UNIVERSITY OF SOUTHAMPTON

The Genetic Diversity of the Sheanut tree (*Vitellaria paradoxa*) in the Farming Systems of Northern Ghana

Peter Nicholas Conrad Lovett M.A. (Oxon), M.Sc. (Aberdeen)

A Thesis Submitted for the Degree of Doctor of Philosophy

Faculty of Engineering and Applied Science

Department of Civil and Environmental Engineering

September 2000

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF ENGINEERING AND APPLIED SCIENCE

CIVIL AND ENVIRONMENTAL ENGINEERING

Doctor of Philosophy

THE GENETIC DIVERSITY OF THE SHEANUT TREE (VITELLARIA PARADOXA) IN THE FARMING SYSTEMS OF NORTHERN GHANA

by Peter Nicholas Conrad Lovett

A broad literature review on 'The sheanut tree' (Vitellaria paradoxa Gaertn.) revealed that this is a multi-purpose species highly valued for oil obtained from its seeds and traditionally maintained in the semi-arid agroforestry parklands of sub-Saharan Africa. Despite increasing knowledge of these systems, there is a widely held view that individuals on agricultural land are only relics of natural 'wild' populations. Results show this to be a misleading notion and one that could lead to poor management strategies that aim to improve production and conserve this valuable resource. On agricultural land in Ghana, stands of this species result when local farmers eliminate unwanted woody species during fallow clearance and select specific sheanut trees using criteria based on spacing, health and yield. Characteristics that could affect population dynamics during traditional management and harvesting - short viability seeds and cryptogeal germination - are also discussed with reference to unconscious selection. It is proposed that V. paradoxa has been semi-domesticated following long-term 'natural' and 'unconscious' selection during cycles of traditional fallow and crop cultivation.

The literature review demonstrates that systematic studies on diversity of this species, and methods to undertake them, are scarce and little is known about patterns of phenotypic or genotypic variation. This information is crucial to identifying potential varieties and centres of diversity for tree improvement and conservation programmes. In Ghana, results indicate that all phenotypic characters exhibited a wide range of continuous variation and a high degree of genetic diversity is suggested. Multivariate analysis implied the existence of possible 'varieties' that were not geographically isolated. The latter finding was further supported by high estimates for geneflow from isozyme analysis, confirming previous suggestions that this is a widely outcrossing species. Clinal trends were identified which typically exhibited decreasing size and variation of morphological characters with increasing severity of environmental conditions. The exception to this general rule was the increase of seed width variation to the north east of the study area. Without any other ecological explanation except high predation, it is suggested that this trait is related to integration in traditional agroforestry systems. As the variation was demonstrated to be positively and significantly correlated to the northeast of the study, centres of diversity or origins of incorporation into the farming system, are suggested in this direction.

Since tree improvement is currently constrained, as true to type varieties are difficult to propagate, this study also offers evidence that micropropagation techniques could offer a potential method for capturing genetic diversity of V. paradoxa. Axillary shoot proliferation was induced in apical shoots of seedlings when cultured in vitro with Murashige and Skoog nutrients reduced to half-strength in the presence of a combination of 6-benzyladenine and α-napthaleneacetic acid. Adventitious roots were stimulated when cultured with Murashige and Skoog nutrients reduced to quarter-strength in the presence of indolebutyric acid. Further research on appropriate propagation techniques is recommended to allow 'methodical' selection and continuation of a tree improvement process first started by African farmers thousands of years ago.

List of contents

Chapter 1. Introduction

Chapter 2. Literature review of the Sheanut Tree (*Vitellaria paradoxa*)

Chapter 3. Materials and Methods

Chapter 4. Anthropic influences on vegetation

Chapter 5. Diversity of the Sheanut tree in Ghana

Chapter 6. *In vitro* propagation of *V. paradoxa*

Chapter 7. Conclusions and recommendations

List of tables, figures and plates

Tables

Figures

Plates

Acknowledgements

During the last six years of part-time study I have been fortunate enough to receive the assistance and support of countless people and organisations without whom this study would not have been possible. I apologise in advance if you are not mentioned here and hope you still accept my gratitude.

Firstly I would like to extend my special thanks to my supervisor Dr Nazmul Haq who obtained funding from the Leverhulme Trust, introduced me to the Sheanut tree and gave many hours of guidance and support. I would also like to extend my thanks to those who also helped financially: My wife and parents; Sarum St Michael Educational Charity; Richard Newett's grant and my employers during this period - the University of Southampton and COVOL Uganda.

Secondly I would like to express my sincere gratitude to all the staff of the Cocoa Research Institute of Ghana at Tafo and Bole for their kind co-operation and help during fieldwork. I am also deeply indebted to the many farmers, village elders, staff at the Botany Department, Legon and other Ghanaians for granting me permission to visit their country and study this remarkable agroforestry system. I should also like to thank colleagues at the University of Southampton, especially technical and greenhouse staff. I am also grateful to advice and references from staff at ICRAF and FAO.

I would like to individually thank: Kevin & Bernie Gager, Simon Meadows-Smith, Bill & Sue Farmer, Daniel Abbiw, Dr Jean-Marc Boffa, Dr Peter Evans, Claire Brookes, Eliot Masters, Peter Hasler, Dr Kate Shreckenberg, Dr Dan Adomako, Dr Adam Yidana, Dr GK Owusu, Dr Opoku-Ameyaw, Dr Yaw Adu-Ampomah, Peter Griffee, Barry Lockyer, Dr Frimpong, Dr Esther Goody, Dr John Hall, Zhila Taheri, Dr Azad, Dr Krishna Paudyal, Dr Gugan Lushai, Sue Gerty, Suz Neave, Dr Zewge Teklehaimanot, Yelifari, Dr Nick Glossop, Dr Jon Lovett, Philip Desmond, David Young and the Bole Wura.

A final word of thanks goes to my long-suffering wife Mariama Anfawusi and all of her family in Ghana. Finally I would like to dedicate this thesis to our daughter, Suzanna Ayisha, with the hope she too will have the opportunity to visit the people and places which have made this research on the Sheanut tree such an enjoyable experience.

Abbreviations

BA	–	Basal Area
BAP	–	6-benzylaminopurine
bp	–	years before present
cd	–	Canopy diameter
CRIG	–	Cocoa Research institute of Ghana
dbh	–	Diameter at Breast height
FAO	–	Food and Agricultural Organisation of the United Nations
h	–	Height
IBA	–	indolebutyric acid
ICRAF	–	International Centre for Research in Agroforestry
ITCZ	–	Inter-Tropical Convergence Zone
LfL	–	Leaf lamina length
LfP	–	Petiole
LfW	–	Lamina width
m.a.s.l.	–	metres above sea level
MS	–	Murashige and Skoog medium
NAA	–	α-napthaleneacetic acid
NDVI	–	Normalised Difference Vegetation Index
NTFPs	–	Non-timber forest products
NtL	–	Seed length
NtW1	–	Seed breadth
NtW2	–	Seed depth
Stems	–	Number of stems
V. paradoxa	–	*Vitellaria paradoxa* (either sub-species)
Fruit	–	Whole drupe or berry (including 'pulp' and 'seed')
Pulp	–	Fleshy mesocarp section of the 'fruit' pericarp
Seed	–	Whole 'de-pulped Sheanut' – testa, embryo and food store
Shell	–	Hardened testa surrounding the 'kernel'
Kernel	–	Embryo and food store after removal of testa
Hilum	–	Endocarp attachment scar on 'seed' (including fibrous material)

Chapter 1. Introduction

1.1. General

The sheanut tree, *Vitellaria paradoxa* Gaertn. (Family - Sapotaceae, syn. *Butyrospermum paradoxum*), is a major component of the woody flora of the Sudan and Guinea savannah vegetation zones of sub-Saharan Africa. This species occurs in the semi-arid regions of 18 countries and its range forms an almost unbroken belt approximately 5,000km long by 500km wide from Senegal to Uganda (Bonkoungou, 1987). *V. paradoxa* is an important source of many non-timber products - the nutritious fruits are eaten, the bark is used for traditional medicines and the latex is used for making glue. The wood is used for firewood, charcoal, construction, for furniture and as pounding mortars (Dalziel, 1937; Abbiw, 1990). The most valued product (usually harvested, processed and sold by women) is the shea butter (francophone: le beurre de Karité) extracted from the dried kernels, which is the main oleaginous product in many areas of this species semi-arid range (Lamien et al., 1996). Shea butter is widely utilised for local domestic purposes such as cooking, as a skin moisturiser or as an illuminant. This oil or fat is also commonly utilised commercially worldwide (particularly the stearate fraction) as an ingredient in cosmetic, pharmaceutical and edible products (Abbiw, 1990).

In recent years much attention has been paid to those woody plants termed as 'Cinderella' species which provide economic or environmental benefits through traditional utilisation but have been often been neglected by mainstream domestication (Leakey & Newton, 1994). The focus of this study is just such a species, and due to the importance of its non-wood products, *V. paradoxa* was included on the list of tree species constituting African forest genetic resource priorities for *in situ* conservation at the fourth session of the FAO Panel of experts on Forest Genetic Resources in 1977. Since 1984, recommended action has included botanical and genecological exploration and in 1988, an appeal for provenance trials to be implemented was voiced by the panel's sixth session (FAO, 1977; 1984; 1988). During the last decade there has been renewed interest in this species (Boffa, 1996) and researchers at ICRAF have determined that this is one of the main priority species for tree improvement in the Sahel (Bonkoungou, 1997).

1.2. Constraints to production

With an ever-growing population of increasing affluence, worldwide oil and fat production rose dramatically from 75.6 to 96 million tonnes between 1987 and 1997. World demand for oilseeds continues to increase and new agricultural methods, new varieties and new crops are continually being sought after to meet this demand (Anon, 1997). The potential of *V. paradoxa* to meet some of this demand for vegetable oil is immense and its occurrence in some of the poorest countries of sub-Saharan Africa also offers numerous opportunities for development. Local production methods, however, remain unchanged and the full potential of this valuable resource has yet to be realised despite widespread commercial interest in shea butter throughout the 20th Century (Abbiw, 1990).

It is clear that many environmental and processing constraints affect the supply of this potentially valuable product. Fire during flowering is said to result in low fruit set and high winds, prior to the harvesting season, induce immature fruit drop (Fleury, 1981; Abbiw, 1990; Anon, 1991). Although the majority of shea butter for commercial use is extracted in developed countries under carefully controlled conditions, a common complaint is that many sheanuts are inadequately dried before leaving Africa and contain a high free fatty acid content (J. Bisgaard, pers. comm., 1999). If aflatoxins occur during processing, as suggested by Kershaw (1982), these methods are also of concern to the food industry. Effective low technology extraction methods would also improve the possibility of adding value at source through local production (Niess, 1983).

With regard to current agroforestry requirements, *V. paradoxa* has many undesirable characteristics. Trees take up to 20 years to mature, the seeds are recalcitrant and the heterozygous populations produce a highly variable crop in terms of quantity and quality. Unfortunately, superior 'true-to-type' individuals are currently difficult to maintain as the species has an out-crossing breeding system and potential vegetative propagation methods have only recently been developed (Bonkoungou, 1987; Grolleau, 1989; Lovett et al., 1997).

All recent reports on *V. paradoxa* emphasise the need for tree improvement and knowledge on what genetic resources are available (Sallé et al., 1991; Hall et al., 1996). Research into tree improvement, conservation and shea butter processing, has been undertaken in many countries including Burkina Faso, Ghana, Mali, Nigeria and Uganda. Despite a number of breakthroughs in propagation techniques, *V. paradoxa* is still a challenging species and has even been called "untameable" (Anon, 1991). Many attempts have also been made to improve processing methods, though most 'new' technology is based on mechanised traditional methods. In Uganda it has been possible to use modified oil presses originally developed in West Africa more effectively, probably because the oil from sub species *nilotica* has a lower melting point (Masters & Puga, 1994; Harris, 1998).

1.3. Has *V. paradoxa* already been domesticated?

Major sources of indigenous vegetable oil are rare across the species range and the importance of shea butter can be compared to that of Olive oil or Palm oil in their respective ecological regions. Travellers in West Africa have noted the traditional management of sheanut trees and trade in shea butter since the 14th Century and although it is likely that this species has been utilised for millennia prior to these observations, evidence is still limited. *V. paradoxa* is often described as a major component of the indigenous farming systems and in the 'agroforestry parklands' of West Africa, intercropped stands of up to 50 sheanut trees per hectare are a common sight (Pullan, 1974; Clark, 1980; Harlan, 1992; Kessler, 1992; Boffa, 1995, 1999). Although the best specimens are found on farmed land and evidence suggests that sheanut trees have been managed in traditional systems for at least 1000 years, this species has usually been described as a 'wild' species as opposed to having been domesticated (Baker, 1962; Abbiw, 1990; Hall et al., 1996).

Before discussing this topic further, it is important to clarify what is meant by the term 'domestication', how it can occur and to briefly summarise what is already known about West African agriculture. Many definitions for domestication exist, but that summarised from Harlan (1975, in: Simons, 1997) provides a good overview of the concept:

> Domestication is human-induced change in the genetics of a plant to conform to human desires and agro-ecosystems, culminating in the plant's loss of its ability to survive in natural ecosystems.

