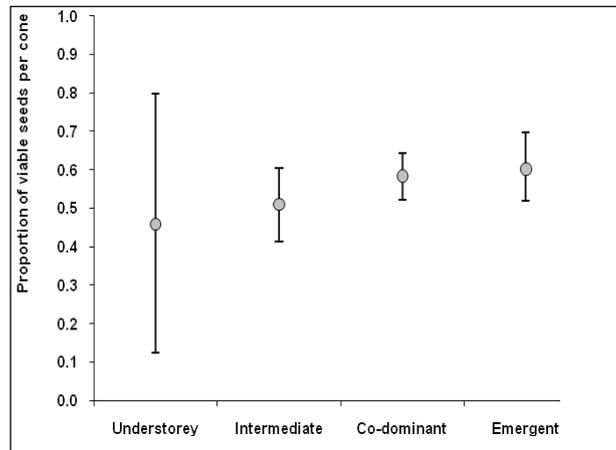


Figure 2.7: The relationship between the proportions of viable seeds per cone and crown position in *W. whytei* on Mulanje Mountain, Malawi

2.3.4 Effect of stem diameter on viable seed output

The parabolic regression was not significant (Figure 2.8a; Table 2.3). Residual plots indicated only a slight heteroscedastic behaviour (Figure 2.8b).

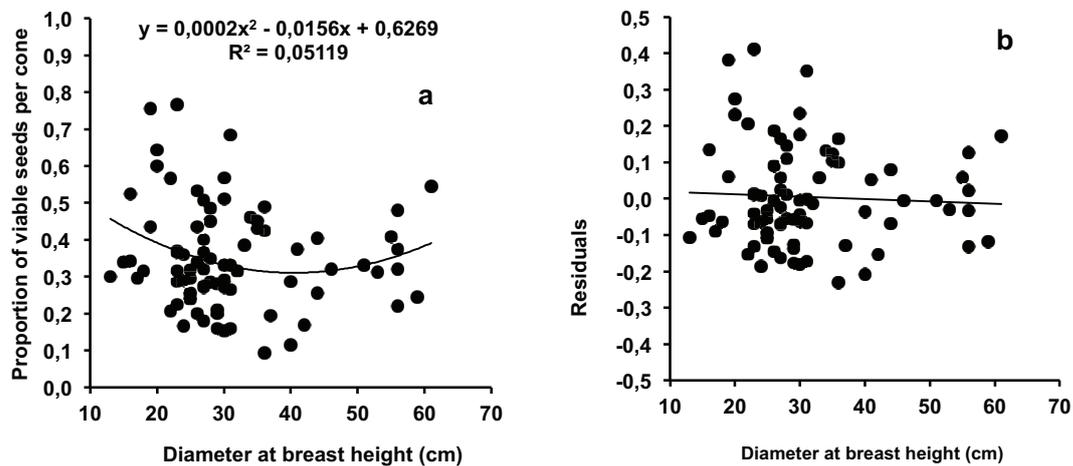


Figure 2.8: The relationship between (a) the proportion of viable seeds per cone and tree stem diameter at breast height (dbh) in *W. whytei* on Mulanje Mountain. Data points present the mean PVS per tree and (b) residual plots fit.

Table 2.3: Parabolic regression tests for effect of dbh on viable seed output in *W. whytei*

Variable	DF	Parameter Estimate	Standard Error	t value	Pr > t
Intercept	1	0.5072	0.1388	3.65	0.0005
Dbh	1	-0.0092	0.0081	-1.13	0.2615
Dbh ²	1	0.0001	0.0001	1.06	0.2934

2.4 DISCUSSION

2.4.1 Relationship between seeds produced and viable seeds

The study found that *W. whytei* cones produce 6 seeds on average, supporting the findings of Pauw and Linder (1997). The study also found that the proportion of viable seeds per cone was generally very low and this is evidence as to why *W. whytei* seed collection expeditions turn out to be very expensive. The observed low proportion of viable seeds per cone could probably result from pollination failure due to lower pollen quantity or quality produced by this species. Field observations on the flowering phenology of *W. whytei* indicated that male cone production was irregular on most trees with highest number of cones being produced during the months of November and December whilst female cone production was throughout the year. In addition, male cones were observed on very few branches during the same period. This suggests that limited quantity of pollen is produced in this species. Low pollen production was associated with low proportion of viable seeds per fruit in naturally fragmented populations of *Parnassia palustris* (Bossuyt 2006). The most important attributes

determining pollination efficiency are the timing, duration and intensity of flowering in plants simplified as synchronous and asynchronous flowering (Bronstein 1995). However, little is known on the flowering pattern of *W. whytei*. Further research is therefore required to establish whether synchronous or asynchronous flowering pattern occurs in this species.

2.4.2 Effect of fragment size on viable seed output

The study found that the proportion of viable seeds per cone was not related to population size and thus might be governed by different ecological processes. Studies conducted elsewhere reported similar findings (Cascante et al. 2002, Dick 2001). These results and earlier studies (Aizen and Feinsiger 1994, Bossuyt 2006, Wang et al. 2010) highlight the complex nature of predicting the outcome of fragmentation on seed viability in fragmented plant populations. Regarding variation in the number of cones producing viable seeds amongst population sizes, isolated trees produced the highest number of cones producing viable seeds compared with cones from all population sizes. In contrast, cones from medium-sized populations produced the highest number of cones producing empty seeds compared to all population sizes. These results highlight the importance of including isolated trees during seed collection expeditions.

The study also showed variations in the proportion of viable seeds per cone among sites and this could be attributed to differences in environmental conditions. Sombani has been described as sheltered from strong winds, warmer and drier while Chambe

and Lichenya are exposed to stronger, colder and wetter winds (Lawrence et al. 1994, Chapman 1995). Warmer temperatures, sheltered and drier conditions probably favour higher proportion of viable seed output in *W. whytei* cones and this needs further investigations.

Despite the lack of annual variation in the proportion of viable seed output per cone between 2008 and 2009, there was high annual variation in viable seed output in some fragments of the same size within sites. This variation may reflect reproductive isolation among different *W. whytei* populations on Mulanje Mountain. Similarly, Herrerias-Diego et al. (2006) reported that trees in one habitat condition experienced reproductive isolation from trees found within other habitats on the same site each year.

2.4.3 Effect of stem diameter (dbh) and crown position on viable seed output

The study found that tree size was not a key determinant of proportion viable seed output per cone in *W. whytei*. Both larger and smaller trees produced similar proportions of viable seeds per cone. Similarly, Manders (1987) observed no differences in viable seed output per cone between trees of different size classes in *W. cedarbergensis* in South Africa. Although cones produced by emergent and co-dominant trees had higher seed viability, they were not significantly different from those produced by understory and intermediate trees. Similarly, cones from isolated trees that resembled the emergent trees failed to produce higher proportions of viable seeds compared to all

fragment sizes. Again, these findings suggest that other ecological processes may govern the proportion of viable seeds per cone in *W. whytei*. Prevalence of adverse weather such as high winds or storms particularly during key phenological events such as pollination can reduce viable seed output (Stephenson 1981).

2.5 CONCLUSIONS

This chapter investigated the effect of fragment size, tree diameter and crown position in forest canopy on viable seed output per cone in *W. whytei*. The following findings were significant:

2.5.1 The proportion of viable seeds per cone in *W. whytei* is not affected by population size, tree diameter and crown position in the forest canopy. It seems other ecological factors such as pollen limitation and environmental conditions are possible causes of the low proportion of viable seeds per cone observed in this study. Further studies are therefore required to identify the factors that lead to the observed low proportion of viable seeds per cone, and how this influences whole-tree seed production in *W. whytei*.

2.5.2 The proportion of viable seeds per cone varied strongly among sites. A higher proportion of viable seed output per cone was associated with sites located in warmer, sheltered and drier conditions.

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CHAPTER 3

EFFECT OF POPULATION SIZE ON SEED RAIN PATTERN IN *WIDDRINGTONIA WHYTEI*²

3.1 INTRODUCTION

Seed dispersal is a critical process in the life history of plants. It allows plants to colonise new locations and, through heterogeneous distribution of propagules, dispersal influences patterns of seedling regeneration, establishment and survival and ultimately the density and distribution of the next generations of adult plants (Webb 1998, Nathan and Müller-Landau 2000, Wang and Smith 2002). Therefore, to predict how plant species disperse, it is important to measure seed rain (Cottrell 2004).

The sum of all propagules that land on a particular piece of ground is often referred to as the seed rain (Nathan and Muller-Landau 2000, Hardesty and Parker 2002). It is the primary reflection of seed dispersal (Hu et al. 2009). The seed rain is defined by quantity and is normally expressed as seeds per unit area per unit time (m^{-2} or $ha^{-1} year^{-1}$, $month^{-1}$ or day^{-1}), i.e. mean seed density over the area it has been dispersed to. Seed rain is a very important aspect of forest dynamics which has many practical applications but often overlooked. Lack of seeds can limit forest recovery after disturbance (Duncan and Chapman 1999, Holl 1999, Cubina and Aide 2001). In order to determine the rate of forest recovery, the flux of seeds into disturbed sites must be determined and a seed rain study becomes obvious. Furthermore, forest management programs aimed at re-establishing important species have been based on inadequate knowledge of the

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species colonising abilities (Cottrell 2004). Again, there is broad evidence that seed rain studies provide important information on which species need to be assisted with regeneration in revegetation programs (Hardesty and Parker 2002, Cottrell 2004). Stewart et al. (2001), for instance, reported a maximum natural regeneration density of 15,778 seedlings ha⁻¹ in an established white spruce stand in Alberta, Canada. To attain the above natural regeneration density, Green et al. (2002) suggested a minimum seed-rain density of 500,000 white spruce seeds ha⁻¹ for adequate density and stocking. These findings clearly demonstrate that information from seed rain studies can provide answers to some of the factors that limit the re-establishment of functioning forests.

There are several factors that affect seed rain. Plant population size influences the quantity of seed rain dispersed within a specific site supplying the seed (Jules and Rathcke 1999, Cunningham 2000). In Chiloe Island, Chile, for instance, Armestro et al. (2007) observed that large population sizes dispersed higher seed rain density than small population sizes. Similarly, seed rain density was higher in large population sizes of *Ilex aquifolium* than in small population sizes in Spain (Herrera and Garcia 2009). However, the effect of plant population size on seed rain density varies amongst species (Dosch et al. 2007).

Seed rain, in many plant species, is highly variable both in time and space. Some species disperse large amounts of seed at intervals of several months or years with little or no seed dispersal in the intervening periods (Zhang et al. 2008). In China, for

example, *Pinus koraiensis* (Korean pine) dispersed most of its seed in summer (Zhang et al. 2008). In Brazil, on the other hand, Barbosa and Pizo (2006) reported continuous seed rain in tropical plant species *Tectona stans* and *Maclura tinctoria*. Similarly, seed rain is influenced by seed limitation, which can be understood as the failure of seed of a given plant species to arrive at suitable sites in sufficient numbers to establish as seedlings (Müller-Landau et al. 2002, Levin et al. 2003). Thus, the seed rain pattern of tree species is an indicator of its regenerative potential.

Widdringtonia whytei naturally has a scattered (patchy) distribution pattern on Mulanje Mountain but the patches have been impacted further by human resource use, management for fire, colonisation by invasive species and conifer aphid attack (Chapman 1995, Bayliss et al. 2007). In disturbed, fragmented and degraded landscapes many studies have suggested that seed availability is a major limiting factor in vegetation recovery (Duncan and Chapman 1999). The phenological pattern of seed rain and seed limitation in *W. whytei* has hitherto received no attention and is little known. Determining seed rain dynamics and seed limitation may therefore be pivotal in understanding the recruitment dynamics of *W. whytei* on Mulanje Mountain. Therefore, the purpose of the study was to investigate the seed rain and seed limitation patterns specifically, establishing the influence of *W. whytei* population size, time of the year, position within a fragment and site on seed-rain density and seed limitation within and around the *W. whytei* fragments.

3.2 MATERIALS AND METHODS

3.2.1 Study area

This study was conducted on Mulanje Mountain in Malawi at three sites, namely Sombani (15°52'41"S; 35°40'89"E), Chambe (15°62'40"S; 35°30'72"E) and Lichenya (15°62'33"S; 35°30'73"E). The altitude of these sites ranges from 1660–2265 m asl (Lawrence et al. 1994). Minimum and maximum temperatures range from 5–28°C with an annual rainfall of 2859 mm per annum (Chapman 1995). These sites are characterised by *W. whytei* forest fragments of different sizes ranging from 0.8–1779 ha, with most of them confined to ravines and hollows.

3.2.2 Study species

Widdringtonia whytei is a wind-pollinated coniferous tree endemic to Malawi and valued for its fine timber with attractive fragrance (Bayliss et al. 2007). *W. whytei* grows up to 40 m in height and c. 1 m in stem diameter at breast height (dbh). Male and female cones are borne on the same tree (Chapman 1995) and sometimes on the same branch (pers. obs.). Cones are globose, 1.5–2 cm in diameter, dark brown with four scales (Chapman 1995). The number of cones per cluster is much less variable, with generally two cones per cluster. Female cones seldom remain closed for more than 2 years (Pauw and Linder 1997). A cone generally contains 4–8 seeds (Pauw and Linder 1997).

W. whytei seed is 25 mm long and 12 mm wide on average with a wing (Pauw and Linder 1997) and is wind-dispersed (Chapman 1995).

3.2.3 Population selection

The seed rain was characterized in three forest fragments at the three sites. The fragments were selected following a stratified random sampling method. At each study site, all fragments were recorded and grouped into three size categories (small, medium and large). Small fragments consisted of groups of ≤ 10 *W. whytei* cone-bearing (reproductively mature) trees in an area either surrounded by other tree species or grass vegetation. Medium fragments consisted of groups of 11-20 cone-bearing individuals while large forest fragments are those comprising of groups of >20 *W. whytei* cone-bearing trees. Only trees in the small and medium fragments were physically counted. Other factors were considered during forest fragment selection, such as accessibility; distance between forest fragments (1 km was considered the minimum distance between selected forest fragments) and comparable stand characteristics such as orientation, elevation and slope despite most fragments being confined to the ravines (slopes) and hollows. In each size group, after exclusion of potentially unsuitable fragments, the suitable fragments were numbered using a piece of paper and placed in a bag. Only one paper bearing a number of a forest fragment was later picked from the bag after a thorough mixing to ensure randomization. Nine fragments were selected for the study, i.e. one fragment per size category (small, medium and large) at each of the three sites (Chambe, Lichenya and Sombani).

3.2.4 Sampling of seed rain

Most of the study fragments are found in deep and narrow valleys. General wind direction in and around fragments was assessed during September. Wind conditions around the forest patches were calm despite the presence of strong winds during this time of the year. The presence of many small peaks or rocky outcrops tends to change the local pattern of wind direction and shelter the fragments from the winds. Wind direction may have more influence on exposed fragments which are uncommon on the mountain. No specific provision was therefore made for wind direction in the sampling design.

Several seed rain trapping methods are available but two general methods commonly used are trapping on sticky surface and in gravity traps (Cottrell 2004). Sticky traps are commonly used in greenhouses to control insect pests. Seed on these traps are exposed and the seed remains vulnerable to removal by wind and rain. Collections using sticky cards may be problematic. The advantage of this method, however, is the ability to focus trapping on seeds capable of distant travel (Kollmann and Goetze 1998).

Gravity traps, on the other hand, include pit traps, surface-placed trays and funnel traps. Pit traps consist of open-top containers buried in the soil. They are poorly suited to seed trapping during winter due to large volume of litter that may accumulate (Kollman and Goetze 1998). Tray traps consist of shallow containers laid on the forest floor. This method is good for collecting large seeds, fruits and cones. Funnel traps concentrate

seed collections into a small area and depending on the trap construction and position; the seeds can be relatively free from predation. Given the problems associated with other trap methods, funnel seed traps were used to capture seed rain in this study (Kollmann and Goetze 1998, Page et al. 2002, Chabrierie and Alard 2005).

The general sampling design for assessment of the seed rain is shown in Figure 3.1. Due to large variability in the seasonality of seed rain and seed production in forest stands, Cottrell (2004) recommends conducting preliminary seed rain studies in order to determine the required number of traps. A pilot study was carried out during the months of January to April 2007 to determine the number of traps required, based on a 40 cm trap diameter (Figure 3.2). The minimum number of traps (n) required for the whole study was determined following Bonham (1989) formula:

$$n = t^2 s^2 / (k \bar{x})^2$$

where: n is the estimated number of traps needed for a specified probability and precision; t is the t-table value; s^2 is the pilot study sample variance; k is the precision required; and \bar{x} is the pilot study mean of seeds per trap.

From the pilot study ($n = 10$); $t = 1.96$; $s^2 = 0.5$; $k = 0.05$ and $\bar{x} = 0.5$, the minimum number of traps required was 77. This study used a total of 95 (40 cm diameter) seed traps.

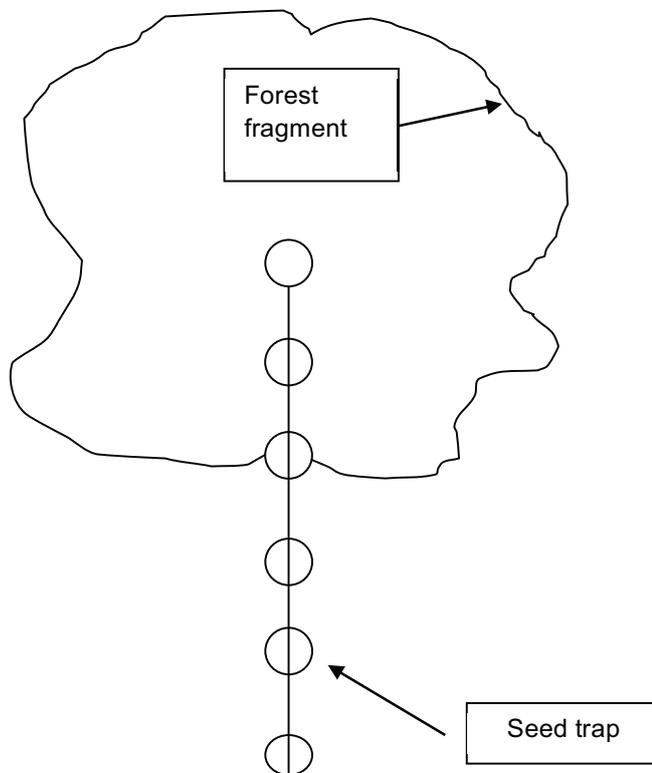


Figure 3.1: Sketch map showing the layout of seed traps in and around a selected forest fragment

Cottrell (2004) recommends using 20-30 cm top diameter funnel seed traps. However, in this study, funnel seed traps, with circular iron wire frame, locally made from high density plastic sheet (Figure 3.2) but with a top of 40 cm diameter (0.1257 m^2) were used. Seed traps with a slightly larger catchment surface area were deliberately used in order to capture more seeds since *W. whytei* tree crowns are generally narrow.

Seed traps were systematically placed from a randomly selected spot on the edge (0 m) of the selected forest fragment. The forest edge was defined as starting at the base of

the first trunks of mature *W. whytei* trees encountered moving into and outside the forest patches. Seed traps were placed at every 10 m up to 30 m outside the forest fragment and inwards up to the centre (Figure 3.1). The distance to the fragment centre varied depending on the fragment size, but 30 m distance was decided as the furthest point seed traps could be placed outside the fragment.

Seed traps were placed 1.5 m above ground (Figure 3.2), as recommended by Kollmann and Goetze (1998). This height was chosen to eliminate seed from taller grasses and herbs which were a common feature in and around the fragments. This height also prevents rodents to enter the seed traps and eat the seed (Cottrell 2004). Eliminating potential seed sources reduces the amount of time required for seed sample processing and decreases the chances of missing the target seeds in large sample volumes (Cottrell 2004).



Figure 3.2: Seed collection from a locally made funnel seed trap placed at 1.5 m above the ground in a *W. whytei* fragment on Mulanje Mountain, Malawi

Seeds were collected from the traps on a monthly basis for a period of 24 months. *W. whytei* seed was easily sorted out from all the trapped fruits, seeds and litter by the data collection team as it was easily identified. This greatly simplified the processing of seed trap collections. Sorting time varied from trap to trap as volume of litter and other plant materials varied between them.

3.2.5 Data analysis

To investigate the effect of fragment size, forest patch position, site, month and year on the seed rain density, a generalised linear model (GLM) with quasi-Poisson and negative binomial link function were tested in the data analysis in order to account for the distribution of count data (Seifert et al. 2010). However, since the quasi-Poisson model was poor as deviances were very high ($5.3 \gg 1.0$), the negative binomial distribution model was used instead. Assessment of goodness of fit of the negative binomial link function distribution model is presented in Table 3.1.

Table 3.1: Assessment of goodness of fit of the negative binomial link function.

Criterion	DF	Value	Value/DF
Deviance	640	175.4404	0.2741
Scaled Deviance	640	175.4404	0.2741
Pearson Chi-Square	640	663.8752	1.0373
Scaled Pearson X2	640	663.8752	1.0373
Log likelihood		7470.7799	

Seed limitation, calculated as the proportion of seed traps not receiving seeds after two years of seed collection (“fundamental seed limitation” sensu Müller-Landau et al. 2002), was also analyzed. Thus, seed limitation was expressed as:

$$\text{Seed limitation index} = 1 - a/n$$

Where, a = the number of seed traps reached by *W. whytei* seed and n = the total number of seed traps. In this study, a seed limitation index >0.80 suggested a very strong seed limitation.

3.3 RESULTS

3.3.1 Seed rain

Seed rain-density was significantly different among *W. whytei* fragment sizes (Table 3.2 and 3.3). Traps in large fragments (>20 trees *W. whytei* cone-bearing trees) collected $10.4 \text{ seeds m}^{-2} \text{ year}^{-1}$ compared to $0.7 \text{ seeds m}^{-2} \text{ year}^{-1}$ for medium fragments (11-20 trees) and $1.1 \text{ seeds m}^{-2} \text{ year}^{-1}$ for small fragments (≤ 10 trees) (Tables 3.2 and 3.4). Seed rain was not significantly different between medium and small fragment (Tables 3.2 and 3.4). The mean annual seed rain density varied greatly between years (Table 3.2 and 3.3). Seed rain density was significantly higher in 2009 ($6.1 \text{ seeds m}^{-2} \text{ year}^{-1}$) than in 2008 ($2.07 \text{ seeds m}^{-2} \text{ year}^{-1}$) (Tables 3.2 and 3.4). Overall seed rain density varied significantly across sites (Table 3.3). Traps at Sombani collected the highest

seed rain density ($7.6 \text{ seed m}^{-2} \text{ year}^{-1}$) whereas those at Lichenya collected the lowest ($1.7 \text{ seeds m}^{-2} \text{ year}^{-1}$) (Tables 3.2 and 3.4).

Table 3.2: Mean seed rain densities ($\text{m}^{-2} \text{ year}^{-1}$) collected for *Widdringtonia whytei* in 2008 and 2009 in and around three fragment sizes at three sites on Mulanje Mountain, Malawi.

Fragment size	Year	Sites			Overall mean: Fragment size
		Chambe	Lichenya	Sombani	
Large (> 20 trees)	2008	3.3 ± 1.6	0.2 ± 0.2	12.2 ± 4.1	10.4 ± 0.9
	2009	13.8 ± 3.6	5.3 ± 2.7	27.6 ± 8.0	
Medium (11-20 trees)	2008	0	0.9 ± 0.3	0	0.7 ± 0.2
	2009	0.7 ± 0.4	2.0 ± 1.2	0.4 ± 0.3	
Small (≤ 10 trees)	2008	0	0.2 ± 0.2	1.8 ± 1.4	1.1 ± 0.4
	2009	0	1.6 ± 0.8	3.3 ± 1.4	
Overall mean: Sites		2.9 ± 0.7	1.7 ± 0.5	7.6 ± 1.7	
Overall mean: Year		2008	2.1 ± 0.6	2009	6.1 ± 1.1

Table 3.3: Generalized linear model (GLM) using negative binomial link function significant tests for the effect of site, year, fragment size and position on seed-rain density in *W. whytei* on Mulanje Mountain.

Source	DF	Chi-Square	Pr > Chi Square
Site	2	6.23	<0.0444
Year	1	4.47	0.0346
Fragment size	2	25.04	<0.0001
Fragment position	2	96.75	<0.0001

Seed rain was highest inside the forest fragments ($12.04 \text{ seeds m}^{-2} \text{ year}^{-1}$) and decreased drastically and very significantly at the edges ($1.0 \text{ seed m}^{-2} \text{ year}^{-1}$) and outside the forest fragments ($0.11 \text{ seed m}^{-2} \text{ year}^{-1}$) (Figure 3.3). Seed rain did not vary between the forest edges and the adjacent non-forest areas. Again the highest seed rain was recorded at Sombani inside the forest fragments ($22.6 \text{ seeds m}^{-2} \text{ year}^{-1}$) then

Chambe (8.6 seeds m⁻² year⁻¹) and Lichenya (5.0 seeds m⁻² year⁻¹) (Figure 3.3) and the differences were significant (Table 3.4).