These 'human-induced' changes, first described by Charles Darwin in his essays on Animal and Plant domestication, may result from three main types of selection (Heiser, 1988):

> *Methodical selection* is that which guides a man who systematically endeavours to modify a breed according to a predetermined standard. *Unconscious selection* is that which follows from men naturally preserving the most valued and destroying the least valued individuals, without any thought of altering the breed; and undoubtedly this process slowly works great changes. Unconscious selection graduates into methodical, and only extreme cases can be distinctly separated; for he who preserves a useful or perfect animal will generally breed from it with the hope of getting offspring of the same character; but as long as he has not a predetermined purpose to improve the breed, he may be said to be selecting unconsciously. Lastly, we have *Natural selection*, which implies that the individuals best fitted for the complex, and in the course of ages changing conditions to which they are exposed, generally survive and procreate their kind. With domestic productions, natural selection comes to a certain extent into action, independently of, and even in opposition to, the will of man.

Archaeological evidence for the domestication of plants in West Africa has been scarce and dates of ca. 5,000 years bp are usually offered (Blumler, 1996), however, linguistic evidence has suggested dates at least 8,000 years bp (Ehret, 1984). What is certain is that many annual crops, including African Rice, Yam, Sorghum, Bulrush Millet, Finger Millet and Tef, all originated in sub-Saharan Africa, a location that Harlan (1971) refers to as a 'non-center' of agricultural origins due to the huge expanse of this area. It has also been suggested, that following long term associations with man, a number of African tree species are also in an intermediary state of becoming domesticated, but since this is an ongoing and iterative process there is no clear point at which a species can be said to be no longer wild. These include the Baobab (*Adansonia digitata* L.), the White-barked Acacia (*Faidherbia albida* (Del.) A.Chev.), the West African oil palm (*Elaeis guineensis* Jacq.), the Cabbage tree (*Moringa stenopotala* Bak.F. Cuf.) and the Locust bean tree (*Parkia biglobosa* (Jacq.) Don.) (Jahn, 1991; Harlan, 1992; Z. Teklehaimanot, pers. comm., 1999).

The question remains as to what ecological or phenotypic characteristics could be used to determine whether individuals of *V. paradoxa* found on farmland are domesticated to any degree, and how can these 'varieties' be differentiated from any truly 'wild' relatives? One word of caution with this approach is that it assumes that undomesticated relatives actually still exist. It is perfectly possible that a similar situation to that found with *Zea mays* subsp. *mays* (Maize) could be encountered, i.e., many difficulties have been encountered when attempting to identify which species, or sub-species, this obviously domesticated crop evolved from (Iltis, 1983).

V. paradoxa is a light demanding gregarious tree species whose monoecious flower clusters are formed during the dry season when the deciduous trees are usually leafless. Cross-pollination by insects, and the wide distribution of fruit by various animals, including humans, suggests an out-crossing breeding system. *V. paradoxa* also has a lengthy maturation period (up to 10-20 years), an irregular fruiting cycle and recalcitrant seeds. The cryptogeal germination of this species has been described as an adaptation to fire (Jackson, 1968; 1974) but in most reports seen by Hall et al (1996), seeds matured early in the rainy season. The first shoots therefore emerge above ground well before any fire risk. Trees are fire resistant when mature, although both mature and immature trees will coppice easily or produce root suckers when severely burnt or cut. From an ecological perspective these characteristics suggest that *V. paradoxa* is capable of surviving high predation and the vagaries of a harsh environment. Although domestication is not immediately apparent it is important to consider what anthropic influences could have driven this Darwinian process with the result that 'domesticated' varieties are more common in the farming system.

Tradition in Africa dictates that the sheanut tree is rarely planted and most individuals result only from natural regeneration. Tree improvement through '*methodical*' selection, is still a laborious option and has probably not been attempted by local farmers who also describe this species as wild. This does not preclude other types of 'human-induced change' and many reports describe *V. paradoxa* populations where larger 'superior' individuals are common on farmland but regeneration abundant only on fallow land (Abbiw, 1990; Hall et al., 1996). Assuming that the selection of healthy and productive trees in a man-made environment - following the removal of unwanted individuals during cyclical fallow clearance - offers an analogous domestication mechanism to that when seeds of annual crops are reserved for planting the following season. These studies could offer support for the domestication of the sheanut tree following '*unconscious*' or '*natural*' selection in environments controlled by anthropic activities. It is therefore feasible that previous reports have failed to appreciate the potential impact of centuries, or even millennia, of anthropic selection on the adaptation of ecological or phenological characteristics.

The limitation with the majority of reports seen (Brookman-Amissah, 1980; Boffa, 1995; Schreckenberg, 1996; Osei-Amaning, 1996), is that they only compare *V. paradoxa* populations on cultivated land with those on fallow land previously disturbed by anthropic activities and not with 'wild' populations in totally unmanaged areas. Although crucial to the system, fallows are only one component of the farming cycle and studies on *V. paradoxa* in these areas can only demonstrate the potential populations from which new 'farmland recruits' are selected. The lack of small diameter trees in West African parklands has also been given as evidence for the degradation of these systems (Gijsbers et al., 1994). Without any 'undisturbed' areas to compare with, this latter study actually only highlights the fact that it is still not possible to distinguish which parklands are under threat from over-exploitation from those being managed sustainably. It is apparent, however, that the environmental situation with regard to tree cover in Africa may now have reached a critical period (Mann, 1990). It is proposed that inventories of different landuses, with similar environmental conditions, together with studies on traditional practices will enable identification of whether anthropic selection is actually occurring or not. Information from these studies should also improve our ability to design future management strategies of this precious resource and identify additional factors that may influence the genetic diversity of this species.

1.4. Diversity of *V. paradoxa*

Despite the twenty years since FAO experts prioritised *V. paradoxa* and over two hundred years since scientific botanical exploration began in West Africa, only sparse information on the phenotypic and genotypic variation of this species is currently available. There has, however, been much debate on the nomenclature of this mono-specific genus and *V. paradoxa* was finally conserved in favour of *Butyrospermum* with the designation of two sub species, subsp. *paradoxa* from the western areas and subsp. *nilotica* (Kotschy) Henry, Chithra et Nair, comb. nov. from the eastern areas of the species range (Henry et al., 1983; Hall & Hindle, 1995). Prior to this, Chevalier (1943) proposed eight varieties based on leaf and fruit characterisation (var. *mangifolia, parvifolia, cuneata, serotina, poissoni, ferruginea, floccosa* and *nilotica*). Dalziel (1937), Aubréville (1950) and Ruyssen (1957) all described variability, noting trees with distinct leaf, seed and canopy forms.

Following extensive research on herbarium samples and using information from all available monographs, Hall et al (1996) clearly show that this species demonstrates a high degree of variation but as they report:

> Until now, research with Vitellaria has been fragmentary and undertaken only on a local basis, notwithstanding over 80 years awareness of the overall range. Little is known on patterns of phenotypic or genotypic variation beyond comments on tree form and fruit and seed size and shape, and the taxonomic differences between the subspecies. The absence of indications of clinal trends from west to east through the range may reflect no more than lack of organised study although it would be consistent with the uniformity of the West African savanna environment within the Sudanian Regional Centre of Endemism. Provenance trials, extended through the range, would improve understanding of variation and lead to closer attention to genotypes with attractive attributes.

Given the potential for economic development of this species and the suggestions that resource degradation is taking place across the species range, it is imperative that centres of diversity are identified and conservation strategies developed. Furthermore, such studies should be capable of identifying superior 'genotypes' which can be included in tree improvement programs which aim to provide germplasm for incorporation into existing agroforestry systems.

1.5. Aims and objectives

The first aim of this study was to conduct a thorough literature review on the African tree species *Vitellaria paradoxa*. Chapter two details the findings of this broad review, covering many aspects on the taxonomy, ecology, management, utilisation and chemical composition of this species. As summarised above, this species is commonly maintained on farmland in the semi-arid areas of many sub-Saharan countries. Despite increasing knowledge on the traditional management of the agroforestry parklands, there is widely held view that individuals on agricultural land are only relics of natural 'wild' populations. It is proposed that this is a misleading notion, and one that could lead to poor management strategies that aim to improve production and conservation of this valuable resource. Furthermore it has been demonstrated that systematic studies on this species, and methods to undertake them, are scarce and little is known about patterns of phenotypic or genotypic variation. As highlighted above this information is crucial to identifying potential varieties and centres of diversity for incorporation into tree improvement and conservation programmes.

In Ghana, the Cocoa Research Institute of Ghana (CRIG) has been undertaking research into the diversity, management and propagation of this species since the early 1980s. The "Sheanut Tree Improvement Project", which commenced in 1994, was a collaboration between CRIG and the University of Southampton, and aimed to continue this work through investigations into the diversity of this species (using morphological, isozyme and chromosome analysis) and the development of vegetative propagation techniques. This project provided the ideal opportunity to conduct the research for this thesis, whose primary objective is to increase our knowledge on this species by evaluating the potential effects on population dynamics during anthropic selection and the resultant diversity of *V. paradoxa* in the farming systems of northern Ghana. It also was hoped to develop *in vitro* methods for capture of genetic diversity.

Having conducted a thorough review of the literature and identified a suitable research area. Observations were made on some of the many factors, which may have potentially influenced the distribution, and diversity of this species in Ghana. These are described in the second half of this introductory chapter and include geology, climate, soil types and ethnic distribution.

Following an ethnobotanical survey of woody perennials in the study area, the second main aim of this study, using methods detailed in Chapter three, was to use a quantitative inventory to compare *V. paradoxa* populations under different intensities of landuse. This was undertaken on sites in an environmentally homogenous area in the West Gonja District of the Northern Region of Ghana. Since the literature review revealed that little is known about populations of *V. paradoxa* in areas without any history of anthropic activity, it was then hoped that the data from these inventories could be used test the currently accepted hypothesis that farmland populations of *V. paradoxa* are only relics of cleared woodland. Farmland sites with different climatic conditions, along a one-degree latitudinal transect, were also studied to allow comparisons with the primary research area. Using participatory interview methods with local farmers, this investigation further aimed to elucidate the role that anthropic activities may have had on the management, conservation and domestication of *Vitellaria paradoxa* subsp. *paradoxa* in the agroforestry parklands of northern Ghana. It was expected that the results from these surveys, as offered in Chapter four could be used to propose a new hypothesis regarding anthropic selection and domestication of this species as described in Chapter seven.

The third aim of the study was to develop simple, cheap and replicable methods for a systematic assessment of genetic diversity of *V. paradoxa* in Ghana using methods also described in Chapter three. Quantitative tree, leaf and seed size measurements were recorded from random samples of farmland trees at study sites throughout semi-arid regions of Ghana. These were selected at 18 localities with the aim of assessing *V. paradoxa* diversity at a representative range of longitudes and latitudes. Germplasm was collected at all sites and seedlings grown in heated greenhouses in Southampton with the intention of performing laboratory-based analysis of isozymes. A small proportion of all samples were also used to determine the fat content of dried sheanut kernels. Results from this study, as given in Chapter five, aimed to show that identification of superior varieties is possible and that data on phenotypic diversity can illustrate the existence of genetic variation thereby justifying tree improvement programmes. The environmental effect on the tree morphology and phenotypic variation was used to investigate the possibility of clinal trends in the study area and to suggest potential centres of diversity. Hypotheses are offered in Chapter seven which attempt to describe some of the possible cause and implications of these results.

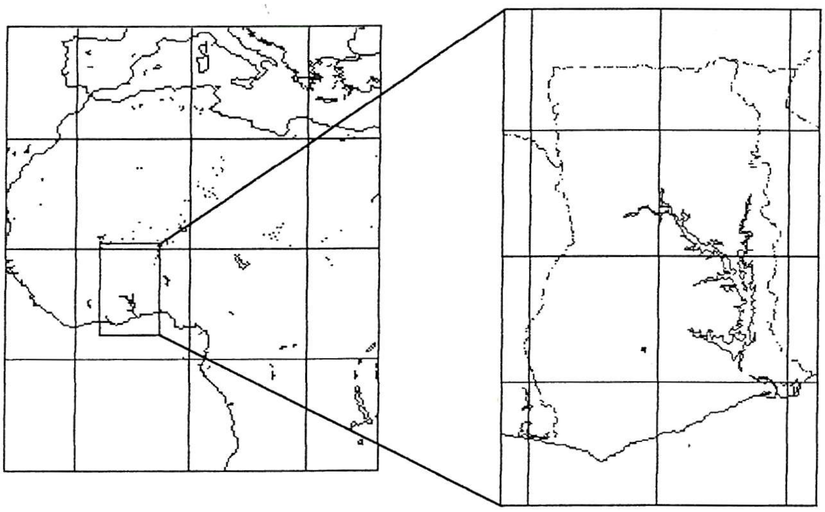
With the aim of developing effective methods of capturing genetic diversity, vegetative propagation work – air-layering, leafy cuttings and *in vitro* regeneration – was also undertaken as part of the "Sheanut Tree Improvement Project". This is the first known report to attempt micropropagation of this species and methods used to develop a protocol for *in vitro* regeneration of *V. paradoxa* seedlings are given in Chapter three. Chapter six describes the successes of this *in vitro* research and Chapter seven includes conclusions and recommendations for future research with regard to the propagation of *V. paradoxa*.

1.6. Background to the Study area

1.6.1. Overview

Ghana is a coastal West African country with approximately the same land area as the British Isles. Bordered by Côte d'Ivoire, Burkina Faso and Togo, it is located between latitude 5 to 11 °N and crossed by the Greenwich meridian (Figure 1.1.). Previously known as the Gold Coast, Ghana was the first African country south of the Sahara to achieve independence from British colonial rule in 1957.

Figure 1.1. Location of Ghana in West Africa



Climatic conditions (temperature, light and rainfall levels) in the southwest third of the country support tropical evergreen vegetation. The remaining land area is semi-arid with savanna woodland as the dominant vegetation type away from cultivated sites. The southern areas are more densely populated with a total country population of about 20 million, an annual growth rate of about 3 %, and a Gross Domestic Product in 1994 of US$25,000 million. The biggest contributions to foreign exchange are generated through exports of gold, cocoa and timber. Many other agricultural exports are also produced (cassava, yams, maize, rice, sorghum, millet, coconut, groundnuts, coffee, tobacco, palm oil and sheanuts) although currently, these only provide minor contributions to export earnings.

1.6.2. Geology

The main geological formations were identified using maps from Wills (1962), and FAO (1968). Further information, particularly on the local geology near Bole, was obtained following discussions with a mineral exploration company, SheaGold Ltd, and access was kindly provided to satellite images and detailed geological maps (Meadows-Smith S., pers. comm., 1997).

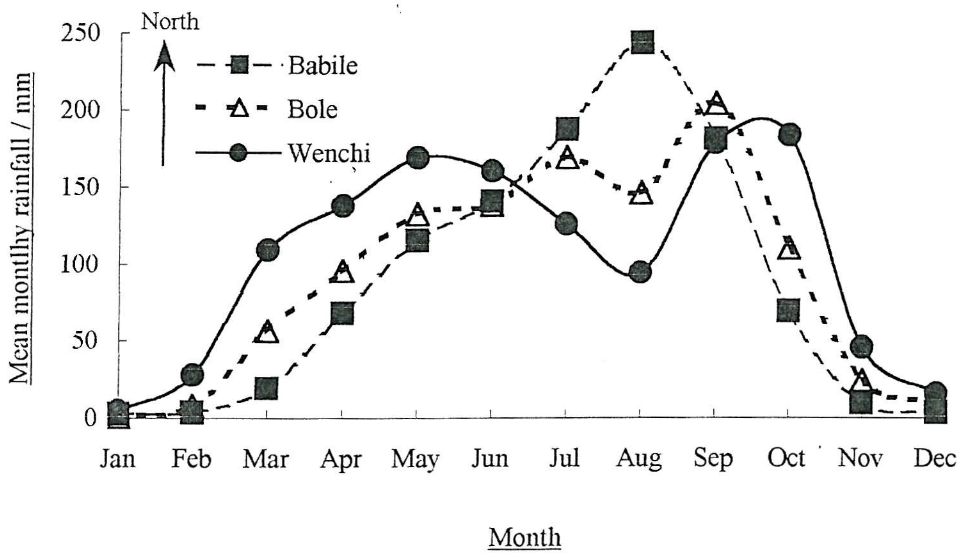
Two main geological formations exist in northern Ghana, those formed in the Pre-Cambrian period (metamorphosed rock such as Granite and Birrimian formations) and those that are of alluvial origins, formed during the Palaeozoic era, (Voltaian sandstones). The latter overlay the older rocks, forming a basin (ca. 100-150 m.a.s.l.) to the southeast, surrounded in a curve to the north and west, with elevated pre-Cambrian formations (ca. 200-300 m.a.s.l). The junction between these two formations, as the granites dip below the Voltaian basin, can be clearly seen in some areas, for example the eroded escarpment at Mole Game Reserve (nr. Damongo) and in the northeast at the Gambaga scarp. Information detailing the properties of these different rock types has been scarce but it is assumed that the impervious granites will drain faster, though with better water retention properties in hollows, and are likely to be mineral rich as compared to the Voltaian formations that have a higher acidity. The Pre-Cambrian uplands are also more likely to influence the climate due to increased elevation and the *massenerbehung* (mass heating) effect of large granite formations (Hall et al., 1996).

1.6.3. Current Climate influences

Copies of many original climatic data forms, for meteorological stations in northern Ghana, were kindly supplied by Meteorological Services Department, Head Office, Legon (Yelifari N.B., pers. comm., 1996). Normalised Difference Vegetation Index (NDVI) dekad images were downloaded (11 year average), from the Africa Data Dissemination Service (http://edcintl.cr.usgs.gov/adds/adds.html). Since NVDI levels are generally recognised as a reliable index of vegetation cover and have also been correlated with rainfall in parts of West Africa (Hess et al., 1996). Africa. These images were used to construct animated 'GIF' images to show the annual movement of rainfall across Africa and Ghana (Please see enclosed 3.5" disc – ghana NVDI.gif)

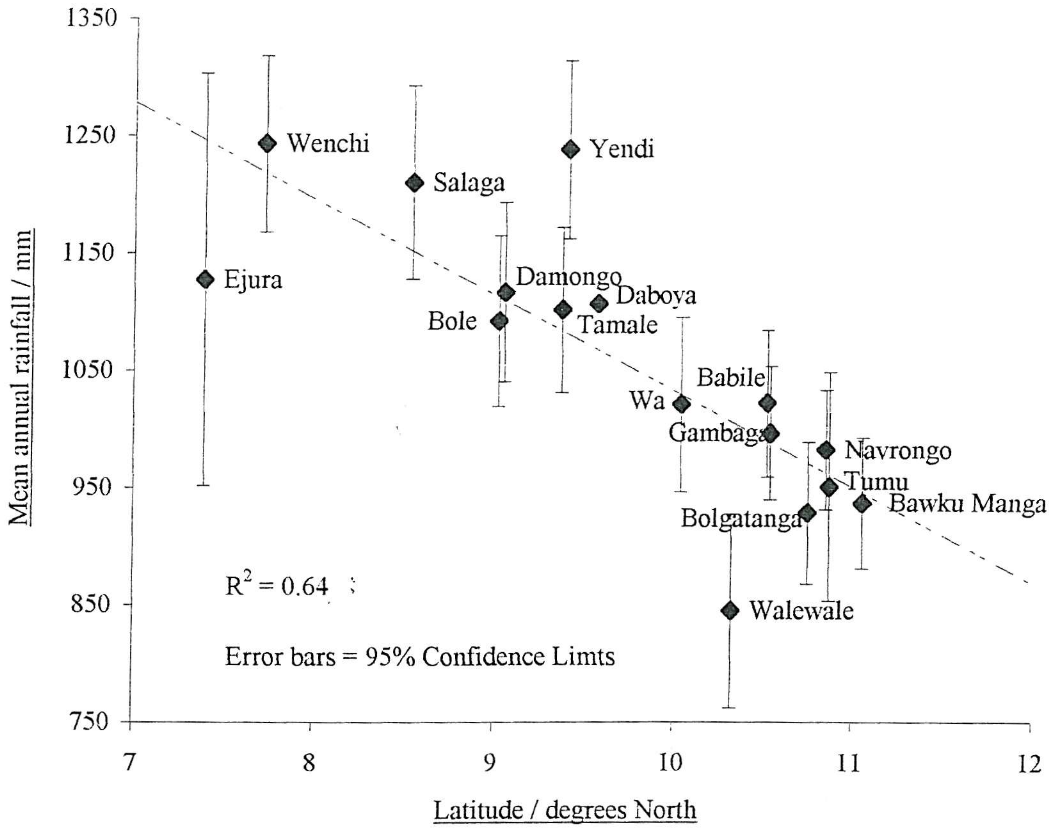
Areas of Ghana, where sheanut trees are common, receive between 800 and 1200 mm of rainfall per annum. The majority falling during a five to seven month wet season (April-September) as the moist airflow of the Inter-Tropical Convergence Zone (ITCZ) moves from the southwest, towards the northeast. This movement of the ITCZ in Africa can be clearly seen using animated NDVI images of Ghana and is also apparent in the rainfall pattern for an area, as the bimodal rainfall in southern parts of the study area clearly becomes mono-modal towards northern sites (Figure 1.2.).

Figure 1.2. Rainfall patterns along a latitudinal gradient in Ghana



During the remainder of the year, winds from the northern Sahara are relatively cold and dry, resulting in severe desiccation of the landscape. It is during this 'harmattan' period that the parched vegetation is commonly burnt to clear land for hunting, farming and to encourage new grass growth. Although intense natural fires do occur, they are uncommon and may occur only every 5-7 years following lightening strikes. The majority of fires are therefore of anthropic origin and are usually set early in the dry season, before *V. paradoxa* flowers. Temperatures range from a monthly average maximum of nearly 40 °C, prior to the rains in March/April, down to a minimum of about 17 °C in January. Humidity levels correlate closely with the ITCZ movement, being highest when cloud cover and the rains are maximal, i.e. at 3pm in Bole humidity was 70 % in July/August, but during the harmattan it dropped to below 30 %.

Figure 1.3. Variation of annual rainfall with latitude, northern Ghana



As Figure 1.3. shows, there is a significant relationship of decreasing rainfall with increasing latitude ($R^2 = 0.64$), and when the 'outliers', Ejura, Yendi and Walewale, were removed from regression analysis, a value of $R^2 = 0.89$ was obtained. Ejura is known to be located in the rain shadow of the hills near Mampong and only a limited number of records for this station exist, as illustrated by the high confidence limits. This ridge of hills (clearly visible from the roadside when travelling from Koforidua to Kumasi) are a continuation of the Voltaian escarpment whose resulting rain shadow may be involved with formation of the dry grasslands of the Afram plains. This latter area also receives less rainfall due to dry climatic conditions resulting from a cold up welling of Atlantic waters in the Bay of Guinea. The so-called 'Dahomey Gap', splits the western and eastern areas of evergreen forest as savanna conditions reach the coast. Yendi probably receives higher rainfall than expected, because of proximity to the highlands in eastern areas where moist forest is common. However, the reasons behind the unexpected low rainfall experienced at Walewale are unclear although proximity to the escarpment at Gambaga may have an effect. As climate can be significantly correlated with latitude, and because the study area had only 16 meteorological stations, latitudinal 'site accurate' figures have been used in place of rainfall when assessing the climatic affect on *V. paradoxa* diversity.

1.6.4. Vegetation zones and past climatic fluctuations

Five main 'natural' vegetation types are described in sub-Saharan West Africa, corresponding to zones of annual precipitation: rain forest (> 1,500 mm), humid savanna (1,600-1,200 mm), tree/shrub savannas (1,500-1000 and 1000-400 mm), densely wooded savanna (1400-900 mm) and arid savannas (<500 mm) (White, 1983; Menaut et al., 1995). Although usually described as distinct latitudinal zones, vegetation composition may well be more influenced by the local affects of fire, than by climatic differences. Fire, and its management by man, has been proposed as a major influence on areas where 'mosaics' of multiple vegetation types exist. Currently accepted mechanisms for formation, however, were determined during the early colonial era and are not always supported by more recent evidence. In northern Guinea (just south of the current range of *V. paradoxa*), research suggests that 'forest islands' surrounded by grasslands, have been formed by generations of deliberate anthropic protection / management and are not forest relics following destruction of extensive forest cover (Fairhead & Leach, 1996).

In addition, there is now increasing evidence, that climate changes have occurred during the last 15,000 years, with corresponding effects on the positions of vegetation types in the study area (Talbot & Johannessen, 1992; Lézine & Vergnaud-Grazzini, 1993; Dupont & Welnelt, 1996; Roberts, 1998; Salzmann & Waller, 1998). Current evidence from these and many other sources, suggest that during the Last Glacial Maximum (LGM), between 15,000 and 9,500 years bp, West Africa was colder and dryer than present with southward movement and possible compression of all vegetation zones. Moist lowland forest species were located in small refugia or along riverine strips, whilst montane and dry woodland Sudanio-Guinean species occupied many areas currently under moist forest. It has been proposed that in some areas the southern limits of the Sahara (as shown by ancient dune formations) may have been up to 400 km further south than today. Following this cold, dry period, pollen studies indicate a rapid expansion of forest during the Holocene 'optimum' as vegetation zones moved northwards. Water level estimates and pollen studies at ancient Saharan lake sites from this period indicate that many woody species (now only found further south) were widespread in areas currently desert. The limits of tropical lowland moist forest are predicted as having expanded northwards by 350 km beyond those found today and the 'Dahomey-gap' has been proposed as being closed as recently as 3,000 years ago. Following this 'optimum' period, pollen and lake level studies imply that the climate became drier and vegetation zones moved south to their current positions.

Since evidence is still scarce and often difficult to decipher, caution is advised and it is still not possible to accurately predict positions of past vegetation zonation. Pollen studies are particularly open to misinterpretation due to the difficulties identifying certain families and separating genera currently found in more than one vegetation type, e.g., pollen from Sapotaceae and Meliaceae is often not identified with certainty although species from these families occur in both humid and semi-arid environments. Widespread woodland clearance for agriculture and metallurgy, during the last few thousand years, may also generate evidence that can be interpreted as a resulting from climatic variation (Schmidt, 1997). What does remain indisputable, is that whatever the cause, positions of the main ecological zones have varied considerably over the last 10,000 years. Animated NDVI images also suggest comparisons with tidal fluctuations, where the annual movement of the ITCZ is analogous to daily tides and larger past fluctuations can be comparable to neap or spring tides. This may have important implications for interpretation of past climates, ecology or biodiversity in the area, particularly for long-lived sessile organisms such as *V. paradoxa*, whose establishment and contraction of populations, will almost certainly lag behind climatic changes.