Seed rain occurred throughout the year, i.e. from January to December during the study period (Figure 3.4). There was no significant difference on overall seed-rain density among months ($p > \text{ChiSq} = 0.6258$). However, the highest seed-rain density was recorded in September (5.0 seeds m⁻² y⁻¹) in 2008 and August (12.7 seeds m⁻² y⁻¹) in 2009 (Figure 3.5).

Table 3.4: Parameter estimates using generalised linear model (GLM) with negative binomial function link on seed-rain density in *W. whytei* on Mulanje Mountain.

Parameter		DF	Estimator	STD Error	Wald 95% confidence Limits		Chi-Sq	Pr>ChiSq
Intercept		1	-3.5304	0.6349	-4.7748	-2.2860	30.92	<0.0001
Site	Chambe	1	-0.3111	0.5158	-1.3220	0.6998	0.36	0.5464
	Lichenya	1	-0.1569	0.4915	-1.1203	0.8064	0.10	0.7495
	Sombani	0	0.0000	0.0000	0.0000	0.0000		
Year	2008	1	-1.2028	0.3675	-1.9231	-04825	10.71	0.0011
	2009	0	0.0000	0.0000	0.0000	0.0000		
Fragment	Large	1	2.8793	0.5137	1.8725	3.8861	31.42	<.0001
	Medium	1	-0.4524	0.5502	-1.5307	0.6259	0.68	0.4109
	Small	0	0.0000	0.0000	0.0000	0.0000		
Position	Edge	1	-0.6331	0.5890	-1.7876	0.5214	1.16	0.2825
	Inside	1	5.1077	0.5375	4.0542	6.1613	90.29	<0.0001
	Outside	0	0.0000	0.0000	0.0000	0.0000		
Dispersion		1	10.5991	1.5772	7.5078	13.6904		

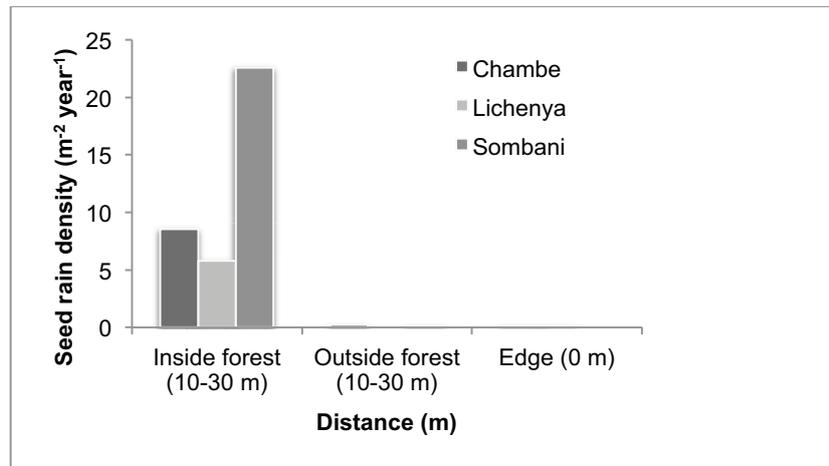


Figure 3.3: Mean seed rain pattern (seeds m⁻² year⁻¹) of *W. whytei* from inside to outside forest fragments on Mulanje Mountain, Malawi.

On the other hand, the lowest seed rain density was recorded in June (0.9 seeds m⁻² y⁻¹) and November (0.6 seeds m⁻² y⁻¹) in 2008 and February (1.5 seeds m⁻² y⁻¹) and May (2.1 seeds m⁻² y⁻¹) in 2009 (Figure 3.5). The lowest seed rain density was generally collected in February and May in both years (Figure 3.4). Again, traps at Sombani collected the highest seed rain density in almost every month (Figure 3.5).

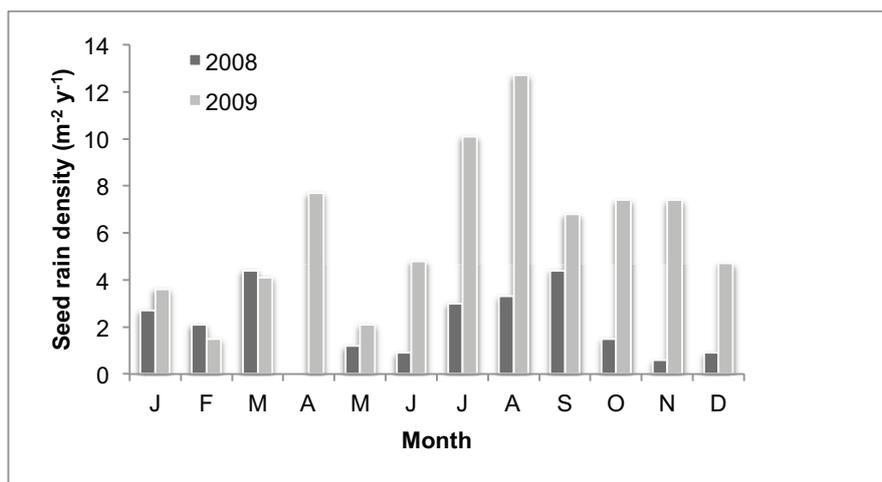


Figure 3.4: Mean monthly seed rain pattern (m⁻² year⁻¹) of *W. whytei* in 2008 and 2009 on Mulanje Mountain, Malawi

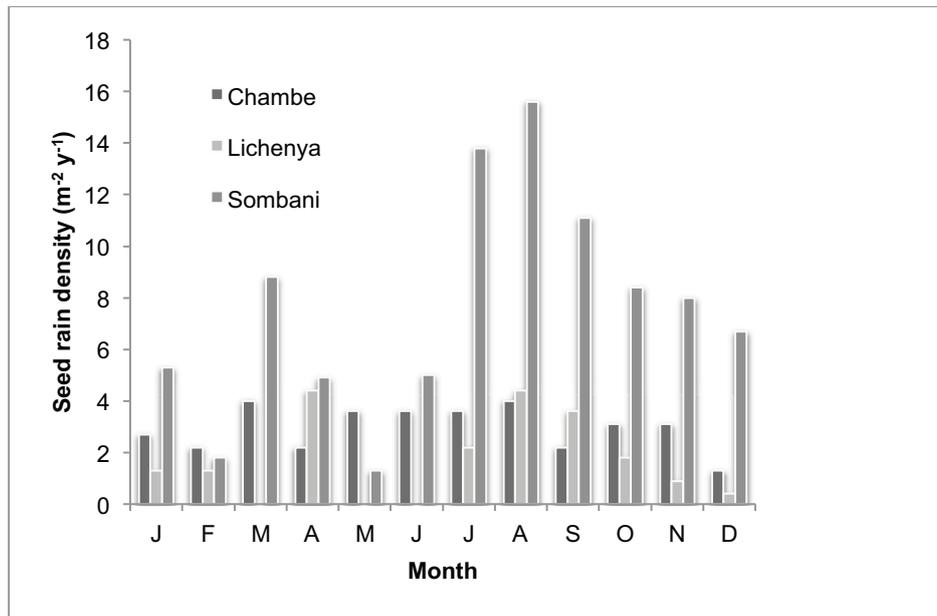


Figure 3.5: Mean monthly *W. whytei* seed rain density sampled in seed traps placed at three sites on Mulanje Mountain, Malawi

3.3.2 Seed limitation

Seed limitation did not vary among *W. whytei* fragment sizes, with 0.95 for large fragments and 0.99 for medium and small size fragments (Table 3.5). Seed limitation was very strong at all sites (0.98), but areas inside forest fragments at Sombani had slightly weaker seed limitation (0.85; Table 3.5). All seed traps placed outside forest fragments (10-30 m) and edge (0 m) of the large fragments failed to receive any seed during the two-year observation period at Lichenya. The same was observed at Sombani, Lichenya and Chambe in the medium and small fragments (Table 3.5).

Table 3.5: Seed limitation indexes for *W. whytei* in three fragment sizes and positions at three sites on Mulanje Mountain, Malawi

Fragment Size	Forest fragment position	Site			Overall means: Fragment size
		Sombani	Lichenya	Chambe	
Large (> 20 trees)	Outside (10-30 m)	0.99	1.00	0.97	0.95
	Edge (0 m)	1.00	0.96	0.99	
	Inside (10-30 m)	0.85	0.95	0.90	
Medium (11-20 trees)	Outside (10-30 m)	1.00	1.00	1.00	0.99
	Edge (0 m)	1.00	1.00	1.00	
	Inside (10-30 m)	0.99	0.96	0.97	
Small (\leq 10 trees)	Outside (10-30 m)	1.00	1.00	1.00	0.99
	Edge (0 m)	1.00	1.00	1.00	
	Inside (10-30 m)	0.97	0.97	1.00	
Overall site means		0.98	0.98	0.98	

3.4 DISCUSSION

The study shows that seed-rain density is highly variable both in time and space and may affect the recruitment of *W. whytei* on Mulanje Mountain. Large fragments contributed significantly higher seed densities than the medium and small fragments. This is not surprising because large *W. whytei* fragments had more seed producing trees (>20 trees) than medium and small fragments. Similar findings were also reported in other studies (Santos et al. 1999; Armesto et al. 2007). Seed rain was inconsistent among *W. whytei* fragments where no seed rain was collected in small and medium fragments at some sites whereas large fragments dispersed seeds at all sites throughout the study period. Certainly, the results point to the importance of large fragment sizes in promoting consistent seed rain in this species. For these reasons, the presence and retention of more cone bearing trees (seed trees) seems to be very important for the continued availability of *W. whytei* seed on the forest floor.

There was a pronounced difference in the amount of seed rain produced between 2008 and 2009. This interannual difference appears to reflect the repeated temporal fluctuations in seed crops that characterise most tree species. Alternate years of high and low seed crops have been reported for many species (Armestro et al. 2007). Similarly, *W. whytei* fragments showed distinct year of high seed-rain density alternated with low or zero seed-rain year. This information provides evidence of mast seeding behaviour in *W. whytei*. Cyclic seed rain has been found in some mast-seeding species (LaMontagne and Boutin 2007). However, two years was too short a period to establish mast and regular years of seed production in *W. whytei*. Longer-term observations should therefore be carried out to better understand the interval of mast years in this species.

Widdringtonia whytei seed rain occurred all year round with relatively high peaks in March/April and July August/September. The occurrence of seed rain throughout the year suggests that *W. whytei* maintains an aerial seed bank and is serotinous. Most species that are serotinous have seed-hold structures (e.g. cones and capsules) that are resinous (Lamont et al. 1991). Opening of such seed-holding structures may occur once there is enough heat to melt the resinous materials that prevents parts of the structure, especially cones, from opening (Helium and Pelchat 1979). Serotiny is an ecological adaptation exhibited by some seed plants, in which seed release occurs in response to an environmental trigger, rather than spontaneously at seed maturation (Lamont et al. 1991). The most common and best studied trigger is fire and the term serotiny is often used to refer to this specific case. *W. whytei* is dependent on fire for

successful regeneration (Chapman 1995, Pauw and Linder 1997). *W. whytei* cones are resinous (pers. obs). This could be one of the reasons as to why high seed-rain densities were collected during the months of August to October during the study period. During this time of the year the mountain experiences slightly warmer temperatures as a result of general temperature rises (Chapman 1995) or increases in fire incidences where the strong upward draughts of air during the fire increases temperatures. For example, Nangoma and Bayliss (2007) observed fire occurrence on the mountain from 2001 to 2006 through the MODIS Rapid Response (MRR) fire alert system. A total of 325 fires were recorded but fire incidence was greatest from the middle to end of the dry season with August having by far the greatest number of fires (132) followed by October (101) and September (60). These observations suggest that seed rain in *W. whytei* is highly adapted to temperature changes. However, it seems to indicate that the species has a weak serotiny; cones seldom remain closed for more than two years (Pauw and Linder 1997). In those species with strong serotiny seed can be retained for many years (e.g. 8 years in *Widdringtonia cupressoides*; Midgley et al. 1995).

W. whytei presented a highly variable seed rain pattern among sites on Mulanje Mountain. The mountain comprises a 'dry region' predominantly on the western side and northern side, and 'wet region' on eastern and southern sides (Chapman 1995). The wetter south-eastern side is due to the prevailing weather fronts from the Mozambique coast and mountains. Sombani is located in the dry north region, whereas Chambe and Lichenya are located in the wetter southern and eastern regions of the

mountain, respectively. Therefore it can be suggested that dry conditions promote high seed rain density in *W. whytei*, hence the high seed-rain density recorded at Sombani.

It is widely recognised that seeds from species that are dispersed by wind can disperse over a longer distance than those dispersed in other ways (Fragoso 1997, Qian 2009). In this study, evidence for longer dispersal distance of the wind-dispersed *W. whytei* was not found. Seed limitation was generally very strong as evidenced by the high number of seed traps that did not receive any seed during the two-year observation period. The major difference in seed-rain pattern was associated with a high concentration of seeds within forest fragments, which suggested that *W. whytei* cannot disperse its seeds for a long distance. This also suggested that the species has a non-random distribution pattern of seed rain (Loiselle et al. 1996). Seed rain declined drastically at the forest edges with seed limitation being highest on the adjacent non-forest habitats which implied that *W. whytei* seeds could not disperse beyond 10 m from the forest edge. Similar results were also found in other studies (Zhang et al. 2005). However, despite the greater concentration of *W. whytei* seed rain within forest fragments, there is strong evidence that they are less likely to germinate and produce viable seedlings in this shady habitat (Chapman 1995). Thus, although it is less numerous, seed falling at the forest margins or away from the forest margin into the grassland due to strong upward draughts of air during fire is most likely to contribute to seedling recruitment of the species. The strong seed limitation observed at all sites may exacerbate the limited distribution of *W. whytei* on the mountain. If seed limitation indeed regulates population recruitment, *W. whytei* has very limited chances to colonise

non-forest habitats. Although this study found strong seed limitation in *W. whytei*, two years was too short a period to draw general conclusions. Clark et al. (1998) recommends long-term observation periods to establish seed limitation in plant species (e.g. 5 years) since results from short-term seed limitation studies are sometimes misleading, especially in pioneer plant species that maintain active long-lasting soil-stored seed banks (Dalling et al. 2002).

3.5 CONCLUSIONS

Seed dispersal is important for understanding the natural regeneration mechanisms of plant species. This chapter assessed the influence of *W. whytei* fragment size on seed rain-density and seed limitation. The following findings were significant:

3.5.1. Seed rain density in *W. whytei* is clearly related to population size. Forest fragments with more than 20 cone-bearing *W. whytei* trees dispersed more seeds per unit area than smaller fragments. Therefore, retaining more trees in a fragment would ensure continued availability of *W. whytei* seeds on the forest floor.

3.5.2. Seed rain in *W. whytei* occurred all year round which is an indication that the species maintains an aerial seed bank. However, the current illegal harvesting rate of *W. whytei* trees with green or live crowns is a great concern because it may reduce the potential seed bank.

3.5.3. Warmer sites, drier and warmer months of the year collected higher seed-rain densities than cooler sites and wetter and cooler months of the year.

3.5.4. In general, this study found strong seed limitation in *W. whytei*; an indication that the species has low chances of colonising the non-forest habitats on the mountain. Because of strong variation in seed rain among sites and years and the on going changing habitat conditions, long-term monitoring of seed rain is essential to obtain a comprehensive understanding of the dynamics of seed rain in this species.

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CHAPTER 4

GERMINATION RESPONSE AND VIABILITY OF *WIDDRINGTONIA WHYTEI* SEEDS TO TEMPERATURE AND LIGHT³

4.1 INTRODUCTION

Charles Darwin in 1855 said "...I have had one experiment some little time in progress which will, I think, be interesting, namely seeds in salt water, immersed in water of 32° - 33° [...], I have in small bottles out of door, exposed to variation of temperature, cress, radish, cabbages, lettuce, carrots and celery and onion seed - four great families. These, after immersion for exactly one week, have all germinated, which I did not in the least expect (and thought how you would sneer at me): for the water of nearly all, and of the cress especially smelt very badly, and the cress seed emitted a wonderful quantity of mucus (the 'Vestiges' would have expected them to turn into tadpoles), so as to adhere in a mass; but these seeds germinated and grew splendidly. The germination of all (especially cress and lettuces) has been accelerated, except the cabbages, which have come up very irregularly, and a good many, I think, dead. One would have thought, from their native habitat, that the cabbage would have stood well. The Umbelliferae and onions seem to stand the salt well." (April 13th, 1855, cited from Darwin 1887).

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T.F. Chanyenga, C.J. Geldenhuys, G.W. Sileshi (2012): Germination response and viability of an endangered tropical conifer *Widdringtonia whytei* seeds to temperature and light. *South African Journal of Botany* 81:25-28. Appendix 1

Charles Darwin's interest in seed germination was a focus within his wider interest in plant development. He published several papers about the above findings in the *Gardeners' Chronicle* and *Agricultural Gazette*. His interest in seed germination was indeed well founded. Seed germination is one of the most critical stages in the life cycle of plants (Thompson and Ooi 2010), and germination requirements are often assumed to be adaptations to the particular habitats where the species occur (Meyer et al. 1990). Each species has particular requirements for seed germination as a result of adaptive radiation into patchy and changing environments (Simons and Johnston 2006). Therefore, understanding these requirements is very important in the conservation of endangered species such as *W. whytei*. Temperature and light are important ecological factors that regulate seed germination of many plant species (Botha et al. 1982, Baskin and Baskin 1998, Jarvis and Moore 2008). Both low and high temperatures have been reported to inhibit seed germination in several species (Teketay 1994, Amri 2010).

The role of light in the germination of seeds has been studied by several authors (Botha et al. 1982, Baskin and Baskin 1998). Some seeds germinate equally well in light and darkness (Baskin and Baskin 1998), while others germinate more readily either only under light (Grime et al. 1981, Baskin and Baskin 1990, Colbach et al. 2002) or darkness (Thanos et al. 1989, Baskin and Baskin 1990). In addition, light requirements for seed germination may vary with changes in temperature (McLemore and Hansbrough 1970, Farmer et al. 1984, Baskin and Baskin 1998). Although many studies have been conducted on the effects of temperature and light on seed germination,

temperature and light germination requirements are relatively unexplored in many plant species (Cony and Trione 1996).

While some studies exist on the effects of temperature on the germination of *W. whytei* seed (Gondwe 2010) no studies exist on the effect of light on the species seed germination. Therefore, the objective of this study was to determine the effect of temperature under both light and dark conditions on the germination of *W. whytei* seeds.

4.2 MATERIAL AND METHODS

4.2.1 Site description

Mature cones of *W. whytei* were collected and bulked in August 2009 from a large population (86 trees of different sizes) at Sombani (15°52'41"S and 35°40'89"E) on Mulanje Mountain. The temperatures on Sombani vary from 5°C in winter to about 28°C in summer. The lowest temperatures usually occur in June/July while the warmest temperatures occur in October. More than 2200 mm of rainfall is received on the site annually. The altitude at Sombani ranges from 1660-2265 m asl (Lawrence et al. 1994). Seeds were obtained after the cones were placed and allowed to open in the shade at the Forestry Research Institute of Malawi, Zomba. In the drying shed, temperature varied between 22 and 30°C and relative humidity ranged between 30% and 38%. Seeds were dried to 5% moisture content (Gondwe 2010). The seed moisture content

(MC) was determined gravimetrically and expressed on a fresh weight basis. The dry mass was measured after heating seeds in an oven for 17 h at 103°C according to the International Seed Testing Association rules (ISTA 2012). Seeds of *W. whytei* are 25 mm long and 12 mm wide with a wing and are dark in colour (Pauw and Linder 1997).

4.2.2 Seed germination and viability

Germination tests included seven temperature regimes with light and dark treatments. Each treatment was replicated five times with 20 seeds per replicate. Seeds were placed in 9-cm diameter Petri dishes filled with Agar-agar, and incubated in a LTGC - 40 growth cabinets at constant temperatures of 5, 10, 15, 20 and 25°C and two alternating temperatures of 15°C night and 25°C day and 10°C night and 20°C day temperatures. These alternating temperatures will be referred to as 15N/25D and 10N/20D in the following discussion. Seeds under the light and dark treatments were exposed to photoperiods of 12 h light/12 h dark hours or continuous dark respectively. Light was provided by the cool-white 40-W fluorescent bulbs with 1000 lux (ISTA 2012). Fluorescent tubes were used because they emit considerable red but little far-red light (Baskin and Baskin 1998). Dark condition was provided by wrapping Petri dishes in three layers of aluminium foil (Baskin and Baskin 1998). Germination counts under light treatments were recorded daily for 30 days while samples in darkness were opened on the final day of the experiment. A seed was considered to have germinated when the radicle was observed.

Complete germination rarely occurs when undertaking seed germination experiments (Scott et al. 1984). Seeds that failed to germinate at the end of the observation period were tested to see if they were viable. Vital staining with 2, 3, 5-triphenyl-2*H*-tetrazolium chloride (Tetrazolium test) was used in this study and was followed by cutting tests for confirmation. Seeds were cleaned with water and soaked in 1% tetrazolium chloride solution for 8 h at 25°C (ISTA 2012). Pink embryos were scored as live and total count was expressed as percentage of viable seeds. The tetrazolium test (Baskin and Baskin 1998) is a rapid biochemical test of seed viability compared to the more slow germination assays (Donald 1994). The tetrazolium test becomes a dormancy test when it is done on the ungerminated seeds left after a standard germination test or as a separate test alongside the germination test.

4.2.3 Statistical analysis

Final germination percentages were calculated for each treatment as the cumulative number of germinated seeds with normal radicles out of the total of 20 seeds per replicate. Since the germination percentage data violated the normality assumptions (Shapiro-Wilk and Kolmogorov-Smirnov tests), data were subjected to the GLM procedure of the SAS system (SAS Institute Inc., USA). Means were separated using Tukey's studentized range test (HSD). A Weibull regression model (Wipfler et al. 2009) was also applied as the germination values are duration observations. The Weibull

model relaxes the exponential model's assumption of constant hazard, and allows the hazard rate to increase or decrease monotonically with respect to elapsed time. The Lifereg procedure of SAS was used to estimate the parameters of regression. This method estimates parameters using a maximum likelihood method.

4.3 RESULTS

4.3.1 Germination response

The highest cumulative germination percentage (100%) was recorded in seeds incubated at a constant temperature of 20°C, followed by alternating temperatures of 15N/25D, which achieved 87% germination (Figure 4.1). Cumulative germination significantly varied ($P < 0.001$) with time (days) at all temperatures (Table 4.1). According to the -2 log likelihoods, the Weibull function poorly fitted the data for temperatures 20, 15N/25D and 25°C (Figure 4.1, Table 4.1).

The cumulative distribution shows that germination reaches 100% within 25 days at a constant temperature of 20°C while at the alternating temperatures of 15N/25D it took almost 30 days to attain 80% germination (Figure 4.1). It took about 16 days for seeds to start germinating at constant temperature of 20°C and alternating temperatures of 15N/25D. It took 21 days for seeds to start germinating at constant temperature of 15°C and alternating temperature 10N/20D (Figure 4.1). Germination remained below 50% either at constant temperatures below or above 20°C.

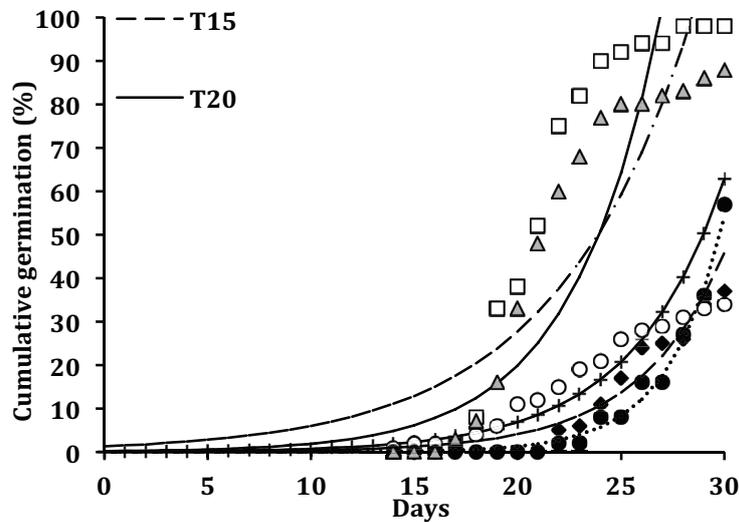


Figure 4.1: Cumulative distribution of germination of *Widdringtonia whytei* seeds over the germination period.

The lowest germination percentage (0%) was recorded at constant temperatures of 5 and 10°C. Across temperature regimes, germination was significantly higher under light than dark conditions (Figure 4.2a). The highest germination was observed at 20°C under both light and dark conditions. However, germination in the dark was slightly higher than in the light under alternating temperatures of 10N/20D.

Table 4.1: Parameters of the Weibull regression analysis of quantitative effect of temperature on germination over time.