1.6.5. Soils

Soils found in the study area result from a combination of climatic effects, landuse and the underlying geological formations (Wilson, 1962; FAO, 1968). According to the FAO world soil map (FAO-UNESCO, 1977), most soils in northern Ghana are Ferric or Plinthic Luvisols with occasional areas of Cambisols, Gleysols and Acrisols. From the literature and field observations, soils found in the study area can be visually and broadly classified into four main groups: Brown deep soils found on granites, Pale brown to red shallow soils on granites, Brown deep soils on Voltaian formations, and Pale brown sandy, shallow soils on Voltaian. The shallow soils are often stony or commonly have associated surface lateritic concretions. Exceptions to these generalisations exist, and black or clayey alluvial soils are found, usually near large rivers or in poorly drained areas. Fertility was assessed by observation on vegetation vigour, frequency of fertiliser application and indicator crop species, e.g. *Discorea* spp. are reported to be nutrient demanding and usually planted first in a bush-fallow rotation.

1.6.6. Ethnic groups

The ethnography of Ghana is extremely diverse with estimates of upwards of 150 languages or distinct dialects within the national boundaries (Bureau of Ghana Languages, 1986). Although impossible to list all, the time spent with people from different ethnic groups in northern Ghana has, from a linguistic point of view, been an extremely challenging period. Most of the groups encountered have significant aspects of their cultures related to the utilisation of *V. paradoxa*. These included the Dagaaba, Dagomba, Gonja, Grussi, Kanjaga, Mamprussi, Mo, Nura, Safalba, Sissala, Vagla and the Wala. For Bole, the site at which most time was spent, evidence supports the arrival of a group of male horsemen from the northwest (possibly Mali), in the 16th century. Following inter-marriage with local women, a new ethnic group (the Gonja) was formed that grew in strength over the centuries and consequently occupied large central-northern areas of Ghana. It is highly likely that agriculture was already being practised in this region prior to this arrival date, since the 'indigenous' Vagla and Safalba ethnic groups still have extensive rights over traditional land and tree management, (Goody, 1954; E. Goody, pers. comm., 1996).

Chapter 2. Literature review of the Sheanut tree (*Vitellaria paradoxa*)

2.1. Taxonomy and species description

2.1.1. Nomenclature

Carl Freidrich von Gaertner first described *Vitellaria paradoxa* in 1807 using a single seed. This specimen was probably from West Africa - an area that was being explored during that period - although collection details and foliage were lacking. Using collections from different localities and dates, other names were also assigned during the 19th century including *Lucuma, Bassia, Mimusops* and *Butyrospermum*. Since seed morphology is not used to differentiate the two sub-species, Hepper (1962) suggested that a more complete epitype be used and that the genus name *Butyrospermum* (butter seed) (Kotschy, 1865) should be used in place of *Vitellaria* (yolk of egg). Despite a lively debate, Hepper's proposal did not convince the committee, failing by seven votes to three with two abstentions (Baehni, 1965; Aubréville, 1966; McVaugh, 1967). The use of *B. paradoxum* subsp. *parkii* (G. Don) Hepper was also incorrect as subsp. *paradoxum* should have been used (Baehni, 1965). A petition for reconsidering the proposal was rejected by one vote (McVaugh, 1970) and Henry et al., (1983) provided the currently accepted nomenclature. The use of the original epithet has also been clarified following recent *Code* changes which allow a specimen collected by Mungo Park in 1797 to be designated as the epitype thereby fixing the West African autonym, subsp. *paradoxa* (Hall & Hindle, 1995).

> *Vitellaria paradoxa* C. F. Gaertn., Fruct. 3: 131, t. 205. 1807 ≡ *Lucuma paradoxa* (C. F. Gaertn.) A. DC., Prodr. 8: 173. 1844 ≡ *Butyrospermum paradoxum* (C. F. Gaertn.) Hepper in Taxon 11: 227. 1962. - Holotype: Single seed of unknown origin (P). - Epitype: "Interior of Africa, *Mungo Park* (BM).
>
> subsp. *paradoxa*
>
> *Bassia parkii* G. Don, Gard. Dict. 4: 36, 1838. *Butyrospermum parkii* (G. Don) Kotschy, Sitzungsber. Kaiserl. Akad. Wiss., Math-Naturwiss. Cl., Abt. 1, 50(1): 359, t. 2. 1865. *B. paradoxum* (C. F. Gaertn.) Hepper, Taxon 11: 227. 1962. *B. paradoxum* subsp. *parkii* (G. Don) Hepper, Taxon 11: 227. 1962.
>
> subsp. ***nilotica*** (Kotschy) Henry, Chithra et Nair, comb. nov. 1983
>
> *Butyrospermum niloticum* Kotschy, Sitzungsber. Kaiserl. Akad. Wiss., Math-Naturwiss. Cl., Abt. 1, 50(1): 358. 1865. *B. parkii* subsp. *niloticum* (Kotschy) Hemsley, Kew Bull. 15: 290. 1961. *B. paradoxum* subsp. *niloticum* (Kotschy) Hepper, Taxon 11: 227. 1962.

(Henry et al., 1983; Hall & Hindle, 1995)

Prior to this designation, Chevalier (1943) proposed eight varieties based on leaf and fruit characterisation (var. *mangifolia*, *parvifolia*, *cuneata*, *serotina*, *poissoni*, *ferruginea*, *floccosa* and *nilotica*). Dalziel (1937), Aubréville (1950) and Ruyssen (1957) also described variability, noting trees with distinct leaf, seed and canopy forms. Apart from subsp. *paradoxa*, typical of West Africa, and subsp. *nilotica* typically found in the eastern areas of this species range, no other subspecies or varietal designations are widely used. Pennington (1991) has, however, suggested that herbarium samples from Cameroun (de Wilde 8751) may indicate the existence of another species of *Vitellaria*.

From an indigenous perspective, nearly every ethnographic group has a distinct name for this tree and its products (Dalziel, 1937; Hall et al., 1996). Despite being from the same landmass and vegetation zone, many of these names are remarkably different, probably indicating the long term and independent utilisation of this species across its range. (cf Ehret, 1984). Linguistic diversity has also been reported for the closely associated and widely utilised species, *Parkia biglobosa* (Jacq.) Benth., an indicator also thought to illustrate ancient utilisation (Ouédraogo, 1995).

2.1.2. Status within the plant kingdom

Aside from the confusion on the correct nomenclature of this species, there has also been some uncertainty as to the subdivision of Sapotaceae and the position of this family in the order of Ebenales. This debate, however, is outside of the range of this study and the following taxonomic location of *V. paradoxa* is offered:

Table 2.1. Taxonomic status of *V. paradoxa*

Taxonomic level		Additional members of same level
Order	**Ebenales**	-
Family	**Sapotaceae**	Symplocaceae. Styraceae, Lissocarpaceae, Ebenaceae
Tribe	**Mimusopeae**	Isonandreae, Sideroxyleae, Chrysophylleae, Omphalocarpeae
Sub-tribe	**Mimusopinae**	Manikarinae, Glueminae
Genus	***Vitellaria***	Mimusops, Vitellariopsis, Autranella, Tiegemella, Baillonella
Species	***paradoxa***	currently monospecific
Subspecies	***paradoxa* & *nilotica***	-

(adapted from Pennington, 1991)

2.1.3. Cytology

Miège (1954) reports 2n = 24, describing four main sizes of chromosome (two pairs at 1.9-2.2 μm; four pairs at 1.5-1.8 μm; three pairs at 1.2-1.4 μm; and, three pairs at 1.0-1.1 μm). Pilz (1980) reported that n = 12, from a pollen sample collected in Kwara, 22 km south of Ilorin, Nigeria. This chromosome number was also confirmed (2n = 24) using root tips from actively growing seedlings from Ghana (P. Lovett, unpubl.). It has been suggested that larger chromosome numbers (2n = 28) represent the more primitive number for African Sapotaceae, whereas 2n = 26 and 2n = 24, are secondary (Arends, 1976). Since most members of Sapotaceae occur in humid areas, this may provide evidence in support of the evolution of *V. paradoxa* during adaptation to semi-arid conditions from a more primitive forest based predecessor.

2.1.4. Botanical description

The following description is based on that given by Hutchinson and Dalziel (1963), Flora of West Tropical Africa; Hemsley (1968), Flora of Tropical East Africa; Maydell (1986), Trees and shrubs of the Sahel; and Keay (1989), Trees of Nigeria:

> Small to medium sized deciduous tree of stout bole and much branched spreading crown, 10 - 15 m high, exceptionally to 25 m, sometimes to 6 m in girth; bark dark grey to black, deeply fissured into square or rectangular corky scales; slash pale pink to red, exuding copious white latex (as do other growing parts). Short shoots with conspicuous cycad-like annular leaf-base scars; young shoots, petioles and flower buds with ferrugineous pubescence of varying intensity (less when mature and on West African taxa). Leaves in large, dense fascicles spirally arranged at ends of shoots. Petiole 5 - 15 cm long; leaf-lamina oblong to ovate-oblong 10 - 25 cm long, 4.5 - 14 cm wide. (to 9 cm in West Africa), rounded at apex, base acute to broadly cuneate, margin thickened and undulate; juvenile leaves rust-red and pubescent, later coriaceous, glabrous (to puberulous in East Africa) and dark green, 20 - 30 pairs of lateral nerves, closely spaced, spreading at right angles to slightly arcuate. Flowers white-green-yellowish, in terminal clusters of 30 - 40 at the generally leafless shoot tips, fragrant. Pedicels 1.5 - 3 cm long, puberulous to densely pubescent. Sepals 8 or 10 in two rows, outer sepals lanceolate, 9 - 14 mm long, 3.5 - 6 mm wide, softly tomentose outside, inner sepals slightly smaller. Corolla tube 2.5 - 4 mm long, 4.5 - 7 mm wide, lobes 8 or 10, broadly ovate, glabrous or pilose externally. Stamens 8 or 10. Filaments 7 - 12 mm long; anthers more or less lanceolate, up to 4.5 mm long. Staminodes up to 8 mm, petaloid, elliptical, apiculate, toothed, Style 8 - 15 mm long (usually smaller in West Africa). Ovary 8 - 10 celled, tomentose. Mature fruit ellipsoid, up to 6.5 cm long, 4.5 cm in diameter, style persistent, sub-glabrous or with pubescence persisting in patches; thick butter-like, mucous pericarp, sweet, containing generally 1, occasionally up to 4, oval or round red-brown seed, up to 5 cm long, 3.5 cm in diameter, with a hard bony testa and a shield-shaped scar or hilum nearly as long and as wide as the seed.

A number of other studies and comparisons (Adu-Amopomah et al., 1995; Awoleye, 1995; Hall et al., 1996) also demonstrate that *V. paradoxa* shows high morphological variability throughout sub-Saharan Africa. It is still unclear, however, whether any clinal trends exist or how morphological variation relates to genetic diversity.

Most reports relate to West African collections and due to the wide variability between individuals of both subspecies, it may sometimes be difficult to differentiate between them. The main morphological distinctions between the two subspecies are given in account by Hemsley (1961) based on a description of the eastern taxon.

> *V. paradoxa* subsp. *nilotica*: "...distinguished from subsp. *parkii* [now *paradoxa*] by the dense ferrugineous indumentum on pedicels and outer sepals, the constituent hairs being long and spreading and imparting a woolly appearance to these parts, especially in the bud stages. In addition, the flowers tend to be larger, with style-lengths from 12.0 - 15.0 mm. (cf. 8.0 - 12 mm. in subsp. *paradoxa*). The indumentum is a conspicuous feature of the younger leaves and persists for a considerable time on the midrib and nerves of the lower surfaces of older leaves which contrasts with the glabrous state found in the older leaves of the West African material."

2.1.5. Tree form

Tree architecture of *V. paradoxa* conforms to Aubréville's model (Hallé et al., 1978), described as dominant for Sapotaceae by Pennington (1991). Although a number of different tree forms have been noted: spherical, erect, spindle-, umbrella- or broom. It has also been suggested that tree size or form is associated with certain agronomic characteristics (Ruyseen, 1957; Desmaret, 1958; Hall et al., 1996). In a study of trees from Thiougou (Burkina Faso), however, Boffa (1995) did not find any significant relationship with crown volume and tree yield. Other results from this study also revealed that that the proportion of fruit pulp increases with tree size but no correlation of stem diameter with fresh nut weight existed. Shreckenberg (1996) classified trees sampled at Bassila, Benin, into 'erect' and 'round' types. A small but significant relationship was found between the ratio of crown height to crown diameter (*cd*) and yield. This finding agrees with reports by Sallé et al., (1991) that round trees are better yielders. A significant relationship was also determined from the former study for canopy and stem diameter using a sample of 60 trees in Benin ($cd = 0.197\text{dbh} + 1.24$, $r = 0.848$, $p = 0.001$).

2.2. Ecology

2.2.1. *Ecogeographical distribution*

Vitellaria paradoxa occurs within several ecological zones including north and south Guinean woodland through to Sudanian and Sudano-Sahelian (Dalziel, 1937; Irvine, 1974; Maydell, 1986; Corbett, 1989; Abbiw, 1990; Hall et al., 1996). Across West Africa, *V. paradoxa* is generally found north of the humid forest zone, occurring near coastal areas only where drier conditions result near Senegal or the Dahomey Gap (Ghana to Nigeria). In Uganda, however, subsp. *nilotica* reaches as far south as 1 °N (Hall et al., 1996). The range for best production has been described as ranging from 11 to 14 °N in West Africa (Bonkoungou, 1987) and the tree is found from 16 °W to 34 °E (Sallé et al., 1991). The rainfall in the species range is seasonal, usually dry for 4 to 8 months usually from November to April, between from 600 to 1,500 mm per year (Maydell, 1986). The average temperature in this area is between 24 and 32 °C with a minimum of 21° C and maximum 36° C (Booth & Wickens, 1988).

V. paradoxa grows in an altitudinal range from 100 - 1,600 m with subsp. *nilotica* more likely to be found at higher altitudes in eastern areas. This species has been found to occur on all soil types and geological formations where suitable climatic conditions exist. *V. paradoxa* appears to prefer well-drained fertile soils with good humus cover and stunted trees often occur on stony or shallow soils. It is a light demanding species preferring open sites in plains and on hillsides typically in the upper part of the colluvial phase away from illuvial conditions or hilltops. (Hall et al., 1996).

Most often found as extensive stands on farmland (Maydell, 1986; Abbiw, 1990; Harlan, 1992), *V. paradoxa* is commonly associated with the woody legume *Parkia biglobosa* (Kessler, 1992). Table 2.2. provides a summary of the 'noteworthy associates' given by Hall et al. (1996) showing only those woody species associated with *V. paradoxa* in at least four of the areas defined. This demonstrates the extensive range of associations commonly found and the widespread occurrence of some of the most notable savanna woody species. Using other studies (Dalziel, 1937; Abbiw, 1990; Peters et al., 1992; Shreckenberg, 1996), the common non-timber forest products (NTFPs) utilised from these species were identified. This summary of widespread associations indicates that *V.*

paradoxa is commonly found with other woody species that are also locally valued and maintained, illustrating extensive indigenous knowledge. This affinity for species providing NTFPs, also offers support to the possible anthropic effects on species composition which have been suggested throughout Africa (Pullan, 1974; Lovett, 1993; Fairhead & Leach, 1996). Despite the lack of information from habitats confirmed as not having any history of anthropic activities, *V. paradoxa* has also been stated to occur in natural open or wooded savannah, albeit at lower densities than on the fertile soils commonly farmed, where it is associated with many other tree species found across its range (Fatubarin, 1985; Franklin, 1991; Jachmann & Croes, 1991; Renes, 1991).

Table 2.2. Woody species associated with *Vitellaria paradoxa*

Associated Species	**Occurrence***	**NTFP utilised**
Annona senegalensis	5	Fruit
Anogeissus leiocarpus	6	Gum
Bridelia ferruginea	6	Root (Fruit?**)
Burkea africana	5	Gum
Combretum collinum	4	Gum
Combretum fragrans	4	Gum?
Combretum glutinosum	5	Gum?
Crossopteryx febrifuga	5	-
Daniella oliveri	8	Fruit, seed and gum
Detarium microcarpum	4	Fruit and seed
Grewia venusta	5	Fruit?
Hymenocardia acida	5	Fruit, young shoots
Maytenus senegalensis	5	Root
Parinari curatellifolia	4	Fruit and seed
Parkia biglobosa	4	Fruit and seed
Pericopsis laxiflora	4	-
Piliostigma thonningii	8	Fruit and leaf
Pterocarpus erinaceus	4	-
Sarcocephalus latifolius	4	Fruit
Terminalia avicennioides	5	-
Uapaca togoensis	4	Fruit?

* Only species which occur in at least four out of eight of the areas described (Bight of Benin drainage - farmland; western basins; Bight of Benin drainage; Lower Niger basin; Benue valley basin; Sanaga/Zaire river basin; Tchad basin; Nile basin)

** ? = Related species commonly utilised though actual species not found in available literature.

(adapted from Hall et al., 1996)

2.2.2. *Phenology and reproductive cycle*

V. paradoxa flowers during the dry season, generally between December to April, whilst there are few leaves on the trees (Maydell, 1986). Day length is apparently not critical for flowering (Booth & Wickens, 1988) though this does not preclude this event from being linked to other influences, e.g. fire through temperature or humidity changes in the soil or air. A wide variation has been noted for the flowering phenology of this species as demonstrated by the comparison of reports in Hall et al., (1996) which may well be explained by climatic influences. Intra-locational variation in phenology was also demonstrated in Benin as Schreckenberg (1996) reported that leaf shedding followed by flowering occurred across a broad timescale, with *V. paradoxa* individuals started flowering as early as October and some as late as March. Although the largest proportion of the population were in full flower between December and February during the peak to end of the dry season. This study also revealed that other phenological events showed intra-locational variation and fruiting started as early as March, finished in July with the peak period between April and May in the first half of the rainy season. Reports seen illustrate that this type of phenology is typical of most deciduous savanna or dry forest tree species which are reported to flower over a wide period while leafless in the dry season (Hopkins, 1968; Menaut et al., 1995). Lieberman (1982), however, reports that in Pinkwae dry forest in Ghana, tree species that flowered in the dry season usually produced dry fruit in constrast to *V. paradoxa* which produces fleshy fruit, a characteristic stated as being typical of trees flowering and fruiting in the rainy season.

Wood samples obtained from *V. paradoxa* from the southern Guinea Savanna of Nigeria demonstrated that wood formation continued all year round, showing no periodicity (except in twigs). It has been proposed that this is due to the presence of some vegetation all year round and an adequate supply of auxins to the trunk keeping the cambium active (Amobi, 1973). In contrast, clear growth rings were visible in individuals of *Vitellaria paradoxa* subsp. *nilotica* that had been cut for charcoal production in northern Uganda (Lovett, 1999), suggesting that phenological events may influenced by local climatic conditions or even genetic variability in the species.

V. paradoxa flowers are monoecious, though only limited self-pollination has been reported. It has been suggested that this may be due to the apparent protogynous nature of flowers (Millogo-Rasolodimby, 1989; Yidana, 1991; 1994). Osei-Amaning (1996) demonstrated that at Bole in Ghana, distances (< 2km) between recipient trees did not influence fruit set, and that *V. paradoxa* was not heterostylous as suggested at other localities by Baker (1877) and Chevalier (1948). In West Africa, insects probably play an important role in night/early morning pollination (Bonkoungou, 1987; Sallé et al., 1991). Bees appear to be particularly important as *V. paradoxa* pollen is commonly found in honey in this area (Millogo-Rasolodimby, 1989; Lobreau-Callen et al., 1986). Wind is thought to play a limited role (Bonkoungou, 1987) and it has even been proposed that this is a bat-pollinated species (Lobreau-Callen et al., 1986), though no other observations have lent support to this view. Observations on subsp. *nilotica* in Uganda (Lovett, 1999; Okullo J.B., pers. comm., 2000) also support insect pollination and many species, notably bees, were recorded as visiting the highly perfumed flowers.

The fruits ripen during the rainy season between May and September (Dalziel, 1937; Sallé et al., 1991). Ripe fruit are generally harvested between 100 to 150 days after flowering (Godin & Spensley, 1971; Bonkoungou, 1987; Shreckenberg, 1996), although the fruiting cycle for individual trees has been stated to be very irregular, sometimes early, other times late with quantities varying (Dalziel, 1937; Anon, 1991). One suggestion is that a three-year cycle exists (Bonkoungou, 1987) or that fruiting irregularities are linked with bush fires (Fleury, 1981). The lack of any firm evidence, however, from long-term yield studies on *V. paradoxa* across the species range, still prevents conclusions on whether fruiting cycles exist or not. During an unusual delay in the rainy season in 1998 in Uganda, trees even aborted fruit and flowered a second time producing a crop two to three months later than usual (Masters E., pers. comm., 1999).

When mature, fruits usually fall to the ground, often assisted by high winds (Booth & Wickens, 1988) and are dispersed by Man, elephants, birds, bats, rodents and primates (Bonkoungou, 1987). Bats are said to take the fruit before it has fallen and eat whilst carrying it on the wing. This has led to the suggestion that on being dropped from the height of a flying bat, the testa is can become cracked thereby stimulating germination

(Jackson, 1968). Reports from Ghana do not support this theory, although de-pulping appears to improve germination rates (Osei-Amaning, 1996).

Natural regeneration can therefore occur following survival of sexually propagated seedlings or via asexual replication from root suckers (Dalziel, 1937). The tree also coppices when cut or burnt, often producing vigorous shoots (Osei-Amaning, 1996), although fire, drought and browsing are suggested to limit natural regeneration (Booth & Wickens, 1988). Jackson (1968) and Bokdam (1977) have described the cryptogeal germination and seedling morphology of this species. On absorbing moisture through the hilum, the embryo expands cracking a circle of testa at the micropylar end of the seed allowing the positively geotrophic pseudo-radicle (formed from fused cotyledonary petioles) to elongate and emerge. This rapidly transports the plumule underground before a swelling develops from which the first true shoot emerges to produce the first leaves. It has been suggested that this germination method is a pyrophytic habit, evolved to safeguard newly established seedlings from the common annual fires (Jackson, 1974). The majority of records summarised by Hall et al., (1996), however, seem to contradict this suggestion as fruiting, germination and sprouting all occur during the rainy season, well before fires are a common occurrence. If viable, seed germination readily occurs within two weeks on damp soil, though many seedlings probably succumb to predation, the dry season or accompanying fires (Bonkoungou, 1987). Other accounts, from higher rainfall areas, describe that seedlings are plentiful (Jackson, 1968; Schreckenberg, 1994).

After germination and formation of a long tap root, a seedling can exist as a suffruticose for several years before becoming a normally growing sapling and many seedlings may actually be six or more years old requiring at least a season free of fire before further growth. Without fire, growth can be rapid, as shown by an eight year tree, near Ibadan, Nigeria, which was 5.5 m high and had four flushes of growth a year (Jackson, 1968). The flowering has been reported to commence after a long vegetative phase of 10 - 12 years (usually 15-20 years), although ontogenetic maturity - when viable seed are produced - may not be reached until later as the flowers are supposedly sterile for the first few years (Dalziel, 1937; Hall et al., 1996). It has also been suggested that 30 - 40 years are required before full productivity is reached. Sheanut trees have been said to live for 300 years, although most reports state that trees are productive for 150-200 years (Busson, 1965; Booth & Wickens, 1988; Raison, 1988).

The above mentioned breeding mechanisms, coupled with the longevity of this sessile species, suggest that during the lifetime of any group of parental trees, both paternal and maternal genes could potentially disperse over enormous distances. This evidence has led to the suggestion that *V. paradoxa* is 'outcrossing' (Hall et al., 1996), a mechanism that would potentially allow high genetic diversity to be maintained and increase the chances of species survival in an ecozone subject to frequent environmental perturbations.

2.3. Tree Management and production

2.3.1. *V. paradoxa* as a crop

Many indigenous crops (Table 2.3.) are cultivated in West Africa, a region that Harlan (1971) has referred to as part of a vast sub-Saharan "non-centre" of agricultural origin. It is still unclear, however, for how long farming has actually been practised in the area. Linguistic evidence suggests at least 8,000 years (Ehret, 1984) though archaeological evidence for domesticated plants is scarce and more recent dates, ca. 5,000 years bp, are usually offered (Blumler, 1996). Sorghum and Millet remains from southern Egypt, 8,000 years old, has led Wendorf et al. (1992) to suggest that African agriculture developed independently of the Levantine Wheat and Barley food production systems. To date, however, the oldest archaebotanical evidence seen for West Africa agriculture (Neumann, 1999; Klee & Zach, 1999), is the abrupt and sychronous appearance of fully domesticated *Pennisetum*, at sites in northern Burkina Faso and northeast Nigeria at around 3000 bp (1300 – 1034 cal BC). Leading to suggestions that agriculture was already being practised prior to these dates in the Sahara and only arrived in the Sahel region after drier conditions become established ca. 3300 bp (Neumann, 1999).

In Ghana, the earliest evidence (Stahl, 1986) seen for plant utilisation, dates from ca. 3,500 - 4,000 years bp at a site near Kintampo (Brong-Ahafo). Interestingly this site is currently within the current range of *V. paradoxa* although no plant material described from the archaeological site offers support for the exploitation of this species during that period. Instead the presence of material identified as *Elaeis guineensis* and *Canarium schweinfurthii* suggests that this area may have been slightly wetter than at present.

The earliest convincing evidence (Neumann et al., 1998; Kahlheber, 1999), for sheanut trees being utilised, is from an archaeological site, known as Saouga, dated to ca. 1000 AD in northern Burkina Faso. The occurrence of numerous shell fragments together the presence of *Vitellaria* charcoal at the site, suggests that it is unlikely this material was imported since only shea butter or de-corticated kernels are usually traded. Additional evidence, from the presence of other plant species at this site, also suggests that Saouga received more rainfall than today, with Millet as the basic crop cultivated in agroforestry parklands, similar to those still being cultivated 100 km south of the site.