Temperature (°C)	-2log likelihood	Intercept	Day	Scale	Weibull shape
15	-1.3	-3.36±1.16	0.24±0.04	0.20±0.05	5.01±1.28
20	39.7	-1.44±1.41	0.23±0.06	0.68±0.15	1.47±0.32
25	12.5	-2.39±0.56	0.22±0.03	0.30±0.06	3.33±0.64
10N/20D	1.7	-7.16±0.99	0.37±0.04	0.23±0.06	4.42±1.21
15N/25D	20.4	0.42±1.02	0.15±0.04	0.39±0.09	2.53±0.59

*Temperatures 5 and 10°C had no germination. Therefore, no parameter estimates exist.

4.3.2. Seed viability

After 30 days of germination assay, seeds that failed to germinate at constant temperatures of 5 and 10°C both under light and dark conditions showed >90% viability (Figure 4.2b). This was followed by seeds that were germinated at 15°C constant temperature under both light and dark conditions.

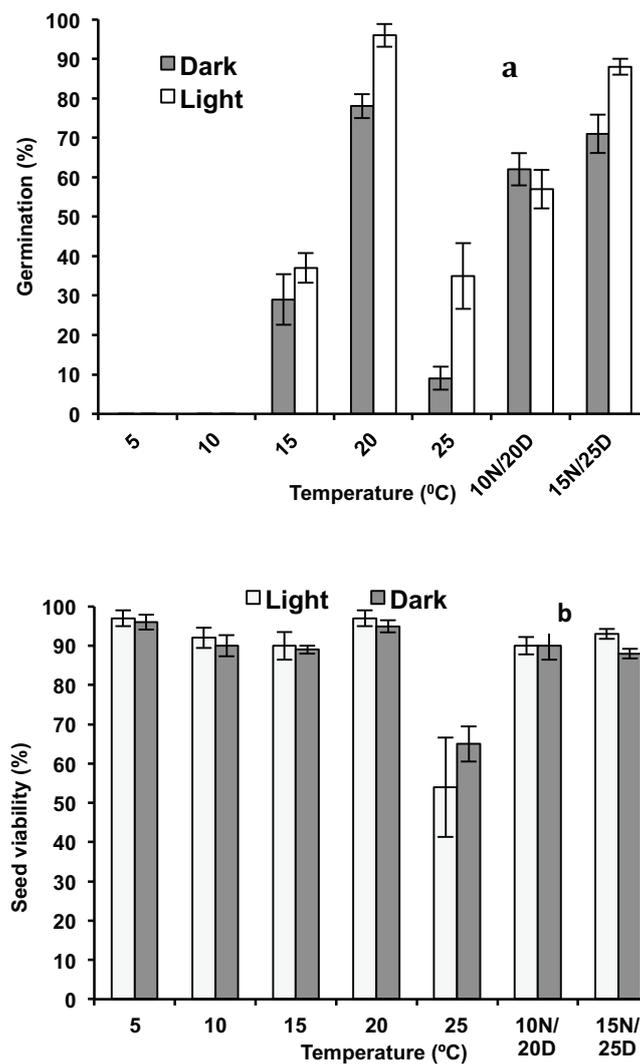


Figure 4.2: The effect of different temperature and light conditions on (a) mean germination and (b) viability of *Widdringtonia whytei* seeds after 30 days. Bars represent 95 % Confidence Intervals.

The lowest overall seed viability was recorded on seeds incubated at 25°C (68%). Many ungerminated seeds under dark conditions remained viable across temperature regimes (Figure 4.2b).

4.4 DISCUSSION

Widdringtonia whytei seed germination exhibited a parabolic relation with the optimal temperature being about 20°C. This agrees well with the mean annual temperature distributional range on Mulanje Mountain. Seeds germinated faster at temperatures between 15-25°C. This behaviour is similar to the germination requirements observed in other tropical montane tree species (Xiao et al. 2010). In the field, this temperature range occurs in early December during the rainy season. This suggests that maximum germination potential of *W. whytei* seeds would occur around December. *Widdringtonia whytei* seed germination was higher at constant than at alternating temperatures. Although seed germination was higher at constant temperatures, alternating temperatures have been found to be more favourable for germination than constant ones (Thompson and Grime 1983), since seeds are exposed to alternating and not constant temperatures in natural habitats (Baskin and Baskin 1998).

The results from viability tests revealed that *W. whytei* seeds which did not germinate at low temperatures of 5 or 10°C were mostly viable. This is in agreement with earlier reports that germination of most tropical species ceases at 10°C (Simon et al. 1976,

Khan and Ungar 1999). Low temperatures (<15°C) experienced on the forest floors of Mulanje Mountain during winter (June and July) (Chapman 1995), could probably restrict seed germination by inducing dormancy. Lack of germination when seeds are exposed to low temperatures also suggests that little or no winter germination would take place in the field, which would consequently reduce the risk of higher seedling mortality later during the dry season. Furthermore, seeds dispersed in winter would partly contribute to the soil seed bank which is dominated by seeds dispersed in the dry season (August to October) (Chanyenga et al. 2011). In contrast, high temperature of 25°C increased seed mortality, suggesting that temperatures higher than 25°C may prevent germination of *W. whytei* as a result of increased seed mortality.

Although seeds germinated in both light and darkness, more seeds germinated under light than dark conditions. Similar responses were reported for other species (Grime et al. 1981). The ability of seeds to germinate under light and dark conditions means that *W. whytei* seeds can germinate either buried or exposed on the soil surface disputing earlier suggestions that *W. whytei* seed requires light to germinate (Chapman 1995). These characteristics suggest that *W. whytei* cannot form a persistent soil seed bank (Pons 2000, Fenner and Thompson 2005).

4.5 CONCLUSIONS

This study was set to investigate the effect of temperature and light on the germination response of *W. whytei* seed. From this study, it can be concluded that:

4.5.1 Temperature is one of the critical factors for the germination of *W. whytei* seed. Germination exhibited a parabolic relationship with temperature, being optimal at about 20°C and it had less success in either direction of its tolerance limits at about 10°C and above 25°C.

4.5.2 Ungerminated seed at high temperature were mostly dead while those at lower temperature were mostly viable.

4.5.3 *W. whytei* seed does not require light to germinate. The present study points to some ecological strategies employed by seeds of *W. whytei* to deal with temperature changes under both buried and exposed conditions.

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CHAPTER 5

NATURAL REGENERATION PATTERNS OF *WIDDRINGTONIA* *WHYTEI* IN FOREST FRAGMENTS

5.1 INTRODUCTION

Widdringtonia whytei (Mulanje cedar) has a dilemma to survive as an endemic coniferous, fire-adapted pioneer tree species within natural fire-fragmented Afromontane forest patches in a very confined area. Its survival is presumably threatened by both natural and anthropogenic factors. Naturally, its own biology and ecology constrain its survival within the environment of the forest patches that have their own constraints within the fire-prone landscape, and by insect attacks. Anthropogenically, it is constrained by uncontrolled harvesting of mature trees for its valuable timber and the uncontrolled lighting of fires. The question is what factors of the biology and ecology of this species hold the key to its successful overcoming the threat to its survival in this confined and constrained landscape? If the species does produce viable seed, then maybe the key lies in successful establishment of natural regeneration in suitable habitats.

Natural regeneration refers to the renewal process of harvested or disturbed forest stands from natural seed fall or from stump or root sprouting (Chen et al. 1992) and involves several stages of the life cycle of plants such as seed production and seedling regeneration and survival (Enrico et al. 2004). Seedlings represent one of the most

vulnerable stages in the life cycle of most plant species, since they have not yet got enough size to face many negative ecological factors occurring in the environment (Silvertown and Charlesworth 2001). Many factors, both biotic and abiotic, exert the primary control of seedling regeneration (Kitajima and Fenner 2000). Availability of seeds and suitable microsites (Schupp 1995) and forest fragmentation (Santos and Telleria 1997, Scariot 2001) have all been considered important factors in the regeneration and survival of tree seedlings.

Studies on the effects of population fragmentation on regeneration dynamics of particular plant species reported contrasting results. Reduced seedling regeneration has been reported in several tropical shade-tolerant tree species in small (<1 ha) relative to large (>10 ha) forest fragments in Brazil (Benitez-Malvido 1998). Some studies have found increased regeneration of successional species (Laurance et al. 1998) while others have found no consistency or clear effects from fragmentation on seedling regeneration (Lehouk et al. 2009). For example, *Leptonychia usambarensis*, an endemic, animal-dispersed tree species failed to regenerate both in small as well as large fragments in Usamabara Mountains in Tanzania (Cordeiro et al. 2009). Thus, the response of seedling regeneration to fragmentation is species-specific.

Regeneration in fragmented landscapes may also be strongly influenced by the existing vegetation cover. Enhanced seedling regeneration and survival has been reported in areas under tree canopies due to shading effect that protected the seedlings against

high irradiance and temperatures (Joffre and Rambal 1988, Breashears et al. 1997). For instance, natural regeneration of *Abies guatemalensis*, an endangered conifer, increased in forested compared to open areas in the highlands of Guatemala as a result of shading effect that maintained humid soil conditions for seedling emergence (Kollmann et al. 2008). Furthermore, fragmentation causes an increase in the proportion of edge-to-interior habitat (Kapos et al. 1997). With humidity increasing, and light availability and temperature decreasing as a function of distance to edge (Kapos et al. 1997), forest fragmentation may ultimately affect seed germination and seedling survival (Bruna 2002). Lopez-Barrera et al. (2006) observed that seedling regeneration and survival of five oak species (*Quercus candicans*, *Quercus crassifolia*, *Quercus laurina*, *Quercus rugosa* and *Quercus segoviensis*) were generally greater along fragment edges than in the forested portion of the gradient in the Chiapas Highlands in Mexico. Although most studies on forest fragmentation have found changes in regeneration and survival of tree seedlings along gradients from edges to the forest interior (Chen et al. 1992; Benitez-Malvido 1998), tree regeneration and seedling survival along these complex micro-environmental gradients is relatively unexplored in many plant species (Meiners et al. 2000, Asbjornsen et al. 2004). Therefore, the study of tree seedling regeneration and survival in fragmented forests, considering the role of different matrices enclosing forest fragments, provides crucial information on forest conservation and management.

Widdringtonia whytei populations are naturally fragmented and have become severely degraded due to anthropogenic disturbances (Chapman 1995). The fragments vary in

vegetation cover and are bordered by a range of modified forest edge microhabitats (pers. obs). Thus, tree seedling regeneration and mortality may respond to fragment sizes, variations in vegetation cover and microhabitats along the forest-edge-exterior gradients (Meiners et al. 2000). Previously, it was found that seed rain distribution pattern of *W. whytei* varied among habitats (Chapter 3; Chanyenga et al. 2011a). Large fragments of *W. whytei* received higher seed rain density ($10.4 \text{ seeds m}^{-2} \text{ year}^{-1}$) compared to medium ($0.7 \text{ seeds m}^{-2} \text{ year}^{-1}$) and small fragments ($1.1 \text{ seeds m}^{-2} \text{ year}^{-1}$); inside forest fragments received higher seed rain densities ($12.04 \text{ seeds m}^{-2} \text{ year}^{-1}$) compared to fragment edges ($1.0 \text{ seeds m}^{-2} \text{ year}^{-1}$) while the open areas, mainly grassland, received almost none ($0.11 \text{ seeds m}^{-2} \text{ year}^{-1}$); seed dispersal limitation was very strong and seed rain occurred all year round with most of it falling at Sombani. Thus, seedling regeneration in *W. whytei* may respond to the seed rain pattern in the forest fragments (Harms et al. 2000). Despite the knowledge on seed rain pattern presented above, *W. whytei*'s seedling regeneration pattern and dynamics are poorly understood. Thus, understanding seedling regeneration dynamics in *W. whytei* may provide additional insights into the conservation and management strategies of the species population on Mulanje Mountain. Therefore, the main objective of this study was to examine whether *W. whytei* population size influences seedling regeneration, survival and establishment and when and where such regeneration and mortality occur. This study addresses the following questions concerning the regenerative characteristics of *W. whytei* in the forest patches: (1) Does *W. whytei* forest fragment size influence seedling regeneration and mortality? (2) When and where does most of the seedling regeneration and mortality occur?

5.2 MATERIALS AND METHODS

5.2.1 Study area

This study was conducted at three sites on Mulanje Mountain, namely Sombani (15°52'41"S; 35°40'89"E; altitude: 1790 – 1960 m asl), Chambe (15°62'40"S; 35°30'72"E; altitude: 1820 – 1900 m asl) and Lichenya (15°62'33"S; 35°30'73"E; altitude: 1660 – 2265 m asl) (Lawrence et al. 1994). These sites are characterized by *W. whytei* forest fragments of different sizes ranging from 0.8 - 177.9 ha with most of them confined to the ravines and hollows on the plateaux and below cliffs and the gorges where the terrain affords some protection from fires (Chapman 1995).

5.2.2 Study species

Widdringtonia whytei is an endemic species to Mulanje Mountain (Chapman 1962). It grows up to 40 m in height and c.1 m in stem diameter at breast height (dbh). A cone generally contains 4 to 8 seeds (Pauw and Linder 1997). *W. whytei* seed is 25 mm long and 12 mm wide on average with a wing (Pauw and Linder 1997) and is wind-dispersed (Chapman 1995). *W. whytei* disperses seed all year round (Chapter 3; Chanyenga et al. 2011a) and the seeds do not require any germination pre-treatment (Gondwe 2010) and light to germinate (Chanyenga et al. 2012).

5.2.3 Study design

5.2.3.1 Population selection

Seedling emergence and mortality were monitored in three forest fragments at the three sites. The fragments were selected following a stratified random sampling method. At each study site, all fragments were recorded and grouped into three size categories (small, medium and large). Small fragments consisted of groups of ≤ 10 *W. whytei* cone-bearing (reproductively mature) trees in an area either surrounded by other tree species or grass vegetation. Medium fragments consisted of groups of 11-20 cone-bearing individuals while large forest fragments were those comprising of groups of > 20 *W. whytei* cone-bearing trees. Only trees in the small and medium fragments were physically counted. Other factors were considered during forest fragment selection, such as accessibility; distance between forest fragments (1 km was considered the minimum distance between selected forest fragments) and comparable stand characteristics such as orientation, elevation and slope despite most fragments being confined to the ravines (slopes) and hollows (plateaux). In each size group, after exclusion of potentially unsuitable fragments, the suitable fragments were numbered using a piece of paper and placed in a bag. Only one paper bearing a number of a forest fragment was later picked from the bag after a thorough mixing to ensure randomization. Nine fragments were selected for the study, i.e. one fragment per size category (small, medium and large) at each site (Chambe, Lichenya and Sombani).

5.2.3.2 Plot layout

Rectangular plots measuring 3 x 15 m were established from a randomly selected edge (0 m) of the selected forest fragment, with the long axis parallel with the forest margin. The forest edge was defined as starting at the base of the first trunks of mature *W. whytei* trees encountered moving into and outside the forest patches. Plots were placed at every 10 m up to 30 m outside the forest fragment and inwards up to the centre (Figure 5.1). The distance to the fragment centre varied depending on the fragment size, but 30 m distance was decided as the furthest point plots could be placed outside the fragment (Figure 5.1).

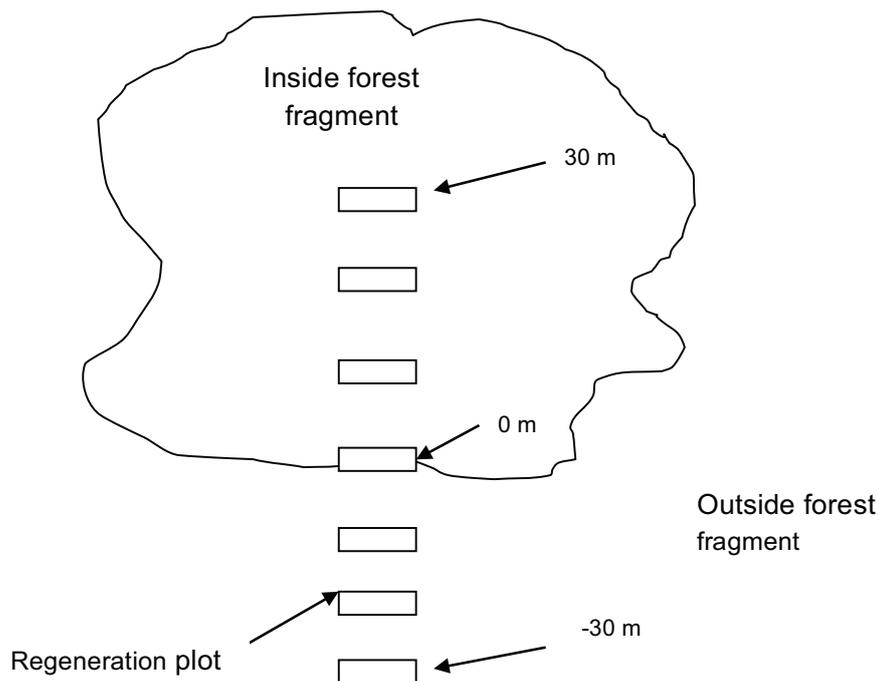


Figure 5.1: Sketch map showing layout of regeneration monitoring plots placed in relation to the forest fragment and edge

In the absence of any studies establishing the age of *W. whytei* seedlings, height was used to establish the regeneration class in this study. Seedlings were classified as

individuals ≤ 30 cm tall. In order to measure seedling density, in 2007, all *W. whytei* seedlings were counted and tagged in all study plots. Thereafter, these plots were visited monthly for 24 months up to 2009. During each visit, newly germinated seedlings were marked for easy identification during the subsequent assessments. Occurrence of adverse agents (such as fires, frost, drought, excess moisture and insects) was also recorded to establish the possible causes of seedling deaths.

5.2.4 Data analysis

The generalized linear model (GLM) of the SAS system was used to analyse the seedling density data. In this model, density counts (Y_i) varying over sampling units ($i = 1, 2, \dots, n$) were assumed to have a specified distribution (either Poisson or negative binomial) whose parameters depend on a vector of linear predictors ($X_i =$ site, year, season, fragment size and patch position) according to a log-linear function of the following form:

$\log \mu_i = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n$ where α and β_i are coefficients to be estimated.

Since the count data (numbers regenerating per plot) showed significant over-dispersion and lack of fit (Deviance $\gg 1$) to the standard Poisson and Quasi-Poisson model, a GLM assuming negative binomial distribution was used (Sileshi 2012).

Seedling mortality was expressed as percentage of number of seedlings that died against the mean seedling density recorded at each site, year, fragment size and

habitat position. A GLM assuming a binary logistic regression was used to analyze mortality (Sileshi 2012).

5.3 RESULTS

5.3.1 Seedling regeneration

Seedling density in *W. whytei* was generally very low (Table 5.1) and not significantly different among fragments (Table 5.2). Seedling density was higher in medium compared to large and small fragments (Table 5.1). Inside fragments presented much higher seedling densities compared to edges or adjacent non-forest habitats and differences were significant (Table 5.2). The highest seedling densities were recorded during the months of January and February (Figure 5.2a). The greater number of seedlings growing in the fragments was recorded at Sombani compared to Chambe and Lichenya and differences were significant (Tables 5.1 and 5.2; Figure 5.2a). Seedling density was greatest in 2008 compared with 2009 and the difference was significant (Table 5.2; Figure 5.2c).

Table 5.1: Mean seedling densities (stems/m²) in 2008 and 2009 in and around three fragment sizes at three sites on Mulanje Mountain, Malawi.

Position	Year	Season	Site								
			Chambe			Lichenya			Sombani		
			Fragment size (L=large; M=medium; S=small)								
			L	M	S	L	M	S	L	M	S
Inside	2008	Rainy	7.0±2.4	-	-	3.5±0.6	-	-	1.8±.6	2.3±0.8	0.8±0.2
		Dry	0.4±0.4	-	-	1.1±0.5	-	-	0.3±0.2	0.8±0.4	0.2±0.3
	2009	Rainy	-	-	-	0.8±.5	-	2.0±1.1	1.5±0.9	1.8±0.8	0.8±0.5
		Dry	-	-	-	-	-	0.1±0.1	-	0.1±0.1	0.1±0.1
Edge	2008	Rainy	-	0.3±0.3	-	-	-	-	-	8.0±2.4	-
		Dry	-	0.1±0.1	-	-	-	-	-	1.3±0.9	-
	2009	Rainy	-	-	-	-	-	2.0±1.2	-	0.3±0.3	-
		Dry	-	-	-	-	-	-	-	-	-
Outside	2008	Rainy	-	5.0±0.3	-	-	-	-	0.3±0.3	3.3±1.1	-
		Dry	-	0.1±0.1	-	-	-	-	0.1±0.1	-	-
	2009	Rainy	-	-	-	-	-	-	-	-	-
		Dry	-	-	-	-	-	-	-	-	-

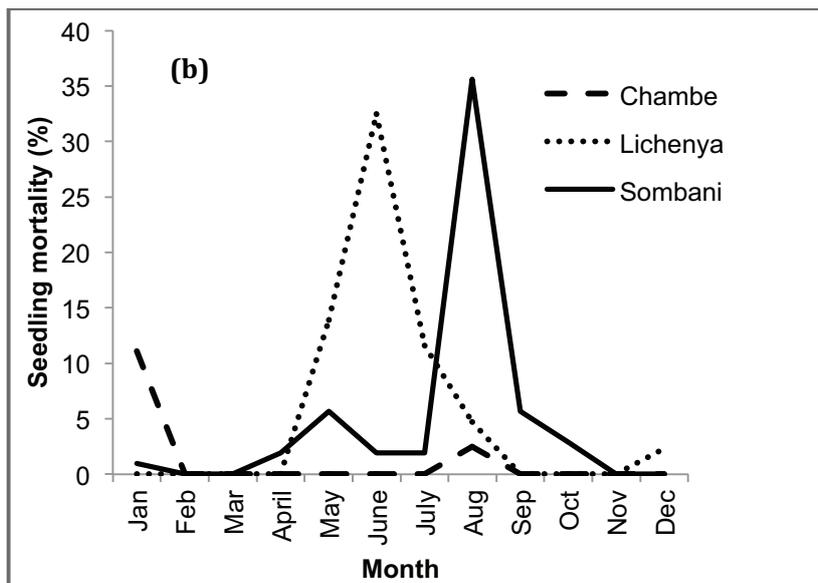
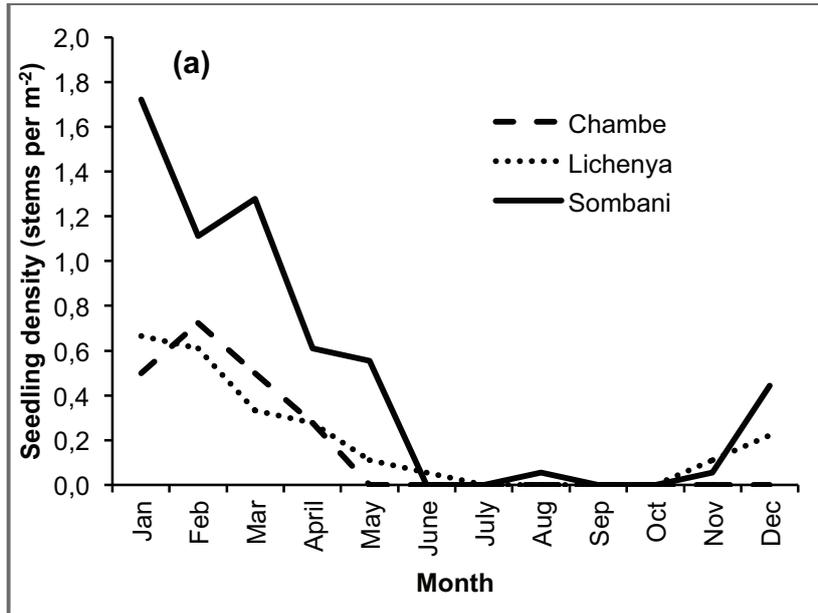
Table 5.2: Analysis of parameter estimates for seedling regeneration using a generalized linear model (GLM) with maximum likelihood.

Parameter		DF	Estimate	STD Error	Wald Chi-Sq	Pr > ChiSq
Intercept		1	2.9717	0.2326	163.2549	<.0001
Year	2008	1	-0.6076	0.1661	13.3784	0.0003
Season	Dry	1	1.0236	0.1662	37.9123	<.0001
Site	Chambe	11	0.9209	0.2763	11.1055	0.0009
	Lichenya	1	0.1869	0.2398	0.6077	0.4357
Fragment	Large	1	-0.2391	0.2156	1.2302	0.2674
	Medium	1	-0.3024	0.2144	1.9897	0.1584
Position	Edge	1	0.5255	0.2650	3.9318	0.0474
	Inside	1	-1.4284	0.2248	40.3711	<.0001

5.3.2 Seedling mortality

Seedling mortality followed the seedling density pattern. Season was not an important determinant in seedling mortality in *W. whytei* (Table 5.3). Seedling mortality percentage was higher in medium fragments compared to large and small fragments and the differences were significant (Tables 5.5 and 5.6).

The highest seedling mortality was recorded along the edges and habitats outside the fragments compared to inside fragments and the differences were significant (Tables 5.3 and 5.4). Seedling mortality varied among sites. The highest seedling mortality was recorded at Sombani compared to Chambe and Lichenya and the differences were significant (Tables 5.3 and 5.4; Figure 5.2b).



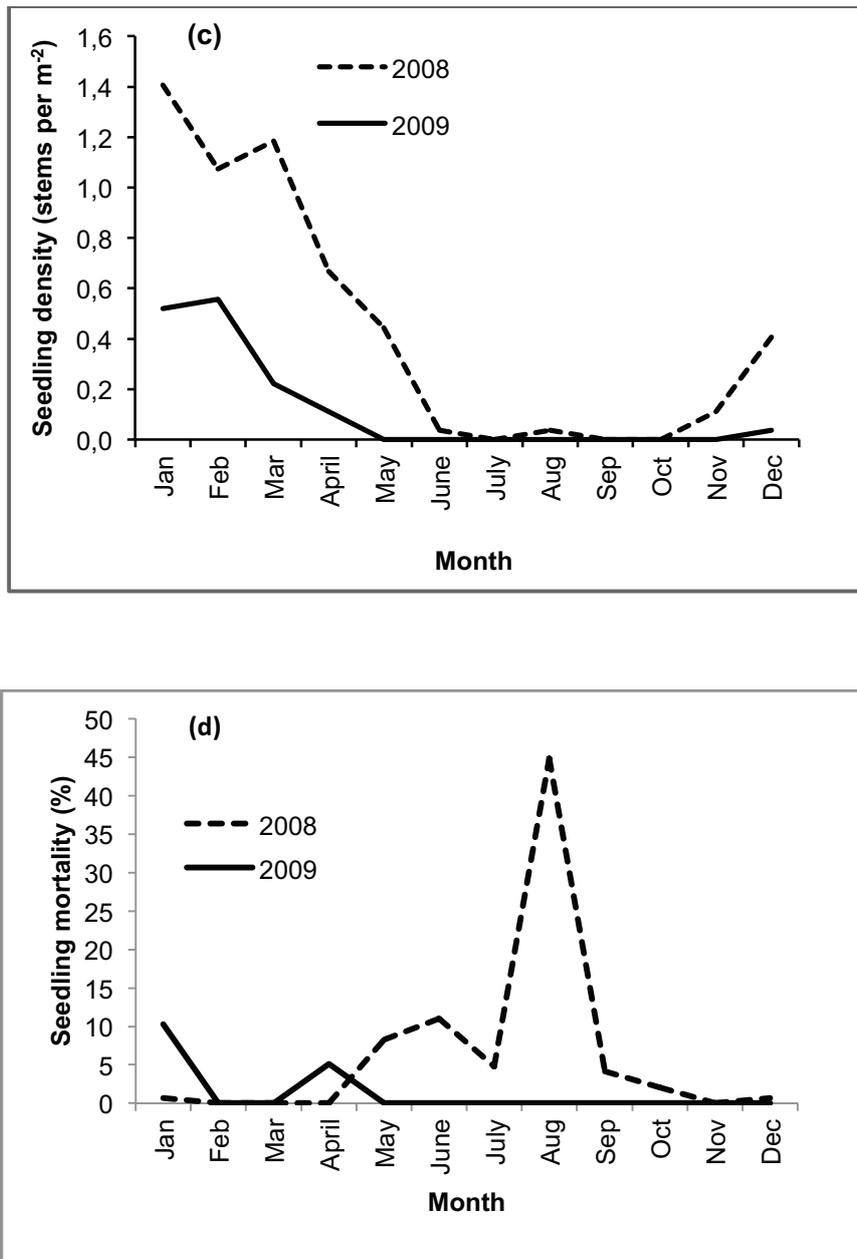


Figure 5.2: Variation in mean seedling density (a and c) and percentage seedling mortality (b and d) in *W. whytei* at three study sites (a and b) during 2008 and 2009 (c and d) on Mulanje Mountain, Malawi

June to September is dry period on Mulanje Mountain (Figure 5.3). At Sombani the highest seedling mortality percentage occurred during the month of August while at Lichenya most of the seedlings suffered deaths in June (Figure 5.2b).

Table 5.3: Seedling mortality (%) in 2008 and 2009 in and around three fragment sizes at three sites on Mulanje Mountain, Malawi.

Position	Year	Season	Site									
			Chambe			Lichenya			Sombani			
			Fragment size (L=large; M=medium; S=small)									
			L	M	S	L	M	S	L	M	S	
Inside	2008	Rainy	4.0-	-	-	5.5	-	-	100	6	-	
		Dry	-	-	-	100	-	-	100	22	-	
	2009	Rainy	-	-	-	100	-	-	100	-	-	
		Dry	-	-	-	-	-	-	-	-	-	15.7
Edge	2008	Rainy	-	-	-	-	-	-	-	100	-	
		Dry	-	-	-	-	-	-	-	100	-	
	2009	Rainy	-	-	-	-	-	100	-	100-	-	
		Dry	-	-	-	-	-	-	-	-	-	
Outside	2008	Rainy	-	100	-	-	-	-	-	100-	-	
		Dry	-	100	-	-	-	-	75	-	-	
	2009	Rainy	-	-	-	-	-	-	-	-	-	
		Dry	-	-	-	-	-	-	-	-	-	

Table 5.4: Analysis of parameter estimates for seedling mortality using a generalized linear model (GLM) with maximum likelihood.

Parameter		DF	Estimate	STD Error	Wald Chi-Sq	Pr > ChiSq
Intercept		1	5.6832	0.6860	68.6356	<.0001
Year	2008	1	1.2318	0.3862	10.1718	0.0014
Season	Dry	1	-0.6174	0.3353	3.3908	0.0656
Site	Chambe	11	1.0570	0.5222	4.0980	0.0429
	Lichenya	1	0.0587	0.4140	0.0201	0.8873
Fragment	Large	1	-1.0312	0.4314	5.7147	0.0168
	Medium	1	-0.6281	0.4430	2.0107	0.1562
Position	Edge	1	1.4918	0.7043	4.4859	0.0342
	Inside	1	-1.5299	0.4320	12.5416	0.0004

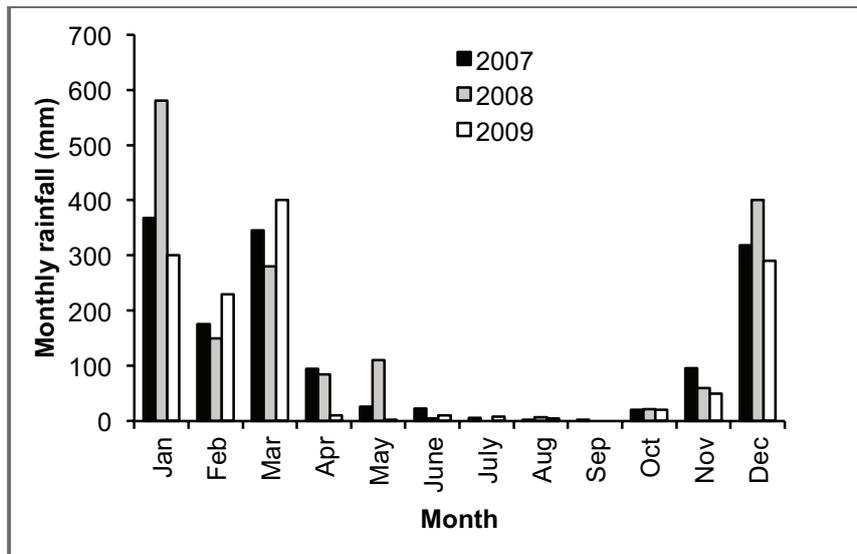


Figure 5.3: Annual rainfall distribution from 2007 to 2009 on Mulanje Mountain, Malawi

Seedling mortality percentage was greater in 2008 than 2009 and the variation was significant (Table 5.4; Figure 5.2c). Out of 184 seedlings recorded and observed during the two-year study period, 142 seedlings died from various causes. Examining the causes of mortality, all 53 recently germinated seedlings (1.0-4.9 cm height size class) died within one month after germination (Figure 5.4). Thick litter layer and excessive shading inside the forest fragments were the main causes of mortality. Litter thickness prohibited seedling rooting into the mineral soil while excessive shading did not allow sufficient light for the newly germinated seedling to get established. Mortality of 84 established seedlings (15.0-19.0 cm height size class) that were mostly found along the forest edges and outside fragments was caused by fire and frost (Figure 5.4). Five seedlings (10.0-14.9 cm height size class) died as a result of dry soils (desiccation) both inside and outside forest fragment (Figure 5.4).

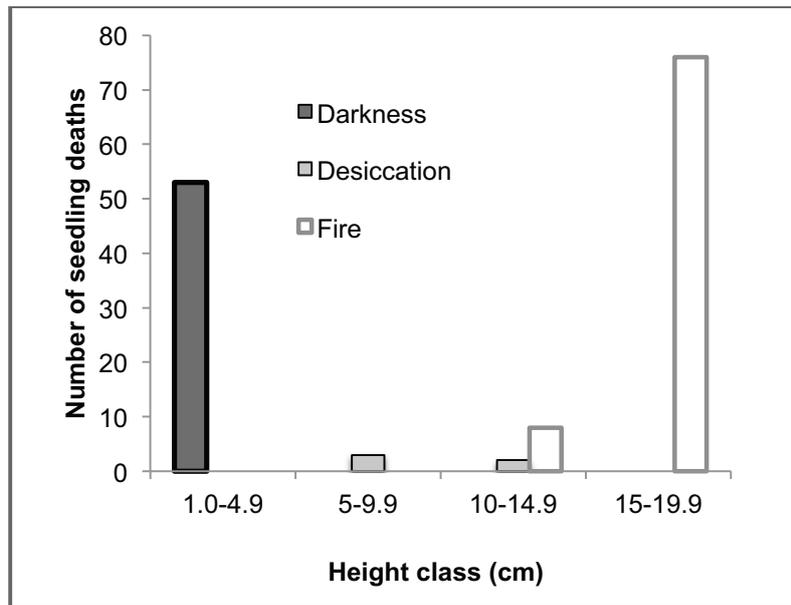


Figure 5.4: Causes of *W. whytei* seedling mortality observed during the study period on Mulanje Mountain.

5.4 DISCUSSION

5.4.1 Seedling regeneration

W. whytei seedling density was extremely low and sparse when averaged over the entire surface surveyed for both 2008 and 2009 although seed rain in some fragments seemed sufficient to allow for some regeneration. This may be explained in part by the generally low seed viability per cone and limited seed dispersal reported in this species. Seed viability was found to be 23% per cone in *W. whytei* (Chanyenga et al. 2011b). Furthermore, *W. whytei* is serotinous and depends on fires for its cones to open in order to disperse large amount of seed (Chanyenga et al. 2011a). However, no fires occurred in the forest patches during the entire study period resulting in strong seed limitation

(Chanyenga et al. 2011a). Thus, low seed viability and strong seed limitation reported in earlier studies may have contributed to the observed extremely low and overdispersed seedling density in *W. whytei* during the study period in the forest fragments on Mulanje Mountain.

While seed rain density was influenced by *W. whytei* population size, seedling density was not. This study showed inconsistencies in seedling densities among fragment sizes in this species. Literature from other studies suggests that conditions in large fragments are in most cases superior for seed germination and establishment in many plant species due to the absence of edge effects (Murcia 1995, Scariot 2001). In this study however, seedling density was higher in medium fragments compared to large and small fragments. A number of possible explanations could be advanced for the observed high seedling density in the medium fragments. Prior to the commencement of the experiment in 2008, fires occurred in parts of the medium fragments at Sombani and Chambe, where the mineral soil was exposed (pers. obs). As a result, a considerable number of seedlings regenerated at the edges and a few outside these patches (pers. obs). These results confirm earlier observations reported by Chapman (1995) and Topham (1936) who described *W. whytei* as a pioneer tree species which is unable to regenerate under closed canopy and that the seedlings are sporadically found at the edges of the forest where litter layer is minimal once fire has destroyed the vegetation. The importance of bare mineral soil for successful germination and early survival of tree seedlings has been demonstrated in numerous studies (Stewart and Rose 1990, Cornett et al. 1997). Furthermore, medium fragments may have had slightly larger

postfire seed reserve on the forest floor as a result of increased dispersal from serotinous cones and reduced seed predation. In charred spots, the black coloration of *W. whytei* seed makes detection by birds less likely (Pauw and Linder 1997).

Pattern of seedling regeneration along the forest-edge-open area gradients followed a similar trend to that of seed rain, although seedling density was much lower than seed rain density (Chapter 3). Inside *W. whytei* fragments, which received the highest seed rain density, recorded the highest seedling density while the edge and open areas with the lowest seed rain density had the lowest seedling density. Herrera and Garcia (2010) reported similar patterns of seedling regeneration in *Ilex aquifolium* in forest fragments in Spain where high seedling densities decreased from inside forests along the forest-edge-exterior gradient. Similarly, most of the seedlings regenerated at Sombani forest fragments, which produced the highest viable seed per cone and seed rain densities. Thus, it seems the availability of seeds that could germinate, rather than the environmental differences among locations by themselves, is one of the key factors driving *W. whytei* seedling density in forest fragments.

Vegetation cover had an influence on seedling establishment. High shrub cover and thick litter layer inside the forest patches prohibited seedling establishment (pers. obs.). Out of 184 *W. whytei* seedlings observed in this study, 89 seedlings with height of greater than 5 cm were found in plots along the edges. Seedling establishment success along the edges was probably due to increased irradiance from the adjacent non-forest

patches and presence of mineral soils. Such environmental conditions along the forest patch edges on the mountain are mostly created by fires and fire-breaks circuiting the patches (pers. obs.). These observations and earlier reports confirm that *W. whytei* is a light-demanding pioneer tree species which relies on catastrophic disturbances such as fires for its early growth and successful establishment (Chapman 1995; Pauw and Linder 1997). Considering that the highest seed rain (Chanyenga et al. 2011a) and seedling (in this study) densities occurred inside forest fragments, *W. whytei* seedling establishment inside forest patches could be enhanced by changing forest light conditions through canopy gap formation (Dai 1996). In such conditions, Geldenhuys (2009) recommends the cutting of trees in groups with a gap diameter of about one to two times the height of the canopy of the average trees in the target stand. Manipulated canopy gaps are reported to enhance early seedling growth and establishment in many forest types (Dai 1996, Bebber et al. 2000, Geldenhuys 2009). Apart from changing forest light conditions, exposure of the mineral soil inside forest patches could also enhance seedling density (Eastham and Jull 1999). This could be achieved by creating fire spots on the forest floor after cutting of trees in groups (sensu Geldenhuys 2009).

Rainfall had a marked influence on seedling density since higher seedling density was observed during the rainy season compared to the dry season. Similarly, wetter years promoted seedling regeneration as was the case in 2008 when a higher rainfall was recorded compared to 2009. A study by Lehouck et al. (2009) also showed that successful regeneration in *Xymalos monospora*, an Afromontane forest tree in a threatened fragmented ecosystem in Kenya, was due to years with abundant rainfall.

5.4.2 Seedling mortality

In tropical trees, natural regeneration is often difficult and high seedling regeneration is followed by high mortality in most cases (Harper 1977). In this study, high seedling mortality followed seedling emergence. 142 out of 184 recorded seedlings died due to various causes. The causes of mortality varied according to microhabitat (pers. obs.). Although fire promoted seedling regeneration along the forest edges in medium fragments, it was the main cause of seedling deaths a year later. Fires during the hot dry months (August/September) caused the death of 76 out of 142 seedlings outside the fragments and along the edges. Although forest edges and areas outside forest patches seemed suitable for early establishment of *W. whytei* seedlings, they experience regular intensive fires (fire zones) implying that naturally regenerated seedlings would not be allowed to get established nor would planting of seedlings in such areas be successful in the long-term (Geldenhuys 1994). The findings suggest that frequent fires are the major cause for the lack of *W. whytei* seedling regeneration success on Mulanje Mountain. Restriction of *W. whytei* seedling regeneration by fires could be one of the major causes of the lack of expansion of *W. whytei* forest patches on the landscape of Mulanje Mountain, hence their current location pattern (Geldenhuys 1994).

Mulanje Mountain experiences both cold (May to June) and warm (July to September) temperatures (Chapman 1995). Frost caused three (3) seedling deaths during the cold months (May/June) of the year outside the forest patches. Frost occurrences are reported to cause tree seedling deaths especially on exposed sites in many montane forests (Jobbagy and Jackson 2000, Enrico et al. 2004). Dry season soil moisture stress accounted for five (5) seedling deaths during the warm months (July to September) of the year both inside and outside forest patches. High temperatures, low relative humidity and high irradiance probably caused the seedling deaths as result of soil desiccation (Crawford 1989). The importance of soil moisture in influencing tree seedling survival in forests has been well reported (Schulte and Marshall 1983). Thus, the interaction between weather and microhabitat characteristics seems to be one of the most important environmental components restricting *W. whytei* seedling establishment both inside (the fire shadow areas) and outside (fire zones) forest patches on Mulanje Mountain.

All 53 newly germinated seedlings (<5 cm) recorded inside forest fragments died. The cause of seedling mortality inside forest fragments, while difficult to elucidate in most cases, was apparently due to the combination of several factors. In this study, the rooting substrate on which the germinated *W. whytei* seedlings were found inside fragments was a thick surface of accumulated litter layers on the forest floor (pers. obs.). Forest floor materials can be a barrier for establishment, dry out quite rapidly and reach very high temperatures under direct solar radiation (Gray and Spies 1997). Under such conditions seedlings of most tree species become highly susceptible to

desiccation, and must establish sufficient root depth in time to acquire soil moisture as surface layers dry out (Gray et al. 2005). During the study period, low temperatures and wet conditions persisted inside forest fragments on the mountain especially during the rainy season (pers. obs.) which may contribute to slow rates of litter decay hence its build-up. Thus, the greater abundance of litter on the forest floor in closed-canopy areas may have also acted as a barrier to heating and evaporation in upper soil layers which could also have led to increased pathological problems. Interaction between factors such as excessive moisture and fungal attacks has been documented previously for causing high seedling mortality in forest-interiors (Robin et al. 2001). Furthermore, inside forest fragment areas are extraordinarily shaded habitats (pers. obs.). The 100% seedling mortality recorded in these microhabitats could be due to lack of light. A number of studies have attributed seedling regeneration failure to shading (Davies 2001), particularly in the case of shade-intolerant species such as *W. whytei* (Chapman 1995). While causes of seedling mortality at the edges and adjacent non-forest areas have largely been identified, the causes of seedling mortality inside forest fragments are little known and it is an area that requires further investigation.

5.5 CONCLUSIONS

The purpose of the current study was to determine whether forest patch size, microhabitat and season influence seedling density and survival in *W. whytei*. The following were major findings:

5.5.1 Seedling density was generally very low and sparse. Fragment size did not influence seedling density. Seedling density was highest in medium compared to large and small *W. whytei* forest patches and it decreased from inside forest patches along the forest-edge-exterior gradient.

5.5.2 Emergence of new seedlings was highest during the rainy season compared to the dry season.

5.5.3 Although seedling density was high inside forest fragments compared to edge and adjacent non-forest habitats, seedling establishment was higher along the edges than inside and open areas. Management that promotes partial canopy opening through group tree felling and reduction of thick litter layer through fire spotting to expose the mineral soil may increase *W. whytei* seedling establishment and survival inside forest fragments.

5.5.4 Seedling regeneration during the rainy season was immediately followed by high seedling mortality during the dry season both inside and outside forest fragments. Although *W. whytei* depends on fire to clear the vegetation for its germination and early seedling establishment along the edges and open areas outside forest fragments, these areas experience frequent fires and it was the main cause of seedling mortality during

the dry and hot months of the year. Management that ensures long fire intervals in these areas would promote seedling establishment. During the cold months of the year, seedling mortality was caused by frost especially outside forest patches while reasons for mortality inside fragments were unclear. Investigations are therefore required in order to establish the causes of *W. whytei* seedling mortality inside fragments.

5.5.5 Returning to the question posed at the beginning of this study, it is possible to state that *W. whytei* survival in the forest patches is partly constrained by low cone viable seed output (Chapter 2), insufficient seedfall and limited seed dispersal (Chapter 3) and low and sparse seedling regeneration observed in this study.

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CHAPTER 6

EFFECT OF POPULATION FRAGMENTATION OF *WIDDRINGTONIA WHYTEI* ON ITS REGENERATION STATUS ON MULANJE MOUNTAIN: A SYNTHESIS

6.1 INTRODUCTION

Mulanje Mountain is an example of an inselberg formed from igneous or sedimentary rocks capped by more resistant strata (Crow 1981). Inselbergs are one of the most striking and persistent land types in Africa (Goudie 1996) and are defined as an isolated hill, knob, koppie or mountain which stands alone and rises abruptly, island-like, from the surrounding terrain (Sarhou and Villiers 1998). In the south-eastern corner of Malawi, Mulanje Mountain rises spectacularly above the Phalombe Plains (Chapman et al. 1991). The greatest scientific interest in inselbergs derives from the fact that they belong to the nowadays extremely rare and unspoiled ecosystems (Seine et al. 1998). The unique, high altitude conditions found above 2000 m lead to high levels of endemism in organisms such as bryophytes, plants and animals (Hilliard and Burtt 1987, Van Wyk and Smith 2001). High levels of endemism mean that a large proportion of the available gene pool is unique to that site. Inselbergs and mountains therefore have an important role to play in the maintenance of genetic diversity (Taylor 1996, Mucina and Rutherford 2006).

The dominant forest vegetation found on inselbergs in the eastern parts of southern Africa is afromontane forest, generally composed of fragmented forest patches of

different sizes as found in South Africa (Von Maltitz et al. 2003, Mucina and Rutherford 2006, Brand et al. 2009). Today, these forest patches have suffered reduction as a result of over-exploitation, fire and grazing (Mucina and Rutherford 2006, Brand et al. 2009). Fire in particular is important in shaping the modern appearance and distribution of forest patches on many mountainous areas (Geldenhuys 1994, Mucina and Rutherford 2006). In order to develop effective strategies to protect such inselberg and mountain ecosystems, it is required to learn more about the composition of inselberg communities, their changes in response to variations in the constellation of biotic and abiotic factors at their specific environment and their sensitivity towards anthropogenic influences (Burke 2002). Therefore, studies in inselberg ecosystems are necessary to answer some of the basic ecological questions for instance regarding the minimum size of species populations required to guarantee their survival at a given habitat and the consequences of population fragmentation for their stability (Barthlott et al. 1993). Population fragmentation threatens the survival of many plant species (Lienert 2004; Lawes et al. 2007) because populations become smaller and isolated, factors believed to increase extinction risks of the species originally inhabiting the fragments (Saunders et al. 1991, Weins et al. 1987, Broadhurst and Young 2006).

The vegetation on Mulanje Mountain is dominated by grasses and afromontane forest. The Afromontane forest is largely composed of a number of fragmented forest patches which have been dominated locally by the tropical conifer *W. whytei*. For many people *W. whytei* typifies the mountain (Chapman 1995). *W. whytei* represents one of nine Southern African conifer species (Mustart 2008). It is a pioneer species endemic to

Mulanje Mountain in Malawi (Bayliss et al. 2007). The tree is highly valued for its timber and almost every *W. whytei* forest patch has been visited (Chapman 1995) since its exploitation started over 100 years ago (Pauw and Linder 1997). However, current *W. whytei* fragments are declining in extent (Sakai 1989) and show very poor natural regeneration (Makungwa 2004). *W. whytei* is now categorized as an endangered species that has been given global importance due to it being listed by the IUCN in a list of 43 conifer species worthy special conservation attention (Bayliss et al. 2007). The current population structure of *W. whytei* in these forest patches is a result of interactions between the biophysical factors such as fires, climate and soils, pests and diseases, invasive species and timber harvesting and reproductive biology characteristics of the species such as viable seed production, seed dispersal, seed germination requirements and natural regeneration (the conceptual framework). To date, however, both the biophysical factors and reproductive biology characteristics have not been understood in the context of fragment or forest patch size of the species. Understanding the role of biophysical factors and reproductive biology behaviour of the species in relation to fragmented forest patches is central for the sustainable management of *W. whytei* on the mountain.

This study was initiated to assess the effects of *W. whytei* fragment size and isolation on viable seed output, seed rain and natural regeneration pattern in the forest patches on the mountain in order to formulate *in-* and *ex-situ* conservation strategies for the species (general objective of the study, see subsection 1.4, Chapter 1). Data was collected using field and laboratory experiments and review of secondary information.

Four specific studies, each with a specific objective, were conducted to examine different parts of this overall study (see Chapters 2 to 5): (i) effects of *W. whytei* population size, tree diameter and crown position in the forest canopy on viable seed output; (ii) phenological pattern of seed rain in *W. whytei* and how it is affected by population size; (iii) seed germination requirements of *W. whytei* and (iv) influence of population size on seedling regeneration pattern and how it relates to seed rain pattern. Each Chapter presents detailed results and discussion for each specific study. This chapter (Chapter 6) is a synthesis of information and recommendations from different studies (see Chapters 2 to 5) with specific reference to how *W. whytei* population size influences viable seed output, seed rain and natural regeneration pattern in order to formulate sustainable management and conservation strategies for the species.