Table 2.3. Examples of "indigenous" cultivated crops in West Africa

Crop		**Possible zone of origin**
Cereals		
Pennisetum glaucum (L.) R.Br.	Pearl millet	Sudan to Senegal
Sorghum bicolor (L.) Moench.	Sorghum	Sudan to Chad
Oryza glaberrima Steud	African Rice	W. Africa savanna
Digitaria exilis (Kipp.) Stapf	Fonio	Nigeria to Senegal
Legumes		
Voandzeia subterranea (L.) Thouars	Bambara groundnut	W. Africa savanna
Vigna unguiculata (L.) Walp.	Cowpea	W. Africa forest margins
Parkia biglobosa (Jacq.) Benth	Dawa dawa	W. Africa savanna
Root or tuber		
Discorea cayenensis Lam.	Yam	Ivory Coast to Cameroon
D. rotundata Poir.	Yam	Ivory Coast to Cameroon
Plectranthus esculentus N.E. Br.	Kafir potato	W. Africa
Oil Crops		
Vitellaria paradoxa Gaertn C.F.	Sheanut tree	W. Africa savanna
Elaeis guineensis Jacq.	Oil palm	W. Africa forest margins
Telfairia occidentalis Hook. f.	A gourd, oil from seeds	W. Africa
Fruit and Nuts		
Adansonia digitata Linn.	Baobab	African savannas
Blighia sapida Koenig	Akee apple	W. Africa
Vegetables and spices		
Abelmoschus esculentus (L.) Moench	Okra	W. Africa
Solanum spp.	Garden eggs	W. Africa savanna
Piper guineense Schum et Thonn	Seeds	W. Africa forest
Hibiscus sabdariffa Linn.	Leafy vegetable	Savanna
Stimulants		
Cola nitida (Vent.) Schott et Endl.	Cola nuts for caffeine	W. Africa

(adapted from Harlan, 1992)

Although the reports above are the earliest direct evidence seen for the utilisation of *V. paradoxa*, verification of another suggestion by Wicker (1998) might push these dates even further back. He proposes that 'the land of Punt' – a place ancient Egyptians document were trading with prior to ca. 3500 bp – was actually located in Uganda in East Africa. Since the major waterway of the Egyptians (The White Nile) flows through areas in northern Uganda and southern Sudan, where shea oil is currently the major 'indigenous' source of vegetable oil (Masters, 1992; Belknap & Wagner, 2000). Is it then possible that the product from Punt described as 'hknw (sic.) oil', is actually shea oil from *Vitellaria paradoxa* subsp. *nilotica*?

As a documented commodity of trade, shea butter has had a very long history in West Africa, described in Mali by an Arab traveller, Ibn-Batutta, in 1354 (Lewicki, 1974) and by Labat in 1728 (Dalziel, 1937). In 1797, the Scottish explorer Mungo Park noted that the agroforestry cropping system, described below, and the use of shea butter, was in widespread use throughout sub-Saharan West Africa (Park, 1799):

> The growth and preparation of this commodity appears to me one of the first objects of the local people in this and neighbouring states, as it constitutes the main article of inland commerce. The butter serves also for every domestic purpose in which oil would otherwise be used; the demand for it is therefore great.

The sheanut tree has a sacred status where utilised and has frequently been noted as a major component of the indigenous yam, sorghum or millet based farming systems practised in West Africa (Pullan, 1974; Harlan et al., 1976; Clark, 1980; Kessler, 1992). The best and healthiest populations are nearly always found on land associated with past or present anthropic activities, as Dalziel (1937) states:

> Occurring naturally in dry savannah, it [*V. paradoxa*] is subject to the annual grass burning and is thus frequently stunted and twisted : where subject to some care in the proximity to a village, it attains greater dimensions and better form, fruiting early and with superior yield.

Despite the long and well-documented association with man, most observers usually describe stands of *V. paradoxa* as relict populations of 'wild' trees (Baker, 1962; Abbiw, 1990; Hall et al., 1996). Harlan (1992), however, lists *V. paradoxa* as a cultivated crop and a possible candidate for being in an intermediate state of domestication following the intensive protection and harvesting of this crop.

2.3.2. Traditional cropping system

Vitellaria paradoxa is commonly found in the "agroforestry parklands" of semi-arid Africa, (Pullan, 1974; Raison, 1988; Boffa, 1999) defined by Bonkoungou et al., (1994) as:

> land-use systems in which woody perennials are deliberately preserved in association with crops and/or animals in a spatially dispersed arrangement and where there is both ecological and economic interaction between trees and other components of the system.

According to Benneh (1987) the land tenure for this system is established by the person who first cultivates the land by clearing trees and planting crops, the right to use this land also extends to his descendants, however, many differences exist between tribes. It is at this stage that individual trees are selected and maintained on cultivated land, with reduced competition, these trees often develop into larger trees than those found in the natural woodland (Ruyssen, 1957). Sheanut trees, because of their slow fruiting times and high returns, are usually carefully protected where they occur and prosecutions result from felling these valuable trees in most countries (Anon, 1991), individual trees can even be inherited (Dalziel, 1937).

Sheanut trees are found at a density dependant on environmental conditions and on the farming system employed in a particular area, with densities ranging from only a few individuals to over 100 trees ha^{-1} (Ohler, 1985; Agbahunga & Depommier, 1989; Maydell, 1986; Kater et al., 1992; Kessler, 1992; Boffa, 1995; Osei-Amaning, 1996; Shreckenberg, 1996; Hall et al., 1996). Comparative reports on population structure indicate that regeneration is usually abundant on land that has been left fallow. On actively cultivated land, however, the total number of individuals is often lower, particularly in smaller size classes, as tree cover is selectively reduced by local farmers (Boffa, 1995; Schreckenberg, 1996; Osei-Amaning, 1996). Alongside smaller numbers of other useful trees, sheanut trees are inter-cropped with agricultural crops such as sorghum, yams, maize, millet, cassava, legumes, sesame and cotton (Agbahunga & Depommier, 1989; Kater et al., 1992; Kessler, 1992). The same system is known as "bush fallow" in Ghana where the fields are cleared from woodland leaving only trees of economic importance and the fields are left fallow at intervals to let the soil regain fertility (Benneh, 1987). In the Sudanaian zone, Raison (1988) has noted that a similar system exists called the bush swidden-fallow (long fallow) where about 100 trees ha^{-1} are maintained during cultivation, particularly *V. paradoxa*, from untouched woodland of about 1000 trees ha^{-1}. During the next four years the area is farmed and the firewood from the dead trees utilised, the land is then left for twenty years before re-cultivating. Following 2-3 successive cycles of leaving all *V. paradoxa*, the land is too shaded and the village moves leaving the area for fruit collection. After 150-200 years, the original trees are removed, being too elderly for production, and the cycle starts again.

A number of studies demonstrate that although annual crops yields are reduced (up to 50 %) under the canopy of *V. paradoxa*, these 'costs' are often outweighed by 'benefits' produced from this tree species, e.g. shea butter, nutrient cycling and protection from the sun or wind (Kater et al., 1992; Kessler, 1992; Bagnoud et al., 1995; Jonsson et al., 1999). Boffa (1995) recognizes that it is farmer-based management which maintain these productive economically and environmentally sound agroforestry systems. In Thiougou, southern Burkina Faso, a loss of only 3 % of total field sorghum yield was estimated as attributable to tree canopy shading (mean *V. paradoxa* density = 27 trees ha^{-1}).

The most commonly perceived problems to these traditional agroforestry parkland systems, are related to 'modern' farming practices which are purported to reduce the tree density and regenerative capacity of these sustainable systems (Kater et al., 1992). For example the use of mechanised farming techniques require large fields free of trees for optimised production and restrictions on early burning may result in fiercer, more destructive fires later in the year (Grigsby & Force, 1993). It has also been suggested that soil fertility, erosion and natural regeneration are influenced by decreases in fallow length which in turn are blamed on increased population pressure in sub-Sahara Africa (Benneh, 1987).

It is still currently unclear, however, whether these environmental concerns are justified or if they are as widespread as reported. Links with causal factors are just as vague and it is still uncertain as to what degree modern farming techniques, climatic changes or increasing population pressures are to blame (Gijsbers et al., 1994). It is also possible that the felling of unwanted trees, widespread use of fire, prevention of regeneration and periods of shortened fallows are actually part of a carefully orchestrated and long-term indigenous cycle of land management as suggested by Raison (1988). For example, 'forest islands' in the grasslands of northern Guinée have been regarded as remnants of extensive forest cover, destroyed by anthropic activity. Recent evidence, however, suggests that these forest islands are not 'relics', but are the result of generations of traditional agroforestry management which uses protection from fire and tree planting to encourage the spread of forest cover (Fairhead & Leach, 1996). Although this example may be location specific - set in the transition zone between humid forest and woodland savanna - it does highlight the need to carefully evaluate local perceptions and practices.

2.3.3. Silviculture and propagation methods

Traditionally, cultivation of this tree has relied upon natural regeneration, either from germinated seedlings, coppice shoots or root suckers. The usual care is simply to protect the new growth from fire and browsing damage during cultivation as described above. It has been suggested, however, that benefits can be obtained from a one time addition of fertiliser in trenches e.g. for ten trees, 2.5 kg ammonium sulphate, 1.5 kg calcium phosphate and 1.5 kg potassium chloride, is said to be sufficient (Bonkoungou, 1987). The protection given to the young trees, until they can withstand fire, is a long-term investment involving good forward planning because of the 15 to 20 year wait before any returns can be harvested (Fleury, 1981).

To cultivate from seed, fresh sheanuts should be planted at the start of the rainy season. Using this method, 75-94 % success has been obtained by sowing 15 by 20 cm spacing, at a depth of 5 cm. The germination time [assumed to refer to the appearance of the first shoot] was also reduced from c. 40 to c. 20 days by using a stratified bed of sand and organic compost (Delwaulle, 1979; Bonkoungou, 1987). Seedlings should be transplanted at eight months in the second season and may be kept in the nursery for up to four years. At this time it is recommended that seedlings be transplanted to the field, at a spacing of 15 m by 20 m for agroforestry, (Delwaulle, 1979) or at 1.5 to 2.0 m intervals in rows 8 m apart for plantations (Axtell & Fairman, 1992). If sown directly into large bags they may be transplanted after 14 to 24 weeks (Booth & Wickens, 1988). The main problem with cultivation in a nursery is due to the presence of the long taproot, which causes transplanting difficulties. Picasso (1984) reported that a *V. paradoxa* seedling root was 20 cm at six months with an 8 cm shoot and at eighteen months the root was 65 cm with a 16 cm shoot. It has been suggested that slow vegetative growth or late flowering is due to competition for resources because the root is acting as a photosynthetic sink for carbohydrates (Frimpong, pers. comm. 1996)

If planting *in situ*, 3 to 6 seeds are recommended per site due to the often low, 40 to 50 %, germination rate, which is suggested may be due to attack by rodents (Bonkoungou, 1987). Delolme (1947) planted three to five nuts per hole in a 2 m x 5 m spacing, with only 46 % producing shoots by the end of the first season, of which 33 % died after two years. Jackson (1968) proposed that germination rates are improved when the hard shell-like testa is cracked or the hilum is placed on the soil surface to allow for the uptake of moisture. Osei-Amaning (1996), however, found no evidence to support this 'cracking' method. Pritchard et al., (1996) have also confirmed other studies, demonstrating that seeds of this species are recalcitrant with short viability, and recommended that short term storage may be possible at 16 °C. Their study also showed that pre-sprouted seeds were affected by chilling stress at 6 °C and that the optimum temperature for germination was 36 °C with an estimated minimum of 11 °C.

Although relatively little work has been reported on vegetative propagation, the use of cuttings, grafting and air layering techniques have been attempted (Booth & Wickens, 1988). No information was seen regarding the use of *in vitro* culture, except Sallé et al., (1991) stated that it could be used once specimens with commercial potential were been identified. Encouraging results (Bonkoungou, 1987) were obtained when propagation was attempted using cuttings, as swellings and cracks at the base of hormone treated cuttings were observed. Trials at CRIG have confirmed that rooting of cuttings is a potential technique for propagation of this species (Adomako, 1985; Frimpong et al., 1987, 1993). Air-layering, though possible, has proved difficult and successes were only obtained after wrapping in *Sphagnum* moss following treatment with NAA (Picasso, 1984). Positive results were also reported from budding trails in this study. Approach and splice grafting have given encouraging results although desiccating atmospheric conditions and abundant latex were offered as the major constraints to these techniques (Chevalier, 1948; Grolleau, 1989). 25 % success rates were achieved after maintenance in high humidity propagators after washing excess latex from scions (Grolleau, 1989). More recent work in Mali and Burkina Faso has lead higher success rates (>70 %) using splice and bud grafting techniques (Sanou H., pers. comm. 2000; Sibidou S., pers. comm., 2000)

2.3.4. Diseases, pests and natural hazards

The two main fungi, which affect the sheanut tree, are *Fusicladium butyrospermi* Griff. & Maubl. and *Pestalozzia heterospora* Griff. & Maubl. The characteristic symptoms of an infection is widespread red or brown leaf spot (Sallé et al., 1991). The tree is the natural host of *Aphasmatylenchus straturatus*, which has been observed on the roots 40 to 50 cm below the ground, it may also produce chlorosis in nearby legumes (Germani & Luc, 1982). Two species of epiphytic *Ficus* have been observed on sheanut trees that may eventually cause death (Booth & Wickens, 1988). Other parasitic plants found on this tree include *Tapinanthus* spp. (Loranthaceae) which have been studied in depth in Burkina Faso (Sallé et al., 1987; Boussim, 1991; Boussim et al., 1993). Abbiw (1990) also mentions that the semi-parasite *Globimetula braunii* grows on sheanut trees.