6.2 DISCUSSION AROUND SPECIFIC STUDY OBJECTIVES

6.2.1 Influence of fragment size, stem diameter, crown position on viable seed output

The importance of viable seed output in plant recruitment and establishment cannot be overemphasized (Faast et al. 2011). Two questions were the focus for this part of study:

- (1) Does population size of *W. whytei* within a forest patch influence viable seed output?
- (2) Does tree size and crown position in the forest canopy influence viable seed output?

This study focused on viable seed output per cone. On average, *W. whytei* cones contained 6 seeds supporting the findings of Pauw and Linder (1997). However, the overall proportion of viable seeds per cone was very low (0.23) indicating that over 75%

of the seeds produced in a cone were empty. Thus, a relatively high number of cones in *W. whytei* produce zero or one viable seed. This is enough evidence as to why *W. whytei* seed collection expeditions turn out to be very expensive on the mountain. The observed low viable seed output per cone could be linked to low pollen quantity and quality production in this species and this supports results from other studies that pollen limitation affects viable seed output in fragmented tree populations (Perry and Knowles 1990, Smith et al. 1998, Ashman et al. 2004, Nagamitsu et al. 2006, Pauw 2007).

Fragment size (Aizen and Feinsinger 1994; Wang et al. 2010), tree diameter (Ares and Brauer 2004), and crown position in the canopy (Tomita et al. 2008) affect viable seed output of many plant species. However, this study has shown that the proportion of viable seed per cone was not affected by fragment size, tree diameter and crown position in the forest canopy. Viable seed output per cone in isolated trees was not different from small, medium and large population sizes of *W. whytei*, i.e. small or isolated trees are not at a disadvantage from viable seed output per cone. After the paper on viable seed output was published (Chapter 2, Chanyenga et al. 2011), it was realized that information on tree cone production was very important to be established. Thirty-six (36) trees (isolated, emergent, suppressed or understory, small and large) were therefore assessed for mature cone production at all the three sites (Chanyenga 2012 unpublished data). Isolated trees produced many more mature cones per tree per year (641.2 ± 21.4 cones) compared to trees from large (570.9 ± 24.3 cones per tree) medium (504.9 ± 36.3 cones per tree) and small (506 ± 24.3 cones per tree) fragments. Thus, isolated trees are expected to produce many more viable seeds per tree

compared to trees growing in large, medium and small forest fragments. Currently, however, isolated trees are very few on the landscape compared to the number of trees in the other population sizes. Thus, from demographic perspective, larger population sizes appear to contribute much more viable seeds compared to isolated trees. Although large population sizes appear to contribute much more viable seeds, there is relatively high mortality rate (32.6%) of *W. whytei* standing trees in forest fragments (Sakai 1989, Makungwa 2004, Makungwa and Chanyenga 2007). Furthermore, illegal logging, which targets live trees, is also significant in the forest fragments (Bayliss et al. 2007). Thus, high mortality rate of standing trees and illegal logging of *W. whytei* live trees reduce the number of seed producing trees that in turn seriously limit viable seed output. Reduction in the number of seed producing trees coupled with low viable seed output per cone may limit *W. whytei* population growth on the mountain.

The relationships between tree diameter, crown position in the forest canopy and viable seed output shows that viable seed output in a cone is not influenced by tree size or crown position in the forest canopy. Viable seed output per cone in small trees was not different from large trees. Although there was no significant difference between small and large trees in viable seed output per cone, large trees (41 to 50 cm dbh) produce many more mature cones (873.7 ± 84.4) per tree per year compared to small trees (<20 cm dbh) (114.3 ± 31.5 cones per tree per year, Chanyenga 2012, unpublished data) indicating that large trees are expected to produce many more viable seeds compared to small trees. This confirms results from other studies that seed production in most trees increases with increasing tree diameter (Zuidema and Boot 2002, Snook et al.

2005, Kainer et al. 2007). On Mulanje Mountain, most of the *W. whytei* populations are in the >55 cm dbh class (Table 1.1) and tree harvesting mostly targets these old and large trees (Figure 1.4 right) for increased returns in terms of timber production. Thus, harvesting of older and larger trees has a greater effect on the overall viable seed output from the population since they are the main reproductive individuals in the *W. whytei* populations in the forest fragments. Crown position or ability to access light resources is clearly not an important determinant of viable seed output per cone in *W. whytei*. While Zuidema (2003) states that trees below canopy level do not produce any viable seed, in this study however, emergent, suppressed and intermediate crown positions in the forest canopy produced similar amount of viable seeds per cone. Contrary to these findings, emergent and isolated trees produce many more mature cones in a year (961.4 ± 70 and 641.2 ± 21.4 cones per tree respectively) compared to suppressed trees (40.5 ± 1.0 cones per tree; Chanyenga 2012, unpublished data). Thus, trees with emergent canopies are expected to produce many more viable seeds than trees with suppressed canopies. This confirms results of other studies that seed production is greater in canopy than understory trees (Karlsson 2000; Debain et al. 2003).

Despite limited influence by population size, tree diameter and crown position in the forest canopy on viable seed output per cone, seed viability output per cone varied among sites on the mountain. Cones from warmer, drier and sheltered sites produced more viable seeds than cones from colder, wetter and exposed sites. Warm temperatures are known to promote pollination by stimulating the growth and maturation

of catkins while drier and sheltered (low-humidity) conditions promote successful fertilization in wind-pollinated species (Whitehead 1983, Koenig et al. 2003). In this study, it appears warmer temperatures, and drier and sheltered conditions promote effective pollination in *W. whytei*. High annual variations in viable seed output per cone in some fragments of the same size within sites were also observed in this study suggesting that reproductive isolation occurs among different *W. whytei* populations on Mulanje Mountain (Herrerias-Diego et al. 2006). This variability in viable seed output per cone among populations between years could probably reflect annual changes of environmental variables that affect this process, for example, the amount of precipitation (Herrera et al. 1998) and prevalence of adverse weather conditions such as high winds (Stephenson 1981). This behaviour could also be attributed to the mast seeding strategy, which is common in most tree species (Herrerias-Diego et al. 2006).

6.2.2 Influence of fragment size on seed rain pattern

Three questions were addressed in this part of the study: (1) Does *W. whytei* population size influence seed rain density? (2) Does seed rain occur in a specific season? (3) Does seed limitation differ from within forest patches to the adjacent non-forest patch habitats? Mulanje Mountain is one of the examples of fragmented and disturbed tropical montane landscapes. In such landscapes, seed availability is a major limiting factor in vegetation recovery (Duncan and Chapman 1999, Holl 1999). The pattern of the seed rain in these landscapes is thus an indicator of regenerative potential (Au et al. 2006) and this is why seed rain and seed limitation studies are important to be carried out in *W. whytei* because they help to establish whether the species recruitment is dispersal

limited or not. The quantity of seed rain density received in any given area is mostly influenced by the population size, season, disturbance events such as devastating fires and the spatial pattern of dispersal by the dispersal agents (Jules and Rathcke 1999, Cunningham 2000, Levin et al. 2003, Zhang et al. 2008). The study shows that seed rain density in *W. whytei* is influenced by fragment size (Chapter 3). Large population sizes contributed higher and consistent seed rain densities compared to medium and small population sizes. This is because of the presence of many seed bearing trees. This confirms results conducted on other conifer species in disturbed and fragmented tropical montane landscapes (Santos et al. 1999, Armesto et al. 2007). Certainly, these results point to the importance of larger population sizes in promoting consistent seed rain in this species.

There is need for plant species to colonize new areas if they have to maintain themselves in fragmented ecosystems (Hewitt and Kellman 2004). In this study, a high number of traps did not receive any seed during the observation period. Seed rain was concentrated within forest fragments (Chapter 3). This means that *W. whytei* seeds are not well-dispersed. Although *W. whytei* seed rain concentrated within forest fragments, they are less likely to germinate and produce viable seedlings in this shady habitat (Chapman 1995). Thus, the concentration of seed rain within forest fragments can seriously limit natural regeneration in *W. whytei*. Seed rain density occurred all year round in forest fragments (Chapter 3). Although seed rain occurred throughout the year, higher seed rain densities were recorded during the warmer and drier months of the year. Seed rain densities were even greater on sites that normally experience warmer

temperatures (Chapter 3). The results show that dry and warm seasons favour *W. whytei* seed dispersal. This is because *W. whytei* cones are resinous and their opening requires high temperature in order to release the seed and this characteristic is called serotiny (Helium and Pelchat 1979, Richardson et al. 1987, Lamont et al. 1991). Cone serotiny is a reproductive adaptation found world-wide among woody plants that live in habitats with frequent fires (Lamont et al. 1991). Thus, failure of cones to open due to non-melting of resin result in part of the seed being retained on the mother plant long after seed maturity (Helium and Pelchat 1979). It is probably this type of characteristic that causes seed rain in *W. whytei* to occur all year-round on the mountain. Thus, the observed cone characteristics confirm that the species maintains an aerial seed bank (Lamont et al. 1991). Although the results of this study provide an insight on the seed rain pattern in *W. whytei*, the study was done for 2 years only and this period is too short for this kind of study. Findings from short-term seed rain and limitation studies are sometimes misleading, especially in pioneer plant species that maintain active long-lasting aerial-stored seed banks (Dalling et al. 2002). Thus, the behaviour of *W. whytei* aerial seed bank needs to be investigated for a relatively longer period. Factors that drive seed rain dynamics in plant populations are complex and require long-term observation periods to come up with general seed rain (Cottrell 2004) and limitation patterns (Clark et al. 1998). However, it seems to indicate that the species has a weak serotiny; cones seldom remain closed for more than two years (Pauw and Linder 1997). In those species with strong serotiny seed can be retained for many years (e.g. 8 years in *Widdringtonia cupressoides*; Midgley et al. 1995).

Seed rain density was generally very low in the forest fragments (Chapter 3). This is because seed rain studies were conducted under normal conditions with no fire disturbance events. Serotinous cones such as those of *W. whytei* require temperatures of 45-50°C to melt the resin that binds the cone scales (based on related *W. cedarbergensis*) and such temperatures are associated with moderate to severe fires (Manders 1987). In other species with serotinous cones, seed rain was greater (289-386 total seeds m⁻² year⁻¹) during the first year after fire followed by reduced seed rain (41-77 total seeds m⁻² year⁻¹) in the second and third years after fire (Zasada et al. 1979, Johnstone et al. 2009). This confirms that fire promotes seed rain density in species with serotinous cones. Effective seed rain and release of large amounts of seed may therefore happen after killing of *W. whytei* trees during extreme fire events, an aspect of the ecology of this species that did not happen during this study.

6.2.3 Seed germination studies

One question was addressed in this part of the study: to what extent does temperature and light influence seed germination in this pioneer tree species of *W. whytei*? Temperature and light are important ecological factors that regulate seed germination in many plant species (Baskin and Baskin 1998, Jarvis and Moore 2008). Thus understanding the species' seed germination ecology in relation to environmental conditions such as temperature and light increase the chances for in-situ conservation (Meyer et al. 1990). This is the first study to investigate the effect of temperature and light on seed germination of *W. whytei*. Most *W. whytei* seeds germinated between 15

and 25°C, with the optimal temperature being about 20°C (Chapter 4). This is the temperature range normally experienced during the rainy season on the mountain (Figure 1.10). At these temperatures, seeds took 16 days to start germinating. This implies that successful seed germination can only be achieved if seeds are subjected to such temperatures for 16 successive days, a condition that is rarely attained on Mulanje Mountain. In addition, these temperatures are restricted to the months of December and January implying that many *W. whytei* seeds have to germinate in December and January to escape delayed germination that might be caused by temperature fluctuations in the subsequent months. December and January is also the time when the mountain experiences high rainfall (Figure 1.10). The timing of rainfall is important in controlling the timing of germination after a high number of seeds has been dispersed following fire events that releases abundant seeds from serotinous cones during the hot dry season (August to October; Chapter 3). Seed germination reached 100% within 25 days at constant temperature of 20°C although such temperatures are not experienced in the field and 80% within 30 days at alternating temperature of 15/25°C. This result shows that *W. whytei* seed is non-dormant and germinates easily. This confirms the results of other studies where prolific seed germination was reported on this and a related species (Mayhead and Ofesi 1989, Pauw and Linder 1997, Gondwe 2010).

Although seed viability tests have existed for a long time, the viability of seeds at the end of a germination trial is not always investigated. A number of reasons may justify not conducting viability tests, such as working with small seeds whose embryos are difficult to expose without destroying the seeds. However, in other cases the justification

is not clear. Whatever the reason, it is not uncommon to assume that those seeds that did not germinate did not have the conditions to break the dormancy. However the results of the *W. whytei* seed germination trial indicate that those seeds that did not germinate under low temperatures (below 15°C) were alive while those at 25°C were mostly dead. This shows that low temperatures induce dormancy while temperatures above 25°C are lethal to the seeds and this is not surprising for a tropical conifer species growing under temperate conditions such as *W. whytei* on Mulanje Mountain. This also suggests that in the event of fire, a high number of seeds exposed to lethal temperatures on the soil surface could be killed (Baskin and Baskin 1998). Clearly, these observations highlight the need to characterize the state and fate of all seeds in an experiment, as this information is essential to understand the exact physiological effect of the treatment to which seeds are subjected. *W. whytei* seeds germinated both in light and darkness. This indicates that *W. whytei* seed can germinate while buried in the soil as long as temperature and moisture conditions are optimal for germination. The ability of seeds to germinate in light and darkness reinforces the idea that *W. whytei* forms an aerial seed bank, a strategy which is common in species of habitats exposed to frequent fire disturbances such as the fynbos in South Africa (Geldenhuys 1994).

6.2.4 Influence of fragment size on natural regeneration pattern in *W. whytei*

Two questions were addressed in this part of the study (1) Does *W. whytei* forest fragment size influence seedling density and mortality? (2) When and where does seedling density and mortality mostly occur? In fragmented landscapes, tree recruitment

is critical for forest persistence and understanding the regeneration pattern of plant species in these landscapes is important for their conservation and management (Enrico et al. 2004, Lawes et al. 2007). In this study, seedling density and mortality were not influenced by *W. whytei* population sizes. However, seedling density was extremely low (Chapter 5). This confirms earlier reports that natural regeneration in *W. whytei* is lacking in and around the forest fragments on Mulanje Mountain (Chapman 1995, Makungwa 2004). Low densities of natural regeneration in most forests have led many silviculturists to dismiss natural regeneration on most sites in favour of artificial regeneration (Eastham and Jull 1999). Other studies showed a strong link between viable seed output, seed rain density and seedling regeneration (Eastham and Jull 1999). Findings in this study (Chapter 2) showed that viable seed output per cone was generally very low (23%). So too was the seedling density. Similarly, seedling density was higher inside *W. whytei* forest fragments compared to the edges and adjacent areas and it followed the seed rain pattern (Chapter 3) although seedling density was much lower than the seed rain density. This shows that seedling density is related to viable seed output and seed rain density in *W. whytei*. Rainfall had a remarkable influence on seedling density. This is a well-known characteristic of tropical forests (Whitmore 1996).

This study showed that seedling regeneration during the rainy season was followed by high seedling mortality during the dry season. Although seedling density was higher inside forest fragments, forest interiors suffered 100% mortality rate compared to forest edges. Lack of adequate light on the forest floor may have contributed to the increased

mortality inside forest fragments. The results suggest that seedling recruitment is strongly limited around the forest edges (Chapman 1995) but the few seedlings that do occur have a better chance to survive unless they grow within the fire zone (Geldenhuys 1994). *W. whytei* has been shown to require exposed mineral soil or litter-free surface for successful seedling establishment once the fire has destroyed the vegetation (Topham 1936). The edge environment of *W. whytei* fragments is characterized by these conditions. Thus, edge environments favour more opportunistic light demanding pioneer species (Asbjornsen et al. 2004), such as *W. whytei*.

On Mulanje Mountain, wildfires are very common especially during the dry season (Nangoma and Bayliss 2007). Outside forest fragments fire was the main cause of seedling mortality. This confirms earlier reports that fire is the greatest cause of seedling mortality on Mulanje Mountain (Chapman 1995). Although fires are the main cause of seedling mortality along the edges and adjacent non-forest patches, they are also responsible for postfire abundant seed release from *W. whytei* serotinous cones (Chapter 3, Lamont et al. 1991, Midgley et al. 1995, Johnstone et al. 2009). In addition, fires also expose the mineral soil after destroying the competing vegetation and litter layer in these micro-environments. The occurrence of abundant seeds from the aerial seed banks and exposed mineral soils could create a chance for the dispersed seeds to germinate and become established in large numbers. However, this study did not include fire events as such extrapolation of these results to fire burned *W. whytei* forest patches should be done with caution. Seedling survival, which was promoted by climate variability, varied from year to year. Thus, knowledge on the influence of climate

variability on seedling survival is very critical for successful natural regeneration management in *W. whytei* in the forest fragments.

6.3 IMPLICATION OF THE RESULTS OF THIS STUDY ON THE ECOLOGY AND DYNAMICS OF *W. WHYTEI* IN THE FOREST PATCHES

This study was set out to determine the effects of *W. whytei* fragment size and isolation on (1) viable seed output, (2) seed rain density and (3) natural regeneration pattern. Viable seed production was not controlled by *W. whytei* population size. Even though large, isolated and emergent trees produce many cones in a year (Chanyenga 2012, unpublished data). This study showed that over 75% of seed in these cones are empty. Furthermore, the *W. whytei* population, which includes seed producing trees, is undergoing a drastic decline due to excessive exploitation and the effects of very intense frequent wildfires around the forest patches (Chapman 1995, Bayliss et al. 2007). Taken together, these results suggest that *W. whytei* population growth will be negatively affected in and around the forest patches on Mulanje Mountain.

The results show a clear influence of large *W. whytei* population sizes in contributing seeds on the forest floors in the forest fragments. Indeed seed dispersal has been widely recognized as a key process within fragmented landscapes that is diminished by human and natural disturbances such as resource exploitation and wildfire and which controls plant population and community persistence (Higgins et al. 2003, Pearson and Dawson 2005). The evidence from this study indicates that large population sizes,

which have many seed bearing trees, do matter in terms of seed dispersal. More importantly, results on seed dispersal under different conditions highlight the importance of considering the role played by environmental factors in species with serotinous cones (Bender and Fahrig 2005). Fire is one of such environmental factors that create ideal conditions for heavy seed falls in species with serotinous cones (Lamont et al. 1991, Au et al. 2006). Besides, forest patches are located in sheltered sites that cause calm conditions and lower fire frequency (Geldenhuys 1994). Thus, regular cool fires and calm conditions in the forest patches make *W. whytei* seeds to be poorly dispersed i.e. light seedfalls and limited dispersal distance. Thus, in absence of moderate to severe fires and strong winds in and around the forest patches, one of the most significant findings emerging from this study is that most seed dispersal is currently restricted to inside forest patches and this situation may persist indefinitely and limit future *W. whytei* population expansion on the mountain.

Current natural regeneration of *W. whytei* in and around the forest patches is generally very low and sparse which is similar to what Makungwa (2004) reported. This is the case despite *W. whytei* seed being non-dormant and that it germinates readily when dispersed. Thus, low numbers of seedlings observed in this study suggests a stagnation of *W. whytei* regeneration, which could be translated into an unlikely expansion of the species throughout the forest patches on the mountain. The study has shown that population size of *W. whytei* does not control patterns of seedling regeneration. A key factor determining successful establishment of *W. whytei* seedlings during the initial regeneration phase is whether seedlings encounter favourable microclimate conditions

to enable them to develop sufficiently deep and extensive root systems (Asbjornsen et al. 2004). Evidence from this study indicates that *W. whytei* seedlings do not get established in the forest understorey but along the edges of the forest fragments and this is in agreement to what was reported in literature (Topham 1936, Chapman 1995). Thick litter layer and vegetation cover suppresses seedling regeneration in the forest understorey while the presence of bare mineral soil and partial shade conditions along the forest edges promotes tree seedling establishment in *W. whytei* (Stewart and Rose 1990, Cornett et al. 1997). Moreover, *W. whytei* seedling regeneration has shown to be more dependent upon occurrence of fire around the forest patches because fire removes the litter layer and exposes the mineral soil (Geldenhuys 1994, Chapman 1995). In general, therefore, it seems variation in canopy density and degree of bare mineral soil exposure determines the success of the regeneration and recruitment of this species. Thus, *W. whytei* is a partial shade-tolerant species. The study has shown that most of the seedling regeneration takes place during the rainy season especially in the months of December and January because this is the time when temperatures are optimal for seed germination in *W. whytei* on Mulanje Mountain. This is a relatively short period for all seeds to germinate and get established considering that seed germination conditions are not uniform on the forest floors. This may limit *W. whytei* population growth in the fragments.

W. whytei grows in a high elevation and fire prone area. Even though *W. whytei* seed germination is successful during December and January, seedlings encounter decreasing temperatures during June and July and high temperatures during August

and September, months characterized by frost and wildfires on the mountain respectively. This clearly indicates that the period for ideal growing condition between seed germination and early seedling establishment in *W. whytei* is very confined. The study has shown that fire is responsible for the highest number of seedling deaths compared to frost along the edges and non-forest patch habitats in hot and cold seasons respectively. Thus, fire is one of the major causes of seedling and tree mortality on Mulanje Mountain and modern thought on the dynamics of cedar population ecology is dominated by this factor (Chapman 1995, Bayliss et al. 2007). Taken together, inadequate viable seed output, light seedfalls and limited seed dispersal from the serotinous cones under non-fire circumstances and low early seedling survivorship found in this study suggests that recruitment is insufficient to replace the aging *W. whytei* tree population and may cause local extinctions of this species in the forest patches. Although *W. whytei* is already categorised as Endangered on IUCN red list (IUCN 2006), the information generated from this study has further raised the fears on the species population persistence on the mountain. Thus, the category of *W. whytei* on the IUCN conservation red list should be raised to attract international conservation attention.

Nowadays, interest has grown to predict how long plant species of conservation concern persist on particular sites. Population viability analysis (PVA) has traditionally been used for this purpose. It is a process in which the likelihood that a population will become extinct is assessed, within a specified time and under particular circumstances (Shaffer 1981, Possingham 1991, World Conservation Monitoring Centre 1992, Pfab

and Scholes 2004 Obloh and Isichei 2007). Findings from this study clearly show that *W. whytei* is facing high risk of extinction due to declining population sizes and insufficient recruitment in the forest patches. PVA is therefore needed to predict whether *W. whytei* population will become extinct or not given the current conditions facing the species on the mountain. This information is important because it will assist forest managers to make good decisions when managing this important species. However, to produce accurate predictions on *W. whytei* population, sufficient and long-term data is required (Pfab and Scholes 2004, Obloh and Isichei 2007). Most PVA studies use data collected for many years e.g. 5, 10 or 20 years (Pfab and Witkowski 2000; Keedwell 2004). In this study, it was not possible to carry out the PVA for *W. whytei* because there was insufficient data for this kind of analysis as it was collected for a short period of time (two years only). Use of insufficient data leads to incorrect conclusions and this is detrimental to the species being modeled (Keedwell 2004).

6.4 GUIDELINES FOR FUTURE MANAGEMENT OF *W. WHYTEI* IN FOREST PATCHES

At the beginning of this study two questions were posed: how would one deal with an endemic tree species which has a fragmented distribution on top of an isolated mountain massif but which has shown economic potential as timber tree? What are the ecological characteristics of this species that enabled it to survive on this isolated habitat and how could this be used to implement a management system that would ensure survival of this species on the mountain but enable development of its economic potential? It is now possible to state that inadequate viable seed output, light seed falls

and limited seed dispersal under non-fire disturbance conditions and low overall seedling survivorship documented in this study has certainly shed some light on how *W. whytei* can be sustainably managed in the face of the on-going decline of its population in the forest fragments on Mulanje Mountain. First, it has been seen that *W. whytei* cones produce insufficient viable seeds. With such a low inherent viability, even if small effects of other agents such as seed predation, fire and disease are not added, natural regeneration of the species is expected to be extremely poor. Meantime, it has been observed that cones from warmer sites produce slightly higher number of viable seeds compared to cones from colder sites. Management practices that preserve some of *W. whytei* forest patches for seed collection purposes on such sites should be promoted because seed collection expeditions would turn out to be more productive and economical. To achieve maximum efficiency, seed harvesters could concentrate their collection efforts on large, isolated and emergent trees. Such trees have a higher cone production although seed viability per cone in these trees is similar. However, *W. whytei* exhibits a large degree of yearly variation in viable seed output at population and individual levels due to climatic fluctuations on the mountain. Seed banking at local level is highly recommended to avoid years of reproductive failure.