Two species of beetle attack the shoots: *Curimosphena senegalensis* Haag. and *Glypsus conspicuus* Westw. Insect defoliants include *Cirina butyrospermi* Vuillet (Saturnidae) and *Anacridiam melanorhodon* Walker. *Ceratitis silvestri* Begzi. damages the ripe fruits whilst *Mussidia nigrivenella* Ragonot and *Nephopteryx orphnanthes* Meyrick. attack the nuts (Sallé et al., 1991). Though these are the main species of insect pest, many others also utilise this tree species but are outside the range of this review. Once fallen, many animals will take the ripe fruit, the main ones being rodents (Bonkoungou, 1987).

The main environmental hazards to *V. paradoxa*, and its fruit yield, include the frequent fires found in this tree's range (Jackson, 1968), localised tornadoes which cause immature fruit to fall as well as damaging the rest of the tree (Dalziel, 1937), and insufficient rainfall (Fleury, 1981).

2.4. Traditional Utilisation

2.4.1. Harvesting and yield

Women and children usually collect the ripe fruit from the ground, early in the rainy season (usually between April and August). Where possible, the harvest is collected as quickly as possible due to rapid germination (believed to spoil oil quality) and vigorous vegetation growth at the start of the rains, soon makes collection difficult (Fleury, 1981; Grigsby & Force, 1993; Schreckenberg, 1996). During a good season, it has been suggested that up to 45 kg of fruit can be collected per person per day, although this is dependent on tree density and yield (Booth & Wickens, 1988). Shreckenberg (1996) stated that a collection basin usually held about 25 kg of fresh nuts or fruit, though loads of 43 and 47 kg were also recorded. Women have the main rights to harvest from land cultivated by their family but as the harvest progresses, longer distances must be covered as fruit near homesteads is quickly collected (Fleury, 1981; Schreckenberg, 1996). Open access collection rights are granted in fallow or woodland areas though women usually prefer to harvest from trees on cleared land stating that there are fewer risks from snakes or scorpions on cleared land and trees produce higher yields because fires are started well before flowering (Grigsby & Force, 1993). Usually only those trees within a days walk are visited, e.g. about seven miles each way (Abbiw, 1990). Schreckenberg (1996) found that routes were planned to maximise visits to uncollected trees and found that a total of 46 trips by one 28 year old woman allowed collection of 1150 kg of fruit (approx. 350 kg dried kernels).

Available reports, for the yield from individual sheanut trees, suggest an average of about 5 to 10 kg dry kernels per year (Table 2.4.), however, this can vary by as much as a factor of 18 from one year to the next (Bonkoungou, 1987). Results from different sized trees and sites suggest that variability could be a result of either tree size, environmental or genetic factors (Boffa, 1995) and although the data below may suggest a decline in yield over time, caution is advised interpreting these results without full data on site and tree characteristics.

Table 2.4. Sheanut production per tree

Citation	Country	Site	Sample size	Years	Seeds (tree^{-1})	Dry kernels (kg tree^{-1})
Delolme (1947)	Burkina	Ferkessedougou	49	1944-45	3268	13.8
Ruyseen (1957)	Burkina	Saria	9	1935-44	3149	-
	Burkina	Ferkessedougou	13	1944-48	3753	15.7
	Burkina	Katibougou	20	1911-15	-	3.7
	Burkina	Ina	25	1949-50	2498	12.2
Desmarest (1958)	Burkina	Niangoloko	217	1954-57	-	4.3
Fleury (1981)	Burkina	Bamako	-	1980	-	10 to 22*
Adomako (1985)	Ghana	Yendi	-	-	-	1.8 to 5
Hyman (1991)	Mali	-	-	-	-	4.5
Boffa (1995)	Burkina	Thiougou	54	1993-94	612	1.7
Schreckenberg (1996)	Benin	Bassila	60	1992-93	1007	5.2
Lovett (1999)	Uganda	Otuke county	21	1996	1848	13.2**

* Conversion factor of fruit to dry nuts = 0.4 (Booth & Wickens, 1988)
** Whole dry seeds

(adapted from Boffa, 1999)

2.4.2. *Traditional processing and utilisation*

Although traditional techniques for production of shea butter vary according to the ethnic group and on resources available, a number of distinct steps are common to the methods used by women in West Africa. The following processing descriptions are therefore summarised from information given by: Fleury, 1981; Korthals-Altes, 1982; Niess, 1988; Abbiw, 1990; Hyman, 1991; Puganosa et al., 1991; Hall et al., 1996; Schreckenberg, 1996 and discussions or observations with local processors in Ghana.

Following collection fruits are de-pulped, either by eating, feeding to livestock (pigs or sheep), squeezing the seed from soft pulp, rubbing in sandy soil or by decomposition following burial in pits. Seeds are then usually boiled, a stage said to de-activate germination enzymes, shrink the kernel to facilitate de-shelling or to prevent fungal growth, before being sun-dried or baking in specially designed kilns. Once the moisture content has been sufficiently reduced the shells are removed by cracking with sticks or pounding in a mortar, and winnowing. The kernels are then thoroughly sun-dried, baked or smoked until moisture content is low enough for storage - FAO (1988b) recommends less than 7 % for safe storage. Seeds are then stored for up to two years in traditional silos or sacks (often with added ash) until required for oil production or selling for export.

Once the sheanut kernels have been dried and separated from the shells, the laborious procedure of oil extraction can continue. The kernels are first roughly crushed before being dry fried until dark brown and slightly oozing oil. If no mechanical grinder is accessible, the coarse granules are pounded in a large wooden mortar into a paste. Following this stage, it is important to keep the paste warm, because shea butter usually solidifies below 32 °C. After two hours of pounding the paste becomes liquid, poured into a pan and briefly put onto the fire before being stone ground until the paste freely oozes oil. Where resources are available, women will walk long distances and pay for mechanical grinding, a process that is usually performed during the cooler hours of dawn to prevent clogging of the machinery. The resultant paste is mixed with water (hot or cold combined to correct temperature) and rapidly hand stirred or beaten for about two hours before the paste 'sleeps' or 'cries', when a white emulsion covers the surface. Since this stage determines the yield of oil extraction, it is important to continue until the maximum emulsion has formed. If difficulties are encountered releasing the oil during this process, previously processed oil or plant extracts are sometimes added to 'seed' the mixture and encourage the oil to separate.

More water is then added and the oil allowed to float to the surface. This is skimmed off and poured into luke warm water prior to decanting. This washing process can then be repeated many times before being heated in a cauldron to evaporate the remaining water, often with added plant extracts to improve quality or flavour. The shea butter is then left to solidify into a homogeneous white, cream or light yellow mass. While still warm and soft, individual amounts for sale can be cut and dropped into cold water to form hard round lumps. Alternatively the shea butter can be wrapped in leaves and stored for long periods without going rancid.

Depending on the process and kernel quality, wide ranges of yields from traditional extraction methods have been reported, from 10 to 34 % of kernel weight (Hall et al., 1996). Extraction rates of 30 to 70 % are usual though high rates of 83 % were obtained by Dagomba women of northern Ghana (Wallace-Bruce, 1995), equivalent to industrial processes which are quoted as achieving 80 % (Fleury, 1981). Many attempts have been made to introduce semi-industrial methods for shea butter extraction, because of the supposedly low yielding and resource intensive traditional processes. Unfortunately due to poor technology, unreliable harvests, poor communication and high start-up costs, many of these projects have not been successful (Fleury, 1981; Anon, 1991; Hyman, 1991; Wallace-Bruce, 1993). Though these processes are often quoted as producing comparable yields and qualities to traditional methods (Hyman, 1991), some buyers of the local products still favour the traditional hand made products and rate it as the highest quality product (Schreckenberg, 1996). Most of these methods attempt to use the same methods as traditional ones but using mechanised stages to reduce the labour, i.e. using motor or animal driven grinders, or fuelwood requirements i.e. with solar power or efficient ovens (Hyman, 1991). Researchers at NRI, in conjunction with Ghanaian institutes, have recently developed a mechanical bridge press which offers a shorter processing time (from 9.5 to 3.25 hrs); reduced water use (from 90 to 1.7 litres); and no need for fuelwood, although extraction rates are unknown (Swetman & Hammond, 1999). Similar presses in Uganda, modified from a Ghanaian design for use with kernels from subsp. *nilotica*, have been reported as achieving a minimum extraction rate of 40 % of available oil, giving an output of ca. 33 kg of oil per 150 kg of kernels - the usual daily throughput of a single press (Harris, 1998).

Locally, shea butter is used for a multitude of purposes, the most important is cooking, being one of the cheapest and most accessible sources of vegetable oil in the semi-arid regions of sub-Saharan West Africa (Abbiw, 1990; Lamien et al., 1996). It is particularly important to those people who do not maintain livestock as a main food source and few sheanut trees are found in areas occupied by traditional pastoralists (Dalziel, 1937). Estimates for consumption have been given as high as 150 g a day for a Mali family of seven (Fleury, 1981), though this may vary according to availability. Dalziel (1937) and Abbiw (1990) also describe other uses of shea butter, which include: being used as a base for medicinal and cosmetic ointments; as a pomade; as a hair cream; for soap; as an illuminant and the by-products of production can be used for a house waterproofing. The oil has even been found effective in protecting cowpeas and groundnuts from infestation by the beetle *Callosobruchus maculatus* F., at doses as low as 0.5mg/kg (Pereira, 1983).

2.4.3. *V. paradoxa as a multi-purpose tree species*

Apart from the fat or oil extracted from the kernels, many other benefits and products of *V. paradoxa* are also commonly utilised. The fruit pulp is eaten by humans and other domestic animals e.g. sheep or pigs, when ripe (Fleury, 1981). The caterpillars of *Cirina butyrospermi* are also eaten and are rich in protein (Dalziel, 1937). The wood is durable, and though commonly not cut, can be used for household utensils, such as furniture, tool handles, pestles and mortars or as building poles (Dalziel, 1937; Abbiw, 1990). As a fuelwood *V. paradoxa* is excellent and can be made into reasonable charcoal (Dalziel, 1937; Abbiw, 1990). Dalziel (1937) also noted that hard wood from *V. paradoxa* has been used in the same way as *Pterocarpus erinaceus* in marine workshops in Sudan. The wood has been described as dull red to deep rich red with a purple tinge, very hard and heavy, resistant to termites, difficult to work but taking a good polish, (720-1280 kg per m^3 air dry) (Eggeling, 1940; Bolaji, 1980; Booth & Wickens, 1988; Keay, 1989). The flowers are an important source of nectar for honey bees and in some localities, hives are commonly kept in or near the trees (Millogo-Rasolodimby, 1989). Ashes from burnt wood of this tree are used as a lye in dyeing and it is possible to make a low grade gutta percha from the sap of the tree (Dalziel, 1937). Despite a significant reduction in beneath canopy crop yields, the long term occurrence of *V. paradoxa* on agricultural land has also been shown to be beneficial to land management by reducing wind speeds, preventing soil erosion, providing shade, increasing soil fertility and improving the microclimate near trees (Kater et al., 1992; Kessler, 1992; Boffa, 1995; Jonsson et al., 1999).

2.5. Commercial Utilisation

As mentioned above, industrial processes can yield up to 80 % of the available fat, however, these methods are currently not known to be used in Africa. The usual method used, involves hot pressing in continuous screw or hydraulic presses and then passing through filter presses (Booth & Wickens, 1988). Solvent extraction using organic solvents such as petroleum ether, n-hexane chloroform or benzene can achieve very high rates of extraction (Kar & Mital, 1981), but are likely to be very expensive commercial methods. Latex in the kernels also causes problems for all methods, particularly those using machinery which are likely to get clogged up (Booth & Wickens, 1988). Increased purity can be obtained by hot filtering and addition of propyl gallate improves preservation (Mital & Dove, 1973).

The commercial uses of shea butter include use as a cocoa butter substitute and in margarine or other fat spreads (Fleury, 1981). Although changes to packaging regulations are under discussion, the former use has been controlled in the EC under European food purity regulations as 'chocolate' products may contain only 5 % (by weight) of other, non-cocoa, fats (Hall et al., 1996). It is probable, however, that Westerners often eat shea butter unknowingly, as it may be present in many edible products where just 'vegetable oil or fat' is listed. Although the larger share of shea butter utilisation is in the food industry, at 97 %, the biggest growing market of shea butter use is in the formulation of base creams both in the pharmaceutical and cosmetic industry (Mital and Dove, 1973; Fleury, 1981; Abbiw, 1990). The majority of shea butter sold on western markets, is processed in developed countries from dry kernels exported from Africa. More recently there have been some attempts to purchase locally processed shea butter for use in skin moisturising products. These include Body Shop, which imports 5 t annually from Ghana (Tawiah, 1994; Jones A., pers. comm., 1996) and COVOL Uganda, which aims to add value at source by assisting local farmers to produce an exportable grade shea butter (Masters & Puga, 1994).