Second, the study shows that the amount of seeds dispersed per unit area could be increased by retaining more *W. whytei* seed producing trees in a forest patch if the objective is to promote natural regeneration. *W. whytei* appears to be a semi-serotinous conifer where the cones provide a prolonged storage of seeds within an aerial seed bank. Minimising the current practice of felling trees with live crowns could be beneficial

because removal of such trees reduces the potential seed bank. However, retention of seed trees in forest patches is proving extremely difficult to attain due to illegal logging that targets these trees for economic reasons and easiness to saw (Makungwa and Chanyenga 2007). The Forestry Department is therefore encouraged to re-enforce the existing *W. whytei* licensing policy which allows for utilization of dead cedar trees only. *W. whytei* does not coppice after being burnt and it is restricted to fire protected valleys where it is a forest pioneer (Pauw and Linder 1997). At the same time, being semi-serotinous, its cones depend upon fire to release heavy seed falls and long distant seed dispersal through upward drafts of hot air associated with fires. However, under the current management strategy of total fire exclusion, which has been in place since 1920 (Chapman 1992), seed dispersal, which is also less effective, is restricted to inside forest patches only. Management that allows moderate to severe fires inside forest fragments at intervals would promote heavy seed falls and subsequent natural regeneration. This is important for the persistence of this fire-adapted species, which depends on seedling recruitment from seed. However, fire intervals for this species are yet to be established although Edwards (1982) suggests an interval of 100-200 years for *W. whytei* to re-enter the successional process and this interval appears to be very unrealistic and difficult to attain.

Finally, seedling survival seems to be the most critical phase for the self-maintenance of the whole *W. whytei* population on Mulanje Mountain. This study suggests that the species has a low chance of seedling establishment inside the forest patches because *W. whytei* seedlings are shade intolerant (Topham 1936, Chapman 1995) and their

establishment success depends on moderate to severe fire-disturbance intensity since it is a fire-adapted species. Management that creates large gaps in the forest fragments through group tree felling and fire spotting to expose the mineral soil, increase heavy seedfalls and subsequent number of seedlings should be encouraged and this is essential for the species persistence on the mountain. Although moderate to severe fire promotes seed dispersal and regeneration in *W. whytei*, cool regular fires currently experienced on the mountain (Nangoma and Bayliss 2007) are preventing the establishment of seedlings and threatening the survival of the species. The evidence from this study shows that fire is the greatest cause of seedling mortality in the fire zone i.e. along the edges of the forest patches and scrubland areas within the range of wind-blown seed from *W. whytei* trees. Forest managers are therefore asked to allow as much time as possible between fires to enable the establishment of seedlings. These observations strengthen the importance of establishing appropriate fire regimes for the species. The results also have implications for ensuring adequate *W. whytei* seedling regeneration in the forest fragments. Natural seedling regeneration is not adequate to guarantee future economic exploitation of the species. Foresters are therefore encouraged to carry out artificial regeneration using nursery-reared *W. whytei* seedlings. This could be achieved through the introduction of annual cedar planting events involving school going children, local communities and volunteer participation from environmental organizations. However, seedling transplanting is costly and labour intensive and requires careful planning and on-going commitment, and may introduce diseases from poor nursery hygiene.

6.5 GENERAL CONCLUSIONS

This study was designed to determine the effects of *W. whytei* fragment size and isolation on viable seed output, seed rain and natural regeneration pattern on Mulanje Mountain. It can now be concluded that:

6.5.1 The size of fragments of *W. whytei* population does not influence the number of viable seeds in a cone nor does tree stem diameter and crown position in the forest canopy. *W. whytei* cones generally produce insufficient viable seeds. Thus, seed harvesting either for ex-situ conservation in seed banks or healthy seedling production in the nursery for planting in the forest patches should be considered, but with careful attention to the hygiene of nursery-raised plants.

6.5.2 The seed rain of *W. whytei* varies greatly with fragment size. The presence of many seed producing trees in large fragments promotes seed rain density. Therefore retaining more seed producing trees ensures continued availability of *W. whytei* seed in and around forest patches. *W. whytei* seed is poorly dispersed in the forest patches because its cones are semi-serotinous and depend on fire to facilitate heavy seedfalls and wider dispersal. Carrying out controlled group-felling of trees in small gaps with diameter of 1 to 2 times the height of the stand canopy, and then burn the debris or allowing moderate to severe fires in the forest fragment at specified intervals could increase heavy seedfalls and wider seed dispersal. This approach should be tested in one or two sites.

6.5.3 Temperature is one of the critical factors for the germination of *W. whytei* seed. Seed of *W. whytei* is non-dormant and readily germinates upon dispersal as long as germination conditions are optimal i.e. with temperatures ranging between 15 and 25°C. The seed does not require any pre-treatment.

6.5.4 Fragment size is not a key factor in influencing seedling regeneration in *W. whytei*. For *W. whytei* seedling establishment, availability of both seed and mineral soil appear to be the primary limiting factors in the forest patches. The observed high seedling mortality raises great concerns about the ability of this species to persist in the forest patches. Thus artificial regeneration would appear to be necessary to augment natural regeneration in reaching acceptable stocking levels.

6.5.5 Current biological threats to the conservation status of *W. whytei* include production of cones with insufficient viable seed, light seed rain, limited seed dispersal and inadequate natural regeneration, as demonstrated in this study. This adds to the other threats *W. whytei* is currently facing in the forest patches on Mulanje Mountain (Bayliss et al. 2007) and could push the species into a “critically endangered” status as it is already categorized as Endangered on the IUCN Red List (IUCN 2006). Integrated conservation and management approaches may be needed to maintain viable *W. whytei* tree populations in the forest fragments.

6.6 LIMITATIONS OF STUDY

6.6.1 Seed viability study

A limitation of this study is that it was conducted over two years, which is only a small proportion of the life history of this long-lived perennial species. With a short observation period like this, caution must be applied when interpreting the results.

6.6.2 Seed rain study

Two limitations to this study need to be acknowledged. One source of weakness, which could have affected the measurement of the seed rain, was that hunters vandalized some seed traps during the rainy season in one of the forest fragments. The high-density plastic material from which the traps were made was used to kindle fire. Consequently, data from such disturbed traps were excluded from the analysis. The second limitation is that this study was done during normal conditions without fire events in the forest fragments. *W. whytei* depends on fire for effective seed dispersal, therefore interpretation of the seed rain results must be applied with caution.

6.7 FUTURE RESEARCH

This research has generated many questions in need of further investigation.

6.7.1 Although seed dispersal and seedling establishment depend on moderate to severe fire in *W. whytei*, repeated cool fires are preventing effective seed dispersal and seedling establishment. This is threatening the survival of the species in the forest patches on the mountain. Periodic moderate to severe fires seem to be essential for *W. whytei* regeneration. Data on appropriate fire regimes for the species is lacking. Further research is therefore needed to establish appropriate fire regimes for this species. One approach could be to establish the age of relatively even-aged stands of *W. whytei* in different forest patches and to relate this to historical records, if any, on fire events on the mountain.

6.7.2 *W. whytei* has shown a cyclic seed rain pattern among populations on the mountain. Cyclic seed rain patterns are associated with mast seeding species. This suggests that mast seeding may occur in *W. whytei*. However, the observation period in this study was too short to establish mast and regular seeding years in *W. whytei*. Further experimental investigations are therefore needed to establish the mast seeding interval period in this species and how this would differ from mass seeding events associated with major fire events (see par 6.7.1).

6.7.3 The present study focused on seed viability, dispersal and natural regeneration, but did not explore plant pollination biology. However, causes of low viable seed production are not known and it could be linked to pollen limitation. So far, little is known on the flowering pattern of *W. whytei* and it would be interesting to undertake pollination studies in this species.

6.7.4 In this study, local seed banking has been suggested as one of the conservation strategies. So far, little is known on the behaviour of *W. whytei* seeds in storage. Further research on seed behaviour whilst in storage would be of great help.

6.7.5 This study shows that seedling regeneration is followed by high seedling mortality. While causes of seedling mortality at the edges and adjacent non-forest areas have largely been identified, the causes of seedling mortality inside forest fragments are little known and it is an area that requires further investigation.

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APPENDIX 1
PUBLISHED PAPERS

Chapters 2-4

Effect of population size, tree diameter and crown position on viable seed output per cone of the tropical conifer *Widdringtonia whytei* in Malawi

Tembo F. Chanyenga^{*1}, Coert J. Geldenhuys[†] and Gudeta W. Sileshi[‡]

^{*} Department of Forest and Wood Science, University of Stellenbosch, c/o Forestry Research Institute of Malawi, P.O. Box 270, Zomba, Malawi

[†] Department of Forest and Wood Science, University of Stellenbosch, c/o Forestwood cc, P.O. Box 228, La Montagne 0184, Pretoria, South Africa

[‡] World Agroforestry Centre, P.O. Box 30798, Lilongwe, Malawi

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Abstract: The tropical montane conifer tree *Widdringtonia whytei* is found in small fragments on Mulanje Mountain in Malawi. A study was conducted with the objectives of determining the effect of population size, tree stem diameter and crown position on the proportion of viable seeds per cone produced by *W. whytei* at three sites (Sombani, Chambe and Lichenya) on Mulanje Mountain. Three population sizes, namely small (fragments with ≤ 10 cone-bearing trees), medium (fragments with 11–20 cone-bearing trees) and large (fragments with > 20 cone-bearing trees) and isolated trees were sampled at each study site. In each fragment, four cone-bearing trees were randomly located, 20 mature cones were collected from each tree and the viability of seeds was tested. Only 23% of the seeds per cone were viable but seed viability per cone was highly variable among fragments. Large fragment populations produced the highest proportion of viable seeds per cone (30%), followed by similar proportions in small fragments (28.3%) and isolated trees (26.1%), with the lowest proportions in medium-sized fragments (18.7%), indicating a non-linear relationship between viable seed output per cone and population size. Tree stem diameter and crown position had no effect on the proportion of viable seeds per cone. Further studies are needed to identify the factors that lead to the low viable seed output per cone, and how this influences whole-tree seed production in *W. whytei*.

Key Words: crown position, invasive aliens, population fragmentation, seed viability, tree stem diameter

INTRODUCTION

As forest landscapes are fragmented by natural events and anthropogenic disturbances, populations of some tree species have become more isolated with decreasing population spatial distribution, numbers of subpopulations, stem densities within fragments and increasing distances between fragmented subpopulations (O'Connell *et al.* 2006). Fragmentation, decrease in stem densities within fragments and isolation of plant populations can affect demographic processes as a result of reduced pollen receipt and increased pollen limitation (Ashman *et al.* 2004, Pauw 2007, Wang *et al.* 2010).

Studies on the effects of population fragmentation on viable seed output of particular plant species reported contrasting results, depending on the biological and ecological characteristics of the species concerned. Seed from smaller populations of *Pinus strobus* had a lower

ratio of filled seed than from larger populations in Canada (Rajora *et al.* 2002). Similarly, the percentage of viable seeds per cone significantly decreased with decrease in patch size in *Pinus tabulaeformis* in China (Wang *et al.* 2010). Population fragmentation in the conifer tree *Picea jezoensis* increased the total number of viable seeds per cone, resulting from enhanced pollen movement (Tomita *et al.* 2008).

Widdringtonia whytei naturally has a fragmented distribution pattern on Mulanje Mountain but the fragments have further been impacted on by wildfires and human resource use (Chapman 1995). In these fragments *W. whytei* stands comprise scattered trees of both smaller and larger stem diameters. Both suppressed and emergent tree canopies exist in the stands (pers. obs.). However, very low natural regeneration has been reported in recent surveys (Bayliss *et al.* 2007). To date, no studies have examined viable seed output per cone in *W. whytei*. This study examined the impact of population fragmentation on viable seed output. It also assessed whether tree stem diameter and crown position of trees

¹ Corresponding author. Email: tchanyenga@gmail.com

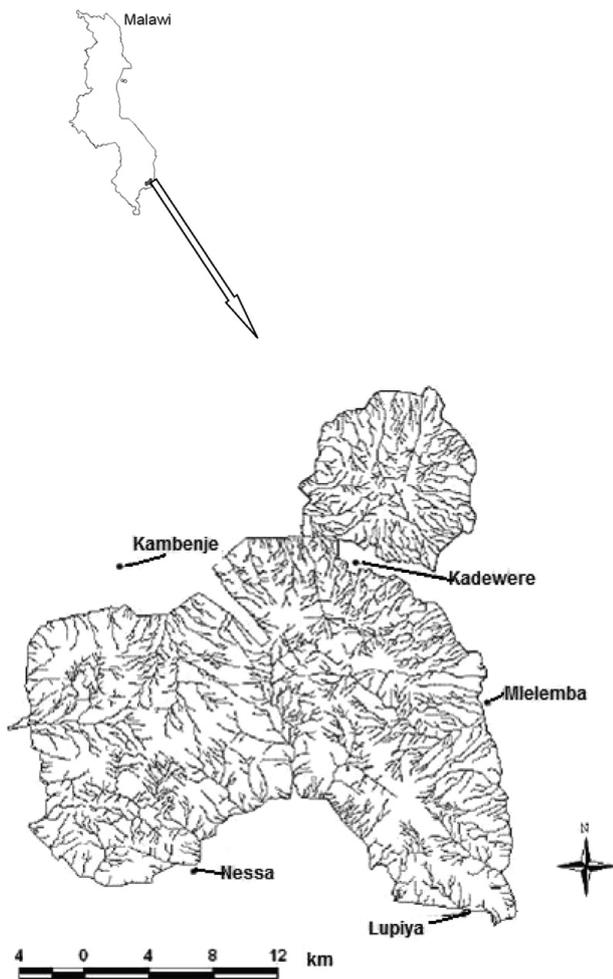


Figure 1. Location of Mulanje Mountain in Malawi.

in relation to the forest canopy affected viable seed output per cone. Our first hypothesis was that viable seed output per cone in *W. whytei* trees decreased with increased population fragmentation. Our second hypothesis was that tree stem diameter and crown position in the forest canopy positively influenced viable seed output per cone.

STUDY SITE

This study was conducted on Mulanje Mountain in Malawi (Figure 1) at three sites, namely Sombani (15°52'41''S; 35°40'89''E), Chambe (15°62'40''S; 35°30'72''E) and Lichenya (15°62'33''S; 35°30'73''E). The altitude of these sites ranges from 1660–2265 m asl (Lawrence *et al.* 1994). These sites are characterized by *W. whytei* forest fragments of different sizes ranging from 0.8–1779 ha, with most of them confined to ravines and hollows.

STUDY SPECIES

Widdringtonia whytei is a wind-pollinated coniferous tree endemic to Malawi and valued for its fine timber and attractive fragrance (Bayliss *et al.* 2007). Its commercial exploitation began in 1898 and continued until 1955, with large areas of forest cleared. Today *W. whytei* has highly fragmented populations on Mulanje Mountain. This species was originally thought to be synonymous with *W. nodiflora* which in turn was thought to be synonymous with *W. cupressoides*. This has been disputed (Pauw & Linder 1997). It is now well established that both *W. nodiflora* (multi-stemmed, narrow crowned tree) and *W. whytei* (taller, wide crowned) occur on Mulanje Mountain (Bayliss *et al.* 2007). In this study the species circumscription follows that of Pauw & Linder (1997). *Widdringtonia whytei* grows up to 40 m in height and *c.* 1 m in stem diameter at breast height (dbh). Male and female cones are borne on the same tree (Chapman 1995) and sometimes on the same branch (*pers. obs.*). Cones are globose, 1.5–2 cm in diameter, dark brown with four scales (Chapman 1995). The number of cones per cluster is much less variable, with generally two cones per cluster. Female cones seldom remain closed for more than 2 y (Pauw & Linder 1997). A cone generally contains 4–8 seeds (Pauw & Linder 1997). *Widdringtonia whytei* seed is 25 mm long and 12 mm wide on average with a wing (Pauw & Linder 1997) and is wind-dispersed (Chapman 1995).

METHODS

Sampling of trees and seed

Two sets of data were collected. In the first study, a stratified random sampling method was followed to select trees to assess the effect of *W. whytei* fragment size on viable seed output per cone. At each selected study site on the mountain, all *W. whytei* population sizes were recorded and grouped into four categories (isolated trees, small, medium and large). Small fragments consisted of groups of ≤ 10 cone-bearing (reproductively mature) trees in an area either surrounded by other tree species or grassland vegetation. Medium-sized fragments consisted of groups of 11–20 cone-bearing individuals while large forest fragments comprised groups of > 20 cone-bearing trees. Only trees in the small and medium fragments were physically counted. A tree was considered isolated if it was separated by more than 500 m from the nearest individual or group of *W. whytei* trees either surrounded by grassland or other vegetation types.

Other factors were considered during forest fragment selection, such as accessibility, distance between forest

fragments (1 km was considered the minimum distance between selected forest fragments) and comparable stand characteristics such as orientation, elevation and slope despite most fragments being confined to the ravines (slopes) and hollows (plateaux). In each size group (small, medium and large) in each site, after excluding potentially unsuitable fragments, one fragment was randomly selected, and one tree from the isolated trees. A total of nine fragments and three isolated trees were selected for the study.

In each selected fragment, four cone-bearing trees were randomly selected, based on accessibility, distance between trees (25–30 m apart), presence of cones and tree health. Cones were also collected from one isolated tree on each study site. Each selected tree was recorded by location, fragment number and tree number for easy identification.

Mature cones which were about to open were collected in 2008 and 2009 following a pre-determined sampling plan. Sample size (number of cones) was determined using the Cochran (1963) formula: $n_o = Z^2 pq/e^2$ where n_o = sample size; z^2 = standard deviation of normally distributed population (1.96); p = maximum variation (0.5); q = confidence interval (0.5) and e^2 = standard error (5%). Based on this formula, 385 cones from 39 trees (approximately 10 cones per tree) were supposed to be evaluated in a year. In this study, however, 20 mature cones were collected and evaluated from each tree giving a total of 780 cones per year. Sample size was deliberately doubled to capture maximum variation in viable seed output per cone.

A tree climber collected the cone using an arm pruner. The crown of each sample tree was divided into north, east, south and west portions. From each portion, the crown was also subdivided into top, middle and lower. Where possible, five cones per branch and one cone per cluster were collected from one or two randomly chosen middle branches of each portion. Cones collected from each tree were bulked.

A separate study was conducted to investigate the effect of stem diameter and crown position on the viable seed output per cone in *W. whytei*. Initially, a subsample of trees at each site was selected to determine the diameter distribution of the population and crown position of each tree to guide the selection of trees to ensure sampling of a broad range of sizes. This study focused only on the Sombani site because the Chambe site had inadequate numbers of accessible small trees and Lichenya lacked large trees. A sample of 86 trees was selected from which 20 mature cones were collected per tree. The stem diameter at breast height (dbh) of sampled trees ranged from 13 to 61 cm. The crown position of each selected tree within the canopy was scored as: (1) understory or suppressed (no direct light) (CP1); (2) intermediate (some overhead or side light) (CP2); (3) co-dominant (full

overhead light) (CP3) and (4) dominant or emergent (full overhead and side light) (CP4) (Snook *et al.* 2005).

Seed viability determination

Each cone was placed in the shade and allowed to open separately in order to determine total number of seeds per cone. Seed viability was determined through germination tests followed by tetrazolium and cutting tests (ISTA 1993). All seeds from each cone were placed in separate plastic Petri dishes filled with solid culture media prepared from agar-agar granules (10 g l^{-1}). Germination experiments were performed in a germinator with a day and night light cycle of 12 h at 20°C . Germination counts were made daily for a period of 30 d. At the end of the experiment, the ungerminated seeds were soaked in water for 24 h and tested in a tetrazolium solution to check if the seeds were still viable and this was followed by cutting tests for confirmation.

Statistical analysis

An exploratory analysis of the effect of fragment size on proportion of viable seed per cone was conducted using SAS version 9.1 (SAS Institute Inc. Cary, NC, USA). This involved plotting the kernel density and normal probability density functions for each fragment. Thus the data were subjected to a linear mixed modelling, where fragment size, site and year were entered in the model as the fixed effects and trees as the random effect. The statistical power of the test was also calculated. The statistical power of a significance test is the long-term probability (given the population effect size, alpha and sample size) of rejecting a false null hypothesis. The statistical power of the test for the fixed effects of site and fragment was sufficiently high (>0.99). While the mixed model analysis shows the mean effect of population size, it does not reveal how seed viability is distributed in each fragment. Therefore, the probability (P) of obtaining a given seed viability value was estimated for each fragment using categorical models. Then the cumulative probability distribution of seed viability was plotted to allow examination of the stochastic dominance of one fragment over the other (Sheather & Jones 1991). In order to evaluate the effect of tree stem diameter and crown position on proportional seed viability, a linear regression analysis was conducted.

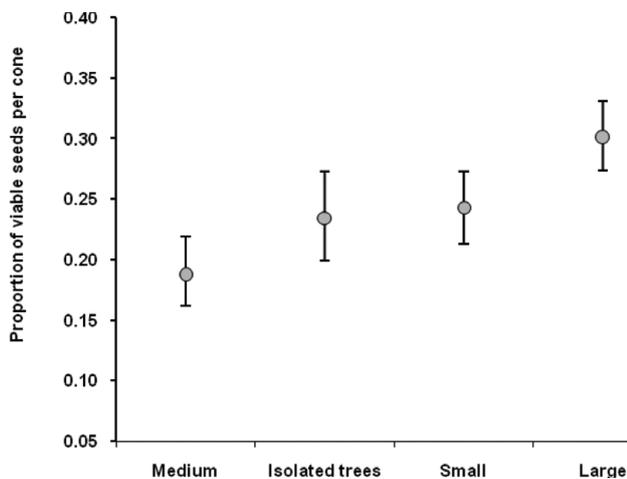
RESULTS

Effect of population size on seed viability

Seed viability was highly variable among fragments ($F = 30.4$; $P < 0.0001$). Large population sizes produced

Table 1. Proportion of viable seed produced per cone in different fragment sizes at three sites on Mulanje Mountain, Malawi. Figures were calculated from 20 cones per tree taken from four cone-bearing trees per fragment.

Fragment size	Year	Chambe	Lichenya	Sombani	Fragment mean
Large	2008	0.26 ± 0.01	0.17 ± 0.01	0.43 ± 0.02	0.29 ± 0.01
	2009	0.31 ± 0.02	0.29 ± 0.02	0.33 ± 0.03	
Medium	2008	0.20 ± 0.01	0.27 ± 0.02	0.11 ± 0.01	0.18 ± 0.01
	2009	0.18 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	
Small	2008	0.21 ± 0.01	0.20 ± 0.01	0.37 ± 0.01	0.23 ± 0.01
	2009	0.15 ± 0.01	0.22 ± 0.02	0.28 ± 0.01	
Isolated	2008	0.19 ± 0.03	0.15 ± 0.04	0.26 ± 0.04	0.23 ± 0.03
	2009	0.26 ± 0.03	0.16 ± 0.03	0.36 ± 0.04	
Site mean		0.22 ± 0.01	0.20 ± 0.02	0.29 ± 0.02	

**Figure 2.** Variability in the mean proportion of viable seeds per cone of different *Widdringtonia whytei* fragment sizes on Mulanje Mountain. Vertical bars indicate 95% confidence intervals.

the highest proportion of viable seeds per cone (30.1%), while medium-sized populations produced the lowest (18.7%) (Table 1; Figure 2). Cones at Sombani produced a significantly higher proportion ($F = 20.7$; $P < 0.0001$) of viable seeds (29%) compared with those at Chambe and Lichenya (22% and 20% respectively). Although the effect of year was not significant (Table 1) some inter-annual variation in the proportion of viable seeds per cone was apparent within fragments of the same size. For example, large fragments at Chambe and Lichenya produced higher proportion of viable seeds per cone in 2009 compared with 2008 whereas the large fragment at Sombani produced higher proportion of viable seeds per cone in 2008 compared with 2009. Isolated trees produced higher proportion of viable seeds per cone in 2009 compared with 2008 at all sites (Table 1).

Effect of stem diameter and crown position on seed viability

The proportion of viable seeds per cone did not significantly vary with tree size ($R^2 = 0.0032$; $n = 86$).

Cones in trees with smaller stem diameters produced slightly higher proportion of viable seeds than cones in trees with larger stem diameters (Figure 3). Similarly, crown position did not have a significant influence on the proportion of viable seeds per cone ($F = 1.02$; $P = 0.382$). Cones from the emergent and co-dominant trees produced higher seed viability (60% and 59% respectively), but they were not significantly different from those produced by understorey and intermediate trees (47% and 51% respectively). Cones from understorey trees had the lowest seed viability, while being the most variable (Figure 4).

DISCUSSION

According to our results, the proportion of viable seeds per cone was not related to population size and thus might be governed by different ecological processes. Studies conducted elsewhere reported similar findings (Cascante *et al.* 2002, Dick 2001). These results and earlier studies (Aizen & Feinsinger 1994, Wang *et al.* 2010) highlight the complex nature of predicting the outcome of fragmentation on seed viability in fragmented plant populations. The proportion of viable seeds per cone was generally very low and this is evidence as to why *W. whytei* seed collection expeditions turn out to be very expensive. The observed low proportion of viable seeds per cone could probably result from pollination failure due to lower pollen quantity or quality produced by this species. Our field observations on the flowering phenology on *W. whytei* indicated that male cone production was irregular on most trees with the highest number of cones being produced during the months of November and December. In addition, male cones were observed on very few branches during the same period. Similarly, low pollen production was associated with low proportion of viable seeds per fruit in a naturally fragmented population of *Parnassia palustris* (Bossuyt 2006). The most important attributes determining pollination efficiency are the timing, duration and intensity of flowering in plants simplified as synchronous or asynchronous flowering (Bronstein 1995). However, little is known on

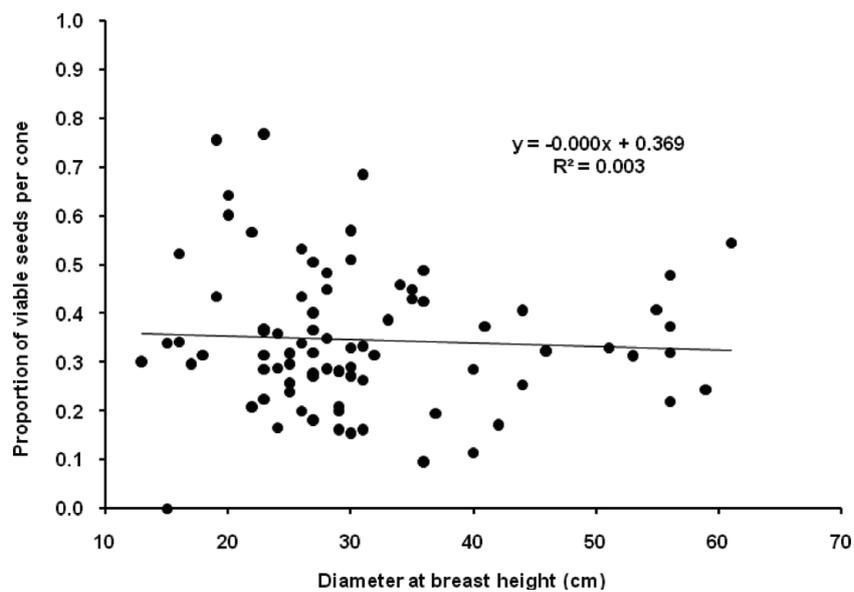


Figure 3. The relationship between the proportions of viable seeds per cone and tree stem diameter at breast height in *Widdringtonia whytei* on Mulanje Mountain.

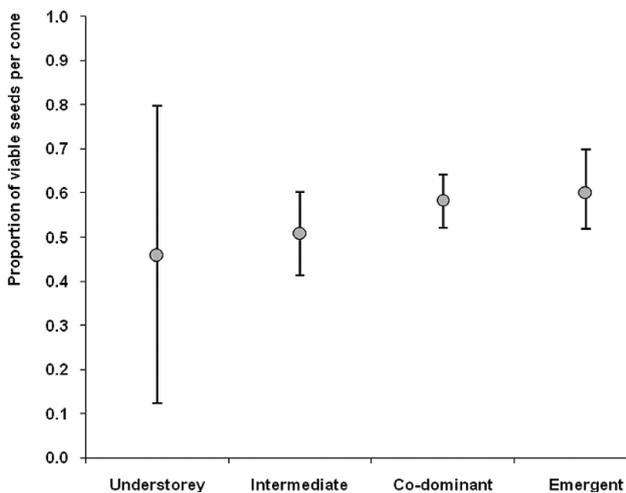


Figure 4. The relationship between the proportions of viable seeds per cone and crown position in *Widdringtonia whytei* on Mulanje Mountain. Vertical bars indicate 95% confidence intervals.

the flowering pattern of *W. whytei*. Further research is therefore required to establish whether synchronous or asynchronous flowering pattern occurs in this species.

Our results showed variations in the proportion of viable seeds per cone among sites and this could be attributed to differences in environmental conditions. Sombani has been described as sheltered from strong winds, warmer and drier while Chambe and Lichenya are exposed to strong winds, and colder and wetter conditions (Chapman 1995, Lawrence *et al.* 1994). Warmer temperatures, sheltered and drier conditions could probably favour a higher proportion of viable seeds

per cone in *W. whytei*, and this needs to be further investigated.

Despite the lack of annual variation in viable seed output between 2008 and 2009, there was high annual variation in the proportion of viable seeds per cone in some fragments of the same size within sites. These observations probably suggest reproductive isolation among different *W. whytei* populations on Mulanje Mountain. Similarly, Herreras-Diego *et al.* (2006) reported that trees in one habitat condition experienced reproductive isolation from trees found within other habitats on the same site each year.

Although cones produced by emergent and co-dominant trees had higher seed viability, they were not significantly different from those produced by understorey and intermediate trees. Cones produced by understorey or suppressed trees produced the lowest number of viable seeds. Cones from isolated trees that resembled the emergent trees failed to produce higher proportions of viable seeds compared to all fragment sizes. Again, our findings suggest that the proportion of viable seeds per cone in *W. whytei* might be governed by other ecological processes. Prevalence of adverse weather conditions such as high winds or storms particularly during key phenological events such as pollination can reduce viable seed output (Stephenson 1981).

Conclusions

Our results have shown that the proportion of viable seeds per cone in *W. whytei* is not affected by population fragmentation, tree diameter and crown position in the

forest canopy. It appears other ecological factors such as pollen limitation and environmental conditions are possible causes of the low proportion of viable seeds per cone observed in this study. Further studies are therefore needed to identify the factors that lead to the observed low proportion of viable seeds per cone, and how this influences whole-tree seed production in *W. whytei*.

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Variation in seed rain from *Widdringtonia whytei* growing in different conditions on Mulanje Mountain in Malawi

T F Chanyenga^a, C J Geldenhuys^b & J Harvey^c

^a Department of Forest and Wood Science, University of Stellenbosch, c/o Forestry Research Institute of Malawi, PO Box 270, Zomba, Malawi

^b Department of Forest and Wood Science, University of Stellenbosch, c/o Forestwood cc, PO Box 228, La Montagne, 0184, Pretoria, South Africa

^c Department of Statistics and Actuarial Sciences, University of Stellenbosch, Private Bag XI, Matieland, 7602, South Africa

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Variation in seed rain from *Widdringtonia whytei* growing in different conditions on Mulanje Mountain in Malawi

TF Chanyenga^{1*}, CJ Geldenhuys² and J Harvey³

¹ Department of Forest and Wood Science, University of Stellenbosch, c/o Forestry Research Institute of Malawi, PO Box 270, Zomba, Malawi

² Department of Forest and Wood Science, University of Stellenbosch, c/o Forestwood cc, PO Box 228, La Montagne 0184, Pretoria, South Africa

³ Department of Statistics and Actuarial Sciences, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa

* Corresponding author, e-mail: tchanyenga@gmail.com

Mulanje Mountain is one of the examples of tropical montane landscapes with plant populations varying in size from small fragments of less than 1 ha to riverine strips and stands of more than 100 ha. Seed availability is a potential limiting factor in vegetation recovery in such landscapes. Field observation on seed rain and seed limitation was conducted in 2008 and 2009 in three sizes of forest fragments at three sites on the mountain to investigate: (1) whether size of *Widdringtonia whytei* forest patches influences seed-rain density; (2) whether *W. whytei* seed rain occurs in a specific season; and (3) if seed limitation differs from within forest patches to the adjacent non-forest patch habitats. The results showed that there was a distinct difference in seed-rain density among fragments where large fragments (>20 trees) collected higher seed-rain density (10.4 ± 0.9 seeds $m^{-2} y^{-1}$) than small fragments (1.1 ± 0.4 seeds $m^{-2} y^{-1}$). The seed-rain density differed significantly between 2008 (2.1 ± 0.6 seeds $m^{-2} y^{-1}$) and 2009 (6.1 ± 1.1 seeds $m^{-2} y^{-1}$). Seed rain was recorded every month of the year but the densities were not significantly different among months. Seed-rain density was highest inside the forest fragments (12.04 ± 2.3 seeds $m^{-2} y^{-1}$) and decreased drastically and very significantly at the edges (1.0 ± 0.8 seed $m^{-2} y^{-1}$) and outside the forest fragments (0.11 ± 0.1 seed $m^{-2} y^{-1}$). Seed limitation was strong (0.98) on all sites; 0.95 in large fragments and 0.99 in medium and small fragments, respectively. Therefore, it can be concluded from this study that the presence and retention of more seed trees ensures the continued availability of *W. whytei* seed on the forest floor within the context of limited seed dispersal.

Keywords: *Widdringtonia whytei*, Mulanje Mountain, seed rain, seed limitation

Introduction

Widdringtonia whytei Rendle is an Endangered species (IUCN Red List) endemic to Mulanje Mountain in Malawi (Bayliss et al. 2007). The tree is highly valued for its fine timber and its commercial exploitation began in the 1890s and continued until 1955, with large areas of forest cleared (Bayliss et al. 2007). However, the species population has undergone a drastic decline due to increased fire incidence and illegal logging. At present, *W. whytei* has a highly fragmented population on Mulanje Mountain. Recent forest inventory reports indicated that natural regeneration of *W. whytei* is lacking in the fragments (Makungwa 2004). Until recently, efforts towards understanding its ecology and conservation have been very limited. Therefore, information is virtually lacking on the ecological behaviour of this species, including its seed dispersal.

Seed dispersal is a critical process in the life history of plants. It allows plants to colonise new locations and, through heterogeneous distribution of propagules, dispersal influences patterns of seedling establishment and survival and ultimately the density and distribution of the next generations of adult plants (Webb 1998, Nathan and Muller-Landau 2000, Wang and Smith 2002). Therefore, to predict how plant species disperse, it is important to measure seed rain (Cottrell 2004).

Seed rain is the total amount of seeds received by any habitat during a certain period of time (Hardesty and Parker 2002). It is normally expressed as seeds per unit area per unit time (m^{-2} or ha^{-1} per year, month or day) for an area, i.e. mean seed density over the area it has been dispersed to. Seed rain is a very important aspect of forest dynamics that has many practical applications but such studies are often overlooked. Lack of seeds can limit forest recovery after disturbance (Cubina and Aide 2001). A seed rain study determines the flux of seeds into disturbed sites. Furthermore, forest management programs aimed at re-establishing important species have been based on inadequate knowledge of the species' colonising abilities (Cottrell 2004). Again, seed rain studies provide important information on which species need to be assisted with regeneration in revegetation programs (Cottrell 2004). Stewart et al. (2001), for instance, reported a maximum natural regeneration density of 15 778 seedlings ha^{-1} in an established white spruce stand in Alberta, Canada. To attain the above natural regeneration density, Greene et al. (2002) suggested a minimum seed-rain density of 500 000 white spruce seeds ha^{-1} for adequate density and stocking. These findings clearly demonstrate that information from seed rain studies can provide answers

to some of the factors that limit the re-establishment of a functioning forest.

Population size influences the quantity of seed rain dispersed within a specific site supplying the seed (Jules and Rathcke 1999, Cunningham 2000). In Chiloe Island, Chile, for instance, Armesto et al. (2007) observed that large plant population sizes produced higher seed-rain density than small population sizes. However, the effect of plant population size on seed-rain density varies amongst species (Dosch et al. 2007).

Seed rain, in many plant species, is highly variable both in time and space. Some species disperse large amounts of seed at intervals of several months or years with little or no seed dispersal in the intervening periods (Zhang et al. 2008). In China, for example, *Pinus koraiensis* (Korean pine) dispersed most of its seed in summer, between July and October (Zhang et al. 2008). Similarly, seed rain is influenced by seed limitation, which can be understood as the failure of seeds of a given plant species to arrive at suitable sites in sufficient numbers to establish as seedlings (Nathan and Muller-Landau 2000, Levin et al. 2003). Thus the seed rain pattern of tree species is an indicator of its regenerative potential.

Widdringtonia whytei naturally has a scattered (patchy) distribution pattern on Mulanje Mountain in Malawi but the patches have been impacted further by human resource use, management for fire, colonisation by invasive species and conifer aphid attack (Chapman 1995). In disturbed, fragmented and degraded landscapes many studies have suggested that seed availability is a major limiting factor in vegetation recovery (also see Duncan and Chapman 1999). The phenological pattern of seed rain and seed limitation in *W. whytei* has hitherto received no attention and is little known. Determining seed rain dynamics and seed limitation may therefore be pivotal in understanding the recruitment dynamics of *W. whytei* on Mulanje Mountain. Therefore, the purpose of the study was to investigate the seed rain and seed limitation patterns in *W. whytei* specifically, establishing the influence of *W. whytei* population size, time of the year, position within a fragment and site on seed-rain density and seed limitation within and around the *W. whytei* fragments.

Materials and methods

Study area

This study was conducted on Mulanje Mountain in Malawi (Figure 1), located at 15°58'35" S and 35°38'00" E, from January 2008 to December 2009. The temperatures on the mountain vary from 5 °C in winter to about 28 °C in summer. The lowest temperatures usually occur in June/July while the warmest temperatures occur in October. More than 2 200 mm of rainfall are received annually. The study sites Sombani (15°52'41" S, 35°40'89" E), Chambe (15°62'40" S, 35°30'72" E) and Lichenya (15°62'33" S, 35°30'73" E) occur at altitudes ranging from 1 660–2 265 m asl (Lawrence et al. 1994). The major vegetation type of the study areas is afro-montane forest dominated by the species *Drypetes gerrardii* and *Ekebergia capensis* (Dowsett-Lemaire 1989). Today, these sites are characterised by *W. whytei* forest fragments of different sizes ranging from 0.8–177.9 ha (Makungwa 2004) with most of them confined to the ravines



Figure 1: Location of Mulanje Mountain in Malawi

and hollows on the plateaux and below cliffs and the gorges where the terrain affords some protection from fires (Chapman 1995).

Study species

Widdringtonia whytei is an endemic species to Mulanje Mountain (Chapman 1962). It grows up to 40 m in height and ± 1 m in stem diameter at breast height (dbh). *Widdringtonia whytei* male flowers are terminal and yellow-brown in colour while female flowers are proximal and white-blue in colour. Male and female flowers are borne on the same tree (Chapman 1995) and sometimes on the same branch (CJG pers. comm., July 2010). Cones are globose, 1.5–2 cm in diameter, and dark brown with four scales (Chapman 1995). The number of cones per cluster is much less variable, with generally two cones per cluster. Female cones seldom remain closed for more than two years (Pauw and Linder 1997). A cone generally contains four to eight seeds (Pauw and Linder 1997). *Widdringtonia whytei* seeds are 25 mm long and 12 mm wide on average with a wing (Pauw and Linder 1997) and are wind dispersed (Chapman 1995).

Population selection

The seed rain was characterised in three forest fragments at the three sites. The fragments were selected following a stratified random sampling method. At each study site, all fragments were recorded and grouped into three size categories (small, medium and large). Small fragments consisted of groups of ≤ 10 *W. whytei* cone-bearing (reproductively mature) trees in an area either surrounded by other tree species or grass vegetation. Medium fragments consisted of groups of 11–20 cone-bearing individuals, whereas large forest fragments are those comprised of groups of > 20 *W. whytei* cone-bearing

trees. Only trees in the small and medium fragments were physically counted. Other factors were considered during forest fragment selection, such as accessibility, distance between forest fragments (1 km was considered the minimum separation distance between selected forest fragments) and comparable stand characteristics such as orientation, elevation and slope, despite most fragments being confined to the ravines (slopes) and hollows. In each size group, after exclusion of potentially unsuitable fragments, the suitable fragments were numbered using a piece of paper and placed in a bag. Only one paper bearing a number of a forest fragment was later picked from the bag after a thorough mixing to ensure randomisation. Nine fragments were selected for the study, i.e. one fragment per size category (small, medium and large) at each of the three sites (Chambe, Lichenya and Sombani).

Sampling of seed rain

Most of the study fragments are found in deep and narrow valleys. General wind direction in and around fragments were assessed during September. The forest patches were calm despite the presence of strong winds during this time of the year. The presence of many small peaks or rocky outcrops tends to change the local pattern of wind direction and shelter the fragments from the winds. Wind direction may have more influence on exposed fragments, which are uncommon on the mountain. No specific provision was therefore made for wind direction in the sampling design.

The general sampling design for assessment of the seed rain is shown in Figure 2. Seed rain was captured using funnel seed traps, recommended as the best traps (Kollmann and Goetze 1998, Page et al. 2002, Chabrierie and Alard 2005). Due to large variability in the seasonality of seed rain and seed production in forest stands, Cottrell (2004) recommended conducting preliminary seed rain studies in order to determine the required number of traps. A pilot study was carried out during the months of January to April 2007 to determine the number of traps required, based on a 40 cm trap diameter. The minimum required number of traps (n) for the whole study was determined following Bonham's (1989) formula:

$$n = t^2 s^2 / (k \bar{x})^2$$

where n is the estimated number of traps needed for a specified probability and precision; t is the t -table value; s^2 is the pilot study sample variance; k is the precision required; and \bar{x} is the pilot study mean of seeds per trap.

From the pilot study ($n = 10$), $t = 1.96$, $s^2 = 0.5$, $k = 0.05$ and $\bar{x} = 0.5$, therefore the minimum number of traps required was 77. Ninety-five funnel seed traps with an aperture of 40 cm were used in this study.

Cottrell (2004) recommended the use of funnel seed traps with top diameters ranging from 20–30 cm. However, in this study, funnel seed traps, with circular iron wire frame, were locally made from high-density plastic sheet but with a top of 40 cm diameter (surface area 0.1257 m²). Seed traps with a slightly larger catchment area were deliberately used in order to capture more seeds since *W. whytei* tree crowns are generally narrow.

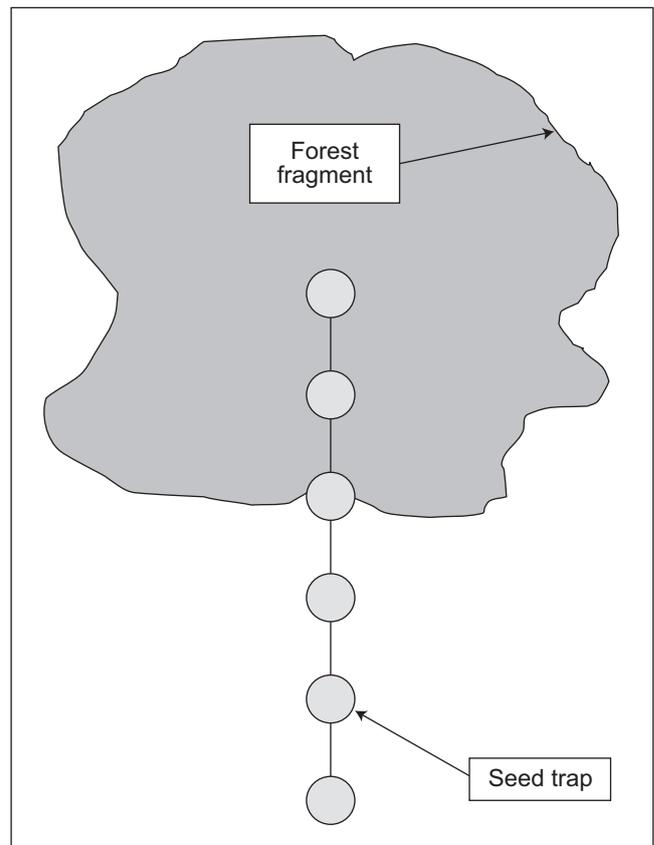


Figure 2: Sketch map showing layout of seed traps in and around any of the n selected *W. whytei* forest fragments on Mulanje Mountain

Seed traps were systematically placed from a randomly selected spot on the edge (0 m) of the selected forest fragment. The forest edge was defined as starting at the base of the first trunks of mature *W. whytei* trees encountered moving into and outside the forest patches. Seed traps were placed at every 10 m up to 30 m outside the forest fragment and inwards up to the centre (Figure 2). The distance to the fragment centre varied depending on the fragment size, but 30 m distance was decided as the furthest point seed traps could be placed outside the fragment.

Seed traps were placed 1.5 m aboveground as recommended by Kollmann and Goetze (1998). This height was chosen to eliminate seed from taller grasses and herbs, which were a common feature in and around the fragments. This height also prevents rodents from entering the seed traps and eating the seed. Eliminating potential seed rain from other species reduces the amount of time required for seed sample processing and decreases the chances of missing the target seeds in large sample volumes (Cottrell 2004).

Seeds were collected from the traps on a monthly basis for a period of 24 months. *Widdringtonia whytei* seed was sorted out in the field from all the trapped fruits, seeds and litter by the data collection team because it was easily identified. This greatly simplified the processing of seed trap collections. Sorting time varied from trap to trap as the volume of litter and other plant materials varied between them.

Data analysis

To investigate the effect of fragment size, forest fragment position and year on the seed-rain density data, mixed model analysis was performed using STATISTICA 9.0 (VEPAC module; StatSoft, Tulsa). Seed counts from each trap were standardised to number of seeds $m^{-2} y^{-1}$ for data analysis. Fragment size, site, year, month and forest fragment position were used as fixed effects. Due to the high number of seed traps not collecting seed both outside and inside the forest fragments, distance was not used as a category; instead, seed rain data were pooled and analysed as outside, edge and inside subcategories. Data were log-transformed to improve normality. For each of these categories and subcategories, *post-hoc* least significance difference (LSD) tests were performed to evaluate the significance of their effects and interactive combinations.

Seed limitation, i.e. the proportion of seed traps not receiving seeds after two years of seed collection ('fundamental seed limitation' sensu Muller-Landau et al. 2002), was calculated, as follows:

$$\text{Seed limitation index} = 1 - a/n$$

where a is the number of seed traps reached by any seed of a given species and n is the total number of seed traps. A seed limitation index >0.80 suggests a very strong seed limitation. However, seed limitation data in this study were dealt with in a comparative, not absolute, manner.

Results

Seed rain

Seed-rain density in large *W. whytei* fragments differed significantly from the medium and small fragments ($F = 32.147$, $p = 0.0000$). Traps in large fragments (>20 *W. whytei* cone-bearing trees) collected 10.4 seeds $m^{-2} y^{-1}$ compared to 0.7 seeds $m^{-2} y^{-1}$ for medium fragments (11–20 trees) and 1.1 seeds $m^{-2} y^{-1}$ for small fragments (≤ 10 trees) (Table 1). Seed rain was not significantly different between medium and small fragments ($p = 0.7255$). Furthermore, the seed rain pattern over the two-year study period varied among *W. whytei* fragments. Traps in the medium fragments collected no seed at Chambe and Sombani in 2008, whereas those placed in the small fragment at Chambe never collected any seed in both years (Table 1).

Seed-rain density was significantly higher in 2009 (6.1 seeds $m^{-2} y^{-1}$) than in 2008 (2.07 seeds $m^{-2} y^{-1}$) ($F = 12.813$, $p = 0.0037$). Overall seed-rain density varied

significantly across sites ($F = 10.133$, $p = 0.0001$). Traps at Sombani collected the highest seed-rain density (7.6 seeds $m^{-2} y^{-1}$), whereas those at Lichenya collected the lowest (1.7 seeds $m^{-2} y^{-1}$) (Table 1) and the difference between these sites was significant ($p = 0.00002$). Seed-rain density at Chambe was significantly different from Sombani ($p = 0.00083$) but not different to Lichenya ($p = 0.3586$) (Table 1).

Seed-rain density was highest inside the forest fragments (12.04 ± 2.3 seeds $m^{-2} y^{-1}$) and decreased drastically and very significantly at the edges (0 m) (1.0 ± 0.8 seeds $m^{-2} y^{-1}$) and outside the forest fragments (0.11 ± 0.1 seeds $m^{-2} y^{-1}$) ($F = 112.41$, $p < 0.0001$). Seed-rain density did not vary between the forest edges and the adjacent non-forest areas ($p = 0.97671$). Again, the highest seed-rain density was recorded at Sombani inside the forest fragments (22.6 seeds $m^{-2} y^{-1}$) then Chambe (8.6 seeds $m^{-2} y^{-1}$) and Lichenya (5.0 seeds $m^{-2} y^{-1}$) (Figure 3) and the differences were significant ($p = 0.0171$).

Seed rain occurred throughout the year, i.e. from January to December, during the study period (Figure 4). There was no significant difference on the overall seed-rain density among months ($F = 0.81$, $p = 0.6258$). However, the highest seed-rain density was recorded in September (5.0 seeds $m^{-2} y^{-1}$) in 2008 and August (12.7 seeds $m^{-2} y^{-1}$) in 2009 (Figure 4). On the other hand, the lowest seed-rain density was recorded in June (0.9 seeds $m^{-2} y^{-1}$) and

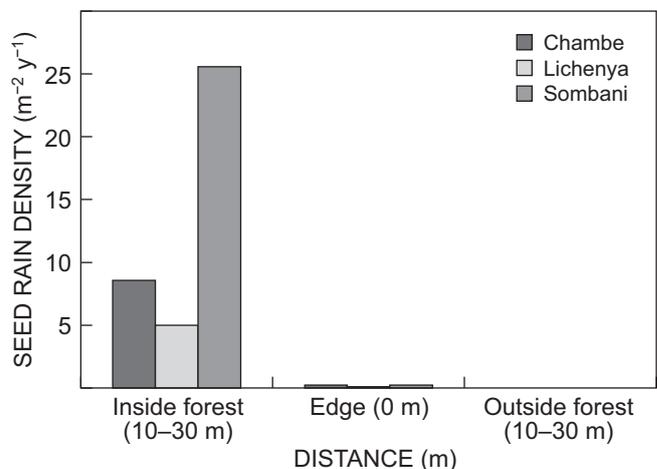


Figure 3: Mean seed rain pattern (seeds $m^{-2} y^{-1}$) of *W. whytei* from inside to outside forest fragments on Mulanje Mountain, Malawi

Table 1: Mean seed-rain densities collected in 2008 and 2009 in three fragment sizes at three sites on Mulanje Mountain, Malawi

Fragment size	Year	Sites			Overall mean: fragment size
		Chambe	Lichenya	Sombani	
Large (>20 trees)	2008	3.3 ± 1.6	0.2 ± 0.2	12.2 ± 4.1	10.4 ± 0.9
	2009	13.8 ± 3.6	5.3 ± 2.7	27.6 ± 8.0	
Medium (11–20 trees)	2008	0	0.9 ± 0.3	0	0.7 ± 0.2
	2009	0.7 ± 0.4	2.0 ± 1.2	0.4 ± 0.3	
Small (≤ 10 trees)	2008	0	0.2 ± 0.2	1.8 ± 1.4	1.1 ± 0.4
	2009	0	1.6 ± 0.8	3.3 ± 1.4	
Overall mean: site		2.9 ± 0.7	1.7 ± 0.5	7.6 ± 1.7	

November ($0.6 \text{ seeds m}^{-2} \text{ y}^{-1}$) in 2008 and February ($1.5 \text{ seeds m}^{-2} \text{ y}^{-1}$) and May ($2.1 \text{ seeds m}^{-2} \text{ y}^{-1}$) in 2009 (Figure 4). The lowest seed-rain density was generally collected in February and May in both years (Figure 4). Again, traps at Sombani collected the highest seed-rain density in almost every month (Figure 5).

Seed limitation

Seed limitation did not vary significantly among *W. whytei* fragment sizes, (e.g. 0.95 for large fragments and 0.99 for medium and small fragments, Table 2). Seed limitation was very strong at all sites (0.98), but areas inside forest fragments at Sombani had a slightly weaker seed limitation (0.85; Table 2). All seed traps placed outside the forest fragments (10–30 m) and edge (0 m) of the large fragments failed to receive any seed during the two-year observation period at Lichenya. The same was observed at Sombani, Lichenya and Chambe in the medium and small fragments (Table 2).

Discussion

The study shows that seed-rain density is highly variable both in time and space and may affect the recruitment of *W. whytei* on Mulanje Mountain. Large fragments contributed

significantly higher seed density than the medium and small fragments. This was because large *W. whytei* fragments had more seed-producing trees (>20 trees) than medium and small fragments. Similar results were also reported in other studies (Santos et al. 1999, Armesto et al. 2007). Seed rain was inconsistent among *W. whytei* fragments where no seed rain was collected in small and medium fragments at some sites, whereas large fragments dispersed seeds at all sites throughout the study period. Certainly, the results point to the importance of larger fragment sizes in promoting consistent seed rain in this species. For these reasons, the presence and retention of more cone-bearing trees (seed trees) seems to be very important for the continued availability of *W. whytei* seed on the forest floor.

There was a pronounced difference in the amount of seed rain produced between 2008 and 2009. This interannual difference appears to reflect the repeated temporal fluctuations in seed crops that characterise most tree species. Alternate years of high and low seed crops have been reported for many species (Armesto et al. 2007). Similarly, *W. whytei* fragments showed a distinct year of high seed-rain density alternated with a low or zero seed-rain year. This information provides evidence of mast seeding behaviour in *W. whytei*. Cyclic seed rain has been found in some mast-seeding species (LaMontagne and Boutin 2007).

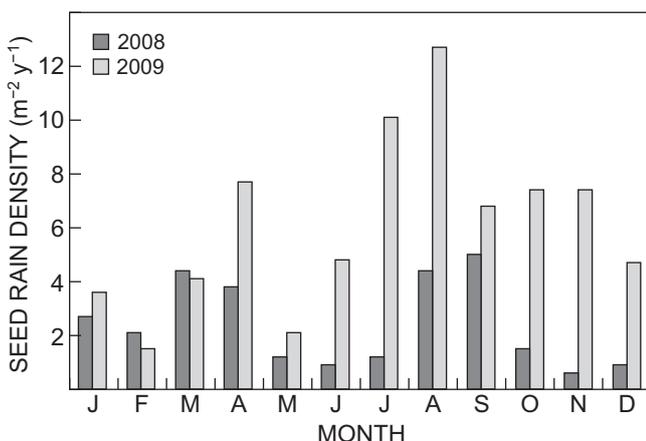


Figure 4: Mean monthly seed rain pattern ($\text{m}^{-2} \text{ y}^{-1}$) of *W. whytei* in 2008 and 2009 on Mulanje Mountain, Malawi

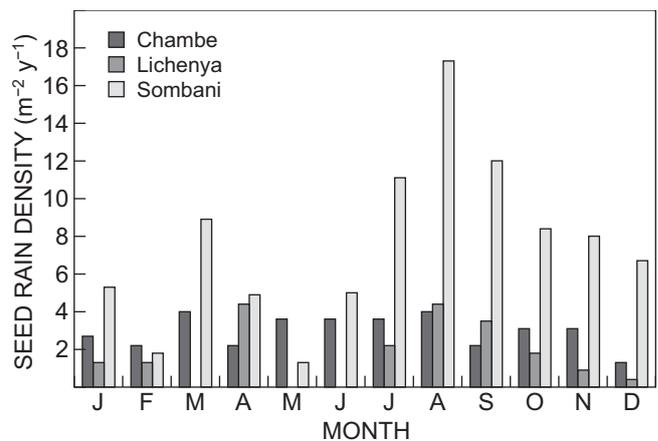


Figure 5: Mean monthly *W. whytei* seed-rain density sampled in seed traps placed at three sites on Mulanje Mountain, Malawi

Table 2: Seed limitation indexes for *W. whytei* from 2008 to 2009 in three fragment sizes and positions at the three sites on Mulanje Mountain, Malawi

Fragment size	Forest fragment position	Site			Overall mean: fragment size
		Sombani	Lichenya	Chambe	
Large (>20 trees)	Outside (10–30 m)	0.99	1.00	0.97	0.95
	Edge (0 m)	1.00	0.96	0.99	
	Inside (10–30 m)	0.85	0.95	0.90	
Medium (11–20 trees)	Outside (10–30 m)	1.00	1.00	1.00	0.99
	Edge (0 m)	1.00	1.00	1.00	
	Inside (10–30 m)	0.99	0.96	0.97	
Small (≤ 10 trees)	Outside (10–30 m)	1.00	1.00	1.00	0.99
	Edge (0 m)	1.00	1.00	1.00	
	Inside (10–30 m)	0.97	0.97	1.00	
Overall mean: site		0.98	0.98	0.98	

However, two years was too short a period to establish mast and regular years of seed production in *W. whytei*. Longer-term observations should therefore be carried out to better understand the interval of mast years in this species.

Widdringtonia whytei seed rain occurred all year round with relatively high peaks in March/April and July/August/September. The occurrence of seed rain throughout the year suggests that *W. whytei* maintains an aerial seed bank. Most species that maintain aerial seed banks have seed-holding structures (e.g. cones and capsules) that are resinous (Lamont et al. 1991). *Widdringtonia whytei* cones are resinous (CJG pers. comm., July 2010). Opening of such seed-holding structures may occur once there is enough heat to melt the resinous materials that prevents parts of the structure, especially cones, from opening (Helium and Pelchat 1979). This could be one of the reasons as to why high seed-rain densities were collected during the months of August to October during the study period. During this time of the year the mountain experiences slightly warmer temperatures as a result of general temperature rises (Chapman 1995) or increases in fire incidences where the strong upward draughts of air during the fire increases temperatures. For example, Nangoma and Bayliss (2007) observed fire occurrence on the mountain from 2001 to 2006 through the MODIS Rapid Response (MRR) fire alert system. A total of 325 fires were recorded but fire incidence was greatest from the middle to the end of the dry season with August having by far the greatest number of fires (132) followed by October (101) and September (60). These observations suggest that seed rain in *W. whytei* is highly adapted to temperature changes.

Widdringtonia whytei presented a highly variable seed rain pattern among sites on Mulanje Mountain. The mountain comprises a 'dry region' predominantly on the western and northern side, and a 'wet region' on the eastern and southern sides (Chapman 1995). The wetter south-eastern side is due to the prevailing weather fronts from the Mozambique coast and mountains. Sombani is located in the dry north region, whereas Chambe and Lichenya are located in the wetter southern and eastern regions of the mountain, respectively. Therefore it can be suggested that dry conditions promote high seed-rain density in *W. whytei*, hence the high seed-rain density recorded at Sombani.

Seed limitation was generally very strong as evidenced by the high number of seed traps that did not receive any seed during the two-year observation period. The major difference in seed rain pattern was associated with a high concentration of seeds within the forest fragments, which suggested that *W. whytei* cannot disperse its seeds for a long distance. This also suggested that the species has a non-random distribution pattern of seed rain (Loiselle et al. 1996). Seed rain declined drastically at the forest edges with seed limitation being highest on the adjacent non-forest habitats, which implied that *W. whytei* seeds could not disperse beyond 10 m from the forest edge. Similar results were found in other studies (e.g. Zhang et al. 2005). However, despite the greater concentration of *W. whytei* seed rain within forest fragments, there is strong evidence that they are less likely to germinate and produce viable seedlings in this shady habitat (Chapman 1995). Thus, although it is less numerous, seed falling at forest margins or away from the forest margin

into the grassland due to strong upward draughts of air during fire is most likely to contribute to seedling recruitment of this species. The strong seed limitation at all sites exacerbates the limited distribution of *W. whytei* on the mountain. If seed limitation indeed regulates population recruitment, the chances of colonisation of non-forest habitats by *W. whytei* are very low.

Conclusion

From the foregoing discussion, it can be concluded that the seed-rain density in *W. whytei* is clearly related to fragment size. This study showed that forest fragments with more than 20 cone-bearing trees of *W. whytei* dispersed more seeds per unit area than smaller fragments. Therefore, retaining more seed trees in a fragment would ensure continued availability of *W. whytei* seed on the forest floor. Seed rain occurred all year round, which is an indication that the species maintains an aerial seed bank. However, the current illegal harvesting rate of *W. whytei* trees with green or live crowns on Mulanje Mountain is a great concern because it may reduce the potential seed bank. Seed limitation is strong in *W. whytei*. The observed strong seed limitation suggests that the species has low chances of colonising the non-forest habitat on the mountain. Management that facilitates transplanting of seedlings and replanting in areas further from the fragments will accelerate recolonisation of this species.

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Short communication

Germination response and viability of an endangered tropical conifer *Widdringtonia whytei* seeds to temperature and light

T.F. Chanyenga^{a,*}, C.J. Geldenhuys^b, G.W. Sileshi^c

^a Department of Forest and Wood Science, University of Stellenbosch, c/o Forestry Research Institute of Malawi, P.O. Box 270, Zomba, Malawi

^b Department of Forest and Wood Science, University of Stellenbosch, c/o Forestwood cc, P.O. Box 228, La Montagne 0184, Pretoria, South Africa

^c World Agroforestry Centre (ICRAF), ICRA Southern Africa Programme, P.O. Box 30798, Lilongwe, Malawi

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Abstract

The tropical conifer *Widdringtonia whytei* Rendle is an endangered species endemic to Mulanje Mountain in Malawi. A study was conducted for the first time under controlled conditions in order to assess the effects of temperature and light on germination and viability of *W. whytei* seeds. Seeds incubated at a constant temperature of 20 °C attained the highest cumulative germination percentage (100%) followed by 87% germination under fluctuating temperatures of 15 °C night/25 °C day. No seed germination occurred at temperatures below 15 °C. Seeds that failed to germinate at temperatures below 15 °C showed the highest (>90%) viability compared to the seeds incubated at 25 °C (60%). Across temperature regimes, germination was significantly higher under light (44.7%) than dark (35.6%) conditions. It is concluded that temperature is one of the critical factors for germination of *W. whytei* seed. The ability of *W. whytei* seeds to germinate both in light and darkness implies that the species would unlikely form a persistent soil seed bank, an attribute which is common in species that survive in habitats frequently disturbed by fires.

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Keywords: Endangered; Germination; Malawi; Photoperiod; Temperature; *Widdringtonia whytei*

1. Introduction

The tropical conifer *Widdringtonia whytei* Rendle represents one of nine Southern African conifer species (Mustart, 2008). It is endemic to Mulanje Mountain in Malawi (Bayliss et al., 2007). Further, *W. whytei* is an endangered species that has been given global importance due to being listed by the IUCN onto a list of 43 conifer species worthy of special conservation attention (Bayliss et al., 2007). The species has undergone a drastic decline due to excessive exploitation and the effects of very intense wildfires (Bayliss et al., 2007; Chapman, 1995). Consequently, *W. whytei* populations are highly fragmented on the Mountain and restricted to ravines and hollows on the plateaus, below cliffs and in the gorges where the terrain affords some protection from fires and logging, to some extent. Current *W. whytei* fragments

show very poor natural regeneration (Makungwa, 2004). Until recently, efforts towards understanding its ecology and conservation have been very limited. To date, no information has been presented on the ecological requirements of this species including the germination of its seeds.

Germination is one of the most critical stages in the life cycle of any plant (Thompson and Ooi, 2010), and germination requirements are often assumed to be adaptations to the particular habitats where the species occur (Meyer et al., 1990). Each species has particular requirements for seed germination as a result of adaptive radiation into patchy and changing environments (Simons and Johnston, 2006). Therefore, understanding these requirements is very important in the conservation of endangered species such as *W. whytei*. Temperature and light are important ecological factors that regulate seed germination of

* Corresponding author.

E-mail address: tchanyenga@gmail.com (T.F. Chanyenga).

many plant species (Baskin and Baskin, 1998; Botha et al., 1982; Jarvis and Moore, 2008). Both low and high temperatures have been reported to inhibit seed germination in several species (Amri, 2010; Teketay, 1994).

The role of light in the germination of seeds has been studied by several authors (Baskin and Baskin, 1998; Botha et al., 1982). Some seeds germinate equally well in light and darkness (Baskin and Baskin, 1998), while others germinate more readily either only under light (Baskin and Baskin, 1990; Colbach et al., 2002; Grime et al., 1981) or darkness (Baskin and Baskin, 1990; Thanos et al., 1989). In addition, light requirements for seed germination may vary with changes in temperature (Baskin and Baskin, 1998; Farmer et al., 1984; McLemore and Hansbrough, 1970). Although many studies have been conducted on the effects of temperature and light on seed germination, temperature and light germination requirements are relatively unexplored in many plant species (Cony and Trione, 1996).

No studies exist on the effects of temperature and light on seed germination of *W. whytei*. Therefore, the objective of this study was to determine the temperature and light requirements for germination of its seeds.

2. Material and methods

2.1. Site description

Mature cones of *W. whytei* were collected and bulked in August 2009 from a large population (86 trees of different sizes) at Sombani (15°52'41"S and 35°40'89"E) on Mulanje Mountain. The temperatures on Sombani vary from 5 °C in winter to about 28 °C in summer. The lowest temperatures usually occur in June/July while the warmest temperatures occur in October. More than 2200 mm of rainfall is received on the site annually. The altitude at Sombani ranges from 1660 to 2265 m above sea level (Lawrence et al., 1994). Seeds were obtained after the cones were placed and allowed to open in the shade at the Forestry Research Institute of Malawi, Zomba. In the drying shade temperature varied between 22 and 30 °C and the relative humidity ranged between 30% and 38%. Seeds were dried to 5% moisture content (Gondwe, 2008). The seed moisture content (MC) was determined gravimetrically and expressed on a fresh weight basis. The dry mass was measured after heating seeds in an oven for 17 h at 103 °C according to the International Seed Testing Association rules (ISTA, 2003). Seeds of *W. whytei* are 25 mm long and 12 mm wide with a wing and are dark in colour (Pauw and Linder, 1997).

2.2. Seed germination and viability

Germination tests included seven temperature regimes with light and dark treatments. Each treatment was replicated five times with 20 seeds per replicate. Seeds were placed in 9-cm diameter Petri dishes filled with Agar-agar, and incubated in LTGC-40 growth cabinets at constant temperatures of 5, 10, 15, 20 and 25 °C and two alternating temperatures of 15 °C night and 25 °C day and 10 °C night and 20 °C day temperatures. These alternating temperatures will be referred to as 15N/25D

and 10N/20D in the following discussion. Seeds under the light and dark treatments were exposed to a photoperiod of 12 h light/12 h dark hours or continuous dark respectively. Light was provided by the cool-white 40-W fluorescent bulbs with 1000 lx (ISTA, 2003). Fluorescent tubes were used because they emit considerable red but little far-red light (Baskin and Baskin, 1998). Dark condition was provided by wrapping Petri dishes in three layers of aluminum foil (Baskin and Baskin, 1998). Germination counts under light treatments were recorded daily for 30 days while samples in darkness were opened on the final day of the experiment. A seed was considered to have germinated when the radicle was observed.

Complete germination rarely occurs when undertaking seed germination experiments (Scott et al., 1984). Seeds that failed to germinate at the end of the observation period were tested to see if they were viable. Vital staining with 2,3,5-triphenyl-2H-tetrazolium chloride (tetrazolium test) was used in this study and was followed by cutting tests for confirmation. Seeds cleaned with water and soaked in 1% tetrazolium chloride solution for 8 h at 25 °C (ISTA, 2003). Pink embryos were scored as live and total count was expressed as percentage of viable seeds. The tetrazolium test (Baskin and Baskin, 1998) is a rapid biochemical test of seed viability compared to the more slow germination assays (Donald, 1994). The tetrazolium test becomes a dormancy test when it is done on the ungerminated seeds left after a standard germination test or as a separate test alongside the germination test.

2.3. Statistical analysis

Final germination percentages were calculated for each treatment as the cumulative number of germinated seeds with normal radicles out of the total of 20 seeds per replicate. Since the germination percentage data violated the normality assumptions (Shapiro–Wilk and Kolmogorov–Smirnov tests), arcsine transformation was applied before subjecting the data to analysis of variance (ANOVA) using the GLM procedure of the SAS software version 9.1 (SAS Institute Inc., USA). Means were separated using Tukey's studentized range test (HSD).

3. Results

3.1. Germination response

Cumulative germination percentage after 30 days significantly differed with temperature ($F=211.1$, $P<0.001$, $df=6$), light condition ($F=26.8$, $P<0.001$, $df=1$) and their interaction effect ($F=5.8$, $P<0.001$, $df=6$). The highest cumulative germination percentage (100%) was recorded in seeds incubated at a constant temperature of 20 °C, followed by alternating temperatures of 15N/25D, which achieved 87% germination (Fig. 1). The lowest germination percentage (0%) was recorded at constant temperatures of 5 and 10 °C. Across temperature regimes, germination was significantly higher under light than dark conditions (Fig. 2a). The highest germination was observed at 20 °C under both light and dark conditions. However, germination in the dark was slightly higher than in the light under alternating

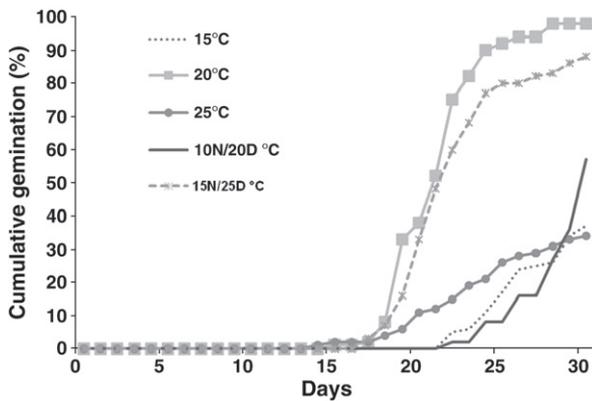


Fig. 1. Cumulative distribution of germination of *Widdringtonia whytei* seeds over the germination period.

temperatures of 10N/20D. The cumulative distribution shows that germination reaches 100% within 25 days at a constant temperature of 20 °C while at the alternating temperatures of 15 N/25D it took almost 30 days to attain 80% germination (Fig. 1). It took about 16 days for seeds to start germinating at

constant temperature of 20 °C and alternating temperatures of 15 N/25D. It took 21 days for seeds to start germinating at constant temperature of 15 °C and alternating temperature 10 N/20D (Fig. 1). Germination remained below 50% either at constant temperatures below or above 20 °C.

3.2. Seed viability

After 30 days of germination assay, seeds that failed to germinate at constant temperatures of 5 and 10 °C both under light and dark conditions showed >90% viability (Fig. 2b). This was followed by seeds that were germinated at 15 °C constant temperature under both light and dark conditions. The lowest overall seed viability was recorded on seeds incubated at 25 °C (68%). Many ungerminated seeds under dark conditions remained viable across temperatures regimes (Fig. 2b).

4. Discussion and conclusions

W. whytei seed germination exhibited a parabolic relation with the optimal temperature being about 20 °C. This agrees well with the mean annual temperature distributional range on Mulanje Mountain. Seeds germinated faster at temperatures between 15 and 25 °C. This behavior is similar to the germination requirements observed in other tropical montane tree species (Xiao et al., 2010). In the field, this temperature range occurs in early December during the rainy season. This suggests that maximum germination potential of *W. whytei* seeds would occur around December. *W. whytei* seed germination was higher at constant than at alternating temperatures. Although seed germination was higher at constant temperatures, alternating temperatures have been found to be more favourable for germination than constant ones (Thompson and Grime, 1983), since seeds are exposed to alternating and not constant temperatures in natural habitats (Baskin and Baskin, 1998).

The results from viability tests revealed that *W. whytei* seeds which did not germinate at low temperatures of 5 or 10 °C were mostly viable. This is in agreement with earlier reports that germination of most tropical species ceases at 10 °C (Khan and Ungar, 1999; Simon et al., 1976). Low temperatures (<15 °C) experienced on the forest floors of Mulanje Mountain during winter (June and July) (Chapman, 1995), could probably restrict seed germination by inducing dormancy. Lack of germination when seeds are exposed to low temperatures also suggests that little or no winter germination would take place in the field, which would consequently reduce the risk of higher seedling mortality later during the dry season. Furthermore, seeds dispersed in winter would partly contribute to the soil seed bank which is dominated by seeds dispersed in the dry season (August to October) (Chanyenga et al., 2011). In contrast, high temperature of 25 °C increased seed mortality suggesting that temperatures higher than 25 °C may prevent germination of *W. whytei* as a result of increased seed mortality.

Although seeds germinated in both light and darkness, more seeds germinated under light than dark conditions. Similar responses were reported for other species (Grime et al., 1981). The ability of seeds to germinate under light and dark conditions

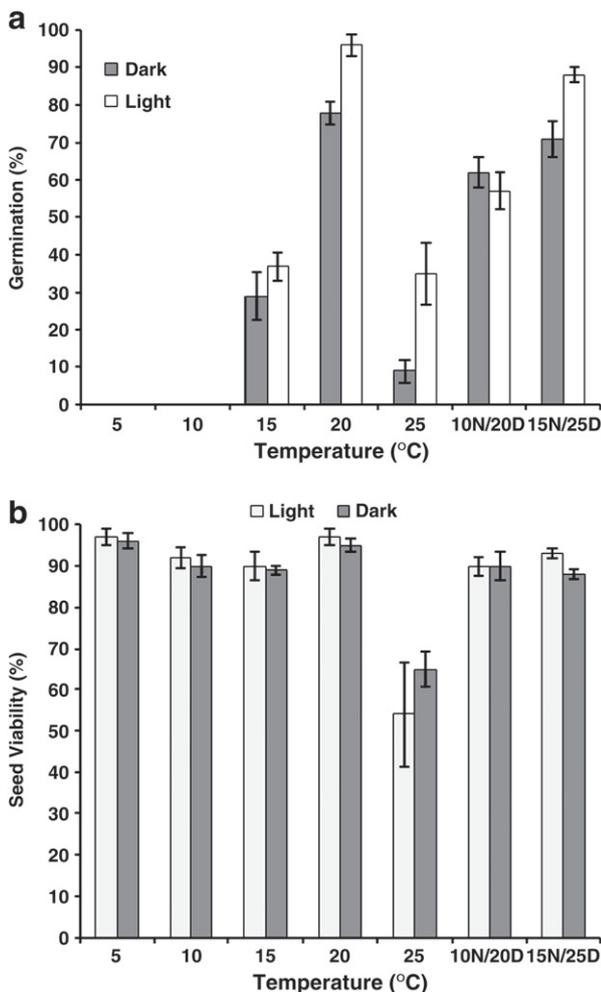


Fig. 2. The effect of different temperature and light conditions on (a) mean germination and (b) viability of *Widdringtonia whytei* seeds after 30 days. Bars represent mean ± standard error.

means that *W. whytei* seeds can germinate either buried or exposed on the soil surface disputing earlier suggestions that *W. whytei* seed requires light to germinate (Chapman, 1995). These characteristics suggest that *W. whytei* cannot form a persistent soil seed bank (Fenner and Thompson, 2005; Pons, 2000). From this study, it can be concluded that temperature is one of the critical factors for germination of *W. whytei* seed and that the seed does not require light to germinate.

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