Total annual production of sheanuts has been suggested as high as 500,000 t (Mendez & Lope, 1991) although gross estimations are extremely variable due to inaccurate assessment of local markets and often only export figures are readily available (Hall et al., 1996). A recent newspaper report from Ghana stated that Kassardjan Industries Ltd exported sheanut products valued at over thirty five and a half million US dollars during the 1997/8 season (Anon, 1998). In the previous season (1996/7) forty-two companies were registered in Ghana as exporters of sheanuts, suggesting that world-wide exports are, or potentially could be, economically very significant to the many sub-Saharan African countries in which this species grows. Shea butter, however, as compared to other agricultural crops, remains of minor importance on the world market. It is possible that uncertainties regarding supply (in terms of quantity and quality of the product) are currently limiting the development of new uses, although many other market structure factors may also be involved. These could include international trade affected by high transport costs relative to value or the lack of good markets for by-products of oil extraction (Delabre et al., 1987).

2.6. Biochemical composition and plant analysis

2.6.1. Sheanut fruit, kernel and butter composition

Few reports describe *V. paradoxa* fruit pulp composition (Table 2.5.), despite being classed as a major product of this tree. It is one of the only fresh foods available at the start of the rains and commonly eaten by people or feed to livestock. (Fleury, 1981; Schreckenberg, 1996). The pulp forms 55 % (range 33-80 %) by weight of the whole fruit which are typically between 20-30 g (Ruyssen, 1957; Booth & Wickens, 1988; Adu-Ampomah, 1995). Prior to ripening, the fruit contains quantities of distasteful latex and few animals will ever consume the enclosed seed, which still contains latex even when ripe (Dalziel, 1937).

Table 2.5. Composition of *V. paradoxa* fruit pulp

Content (100 g^{-1})											
mg									**g**		
							Ascorbic acid				
Ca	Mg	P	Fe	Zn	Mn	Cu	Ripe	Unripe	Glucose	Fructose	Sucrose
36.40	26.30	18.00	1.93	0.47	0.24	0.11	196.1	33.0	1.1-2.0	1.1-1.9	0.7-1.7

(Dako, 1974; Eromosele et al., 1991)

Table 2.6. Chemical composition of dried *V. paradoxa* kernels (%)

Fat	**Protein**	**Carbohydrate**	**Unsaponifiable fat***	**Ca**	**P**	**Fe**	**Thiamine**	**Moisture**
31.3-62.0	6.8-9.0	31.0-38.2	2.5-12.0	0.10-0.11	0.04	0.003	0.00006	5.0-8.0

*Expressed as a percentage of total fat content

(adapted from Hall et al., 1996)

Chemical analyses of sheanut kernels are more numerous and have shown a wide degree of variation (Table 2.6.). Dalziel (1937) suggested this may depend on when and where the kernels were collected or how they have been stored. Kershaw & Hardwick (1981, 1986) reported similar variation between individual kernels, stating that oil percentage varied from 19.7 % to 67.3 %, and recommended that large samples be used for testing commercial deliveries. Hyman (1991) offered a typical fat content of 45 to 55 %, but suggested that this may be much lower later in the harvesting season. Dalziel (1937) also reported that kernels contain 9 % protein (no composition details given). Work by Ata and Fejer (1975) stated that only about 50 % of the total nitrogen content was protein and 25 % consisted of low molecular weight peptides. In addition, their study showed that allantoin, and its intermediary products, make up 24-28 % of the total nitrogen in the water extract of de-fatted sheanut kernel meal.

It is apparent that most analyses have been performed on commercial samples with limited knowledge of where samples were collected. The most extensive study which included the location was undertaken in Ghana (Amponsah et al., 1985; Amponsah and Adu-Ampomah, 1988; 1989; Adu-Ampomah et al., 1990; 1995), and although the inter-related affects of morphological and environmental characters were lacking, this study clearly revealed inter-tree and inter-location variation in fat content (Table 2.7.).

Table 2.7. Variation of *V. paradoxa* kernels in Ghana

Region	Location	Mean Fat %	Max	Min	Region mean
Northern	Kahifehiyili	49.9	62.0	31.3	
	Kukuo	52.3	55.6	46.8	
	Samba	52.6	58.2	46.3	
	Yendi	45.0	-	-	
	Zang	45.9	-	-	**49.12**
Upper East	Binaba	42.5	43.0	41.5	
	Bulungu	58.1	60.1	55.0	
	Ghani	52.0	54.0	48.9	
	Koka	54.9	58.8	51.1	
	Kokobla	54.2	56.8	49.9	
	Kpalungu	51.5	53.5	48.0	
	Kusanaba	55.6	-	-	
	Songo	56.9	61.7	52.1	
	Sumburungu	51.0	55.0	45.4	**52.96**
Upper West	Covent (Wa)	52.4	56.6	49.6	
	Fian	46.5	47.5	45.7	
	Funsi	54.4	60.6	40.3	
	Kon	54.1	55.9	51.5	
	Tamapuo	53.6	65.5	43.9	**52.21**
Total		**51.8**	**65.5**	**31.3**	

(Adu-Ampomah et al., 1990; 1995)

Lipids extracted from *V. paradoxa* kernels have a complex fatty acid composition, though the main components are Stearic (C18:0) and Oleic (C18:1) acid (Table 2.8.)

Table 2.8. Composition of total lipid (45g $100g^{-1}$) from Nigerian kernels

Non-polar lipids (96 ±0.3 % of total lipid)			
Triglyceride	86 ±0.9 % (of non-polar lipids)		
		Stearic acid	46 %
		Oleic acid	41 %
		Palmitic acid	4 %
		Linoletic acid	7 %
		Linolenic acid	1 %
Free fatty acid	1 ±0.6 % (of non-polar lipids)		
Diglyceride	3 ±0.8 % (of non-polar lipids)		
Sterol	10 ±0.6 % (of non-polar lipids)		
		Campesterol	11 %
		Stigmasterol	20 %
		ß-sitosterol	68 %
Polar lipids (3 ±0.4 % of total lipid)			
Phosphatidyl choline	50 ±0.7 %		
Phosphatidyl serine	22 ±0.4 %		
Phosphatidyl ethanolamine	10 ±0.5 %		
Unknown	18 ±0.6 %		
		(Main fatty acids:	Oleic 46-50 % Stearic 36-40 %)
Glycolipids (1 ±0.2 % of total lipid)			
Digalactosyldiglyceride		(main sugars:	galactose 32 % glucose 66 %)

(Badifu, 1989)

The majority of records seen (Hall et al., 1996) have been from West African samples which consist of about 40-45 % Stearic and 40-45 % Oleic acid, with the latter usually forming a slightly higher proportion of the total lipid content. The limited number of reports seen for subsp. *nilotica* (Hemsley, 1961; Masters, 1992) suggests local production of an oil with a lower melting point than from the West African sub-species. As shown in Table 2.9., Harris (1998) has offered further support for these suggestions as a direct comparison of fatty acid composition from subsp. *nilotica* (Uganda) and subsp. *paradoxa* (Ghana) revealed some significant differences.

Table 2.9. Fatty acid (%) comparisons for samples from *V. paradoxa* sub-species

Fatty acid	subsp. *nilotica*	subsp. *paradoxa*
Stearic acid	30.7	43.2
Oleic acid	57.4	44.3
Total saturated acids	36.1	48.6
Total mono-unsaturated acids	57.7	44.7
Total poly-unsaturated acids	6.2	6.7
Total unsaturated acids	63.9	51.4
Total oil content	53.7	51.5

(Harris, 1998)

The actual composition of shea butter, as the traditionally extracted lipid is usually known, is very dependent on the extraction method used. Fat output can vary from about 10 % (of dry kernel weight) using traditional processing to 40 % using modern industrial methods (Dalziel, 1937; Fleury, 1981; Kar & Mital, 1981; Korthals Altes, 1982; Niess, 1988; Masters, 1992; Boffa, 1995; Schreckenberg, 1996; Kisakye et al., 1997). Other differences that result from extraction methods are variations in colour, ranging from colourless to bright yellow, and vitamin E (di-α–tocopherol) content, ranging from zero (water extraction using high temperatures) to about 9 mg per 100 g of shea butter (using low temperature solvent extraction). Vitamin E is an important constituent of the butter as it acts as an antioxidant preventing rancidity, as shown by the low peroxidase values in solvent extracted oil (Kar & Mital, 1981). The melting point of shea butter has been determined as 37.8° C. but this can be altered if required, by the addition of paraffin or bees wax and peanut oil (Mital & Dove, 1971; 1973) particularly if for use as a cosmetic/medicinal ointment or cream. Some detailed analysis by Itoh et al., (1974) as shown in Table 2.10., describe the sterol and methylsterol content of commercially prepared crude shea butter. In comparison with other oils tested during this study, shea butter differed from other plants although the compositional pattern was analogous to that found in *Theaceae*.

Table 2.10. Sterol and methylsterol content of commercial crude shea butter

Saponification value:	196.7		
Iodine value (Wij's method):	63.9		
Unsaponificables (p/100 oil)	5.1		
Fractions (p/100 of total unsaponificables)			
1. less polar	18		
2. triterpene alcohols	7		
(p/100)		Cycloartanol	detected
		β-Amyrin	8
		Butyrospermol	26
		Parkeol	detected
		α–Amyrin	46
		Lupeol	16
		24-methylenelanost-9(11)-enol	detected
		Cyclobranol	1
		Others	3
3. 4-methylsterols	2		
(p/100)		Obtusifoliol	22
		Gramisterol	4
		RRT = 1.28	5
		RRT = 1.36	3
		Others (mainly cycloeucanol)	66
4. sterols	5		
(p/100)		Δ^7-Stigmastenol	37
		Δ^{7-}Avenasterol	11
		24-Methylcholest-7-enol	6
		α-spinasterol	43
		unknown	3

(Itoh et al., 1974)

2.6.2. *Other chemical constituents*

An alkaloid was detected in *V. paradoxa* leaves when a moderate precipitate was produced with Picric acid reagent (Adegoke et al., 1968). Dalziel (1937) also describes the use of a poison made from the root of the sheanut tree and tobacco scourings.

Chapter 3. Materials and Methods

3.1. Study sites

With the aim of obtaining viable germplasm from as many representative areas as possible, the main criteria for study site selection were based on the logistics of field study in northern Ghana bearing in mind the recalcitrant nature of *V. paradoxa* seeds:

- The site was within an one to two hours journey by public transport from suitable overnight accommodation from which de-pulped seed samples could either be taken to Accra to send to the UK, or planted in-country within one week
- Stands of productive *V. paradoxa* on farmland were within easy walking distance from the point at which public transport could be obtained
- Sites were well distributed and all of the main ecozones occurring in northern Ghana – Transition, Guinea Savannah (north and south) and Sudan – should be represented
- 1:50,000 maps of Ghana (1966) were available for the site to enable accurate determination of position and altitude

As described below (Section *3.3.1.*) an attempt was made to characterise at least ten trees per site from a total of 24 different sites around 18 different localities in the Brong-Ahafo, Upper West, Upper East and Northern Regions of Ghana (Table 3.1., Figure 3.1.). These were chosen in each of the five main geographic areas using Nandom/Wa (northwest), Bole (west), Wenchi (southwest), Tamale (central) and Bolgatanga (northwest) as study bases. On arrival at each site, meetings were held with local Chiefs, and senior agricultural extension personnel, prior to proceeding with fieldwork in any area. Although this activity was dictated by politeness and local tradition, it also became one of the biggest constraints to sample collection because of time restrictions and the fact that local community members had already harvested most fresh fruit.

Table 3.1. Survey locations in Ghana

Area	Location	Rainfall (mmyr^{-1})	Latitude (N)	Longitude (W)	Altitude (m.a.s.l)	Geology	Soil unit & phase
Central (C)	1. Daboya	Daboya 1106.2	9° 32'	1° 23'	107	P	Lp2-petroferric
	2. Sankpala	Tamale 1101.0	9° 16'	1° 14'	137	P	Lp2-a petroferric
	3. Tolon		9° 25'	1° 04'	152	P	Lp2-a petroferric
East (E)	4. Yendi	Yendi 1237.6	9° 27'	0° 01'	195	P	Lf26-a petroferric
North East (NE)	5. Disiga	Walewale 845.1	10° 01'	0° 50'	150	P	Lf26-a petroferric
	6. Walewale		10° 22'	0° 48'	152	P	Lf1-1a petric
	7. Navrongo	Navrongo 982.1	10° 52'	1° 04'	198	pC	Lf1-1a petric
	8. Polmakom	Bawku 937.0	11° 04'	0° 01'	274	pC	Lp4 petric
North West (NW)	9. Nandom	Babile 1021.3	10° 50'	2° 45'	267	pC	Lf31-a petric
	10. Tumu	Tumu 950.7	10° 53'	1° 59'	305	pC	Lp6-1a petric
	11. Wa	Wa 1020.3	10° 03'	2° 30'	312	pC	Lf31-a petric
South West (SW)	12. Branam	Wenchi 1243.3	7° 56'	2° 05'	200	pC	Lf31-a petric
	13. Subinso		7° 58'	2° 05'	200	P	Lf31-a petric
West (W)	14. Bole	Bole 1091.8	9° 01'	2° 29'	309	pC	Lf31-a petric
	15. Kiape		9° 02'	2° 24'	320	pC	Lf31-a petric
	16. Mandari		9° 03'	2° 35'	274	pC	Lf31-a petric
	17. Bomburi	Damongo 1116.3	8° 46'	2° 00'	152	pC	Lf31-a petric
	18. Damongo		9° 04'	1° 49'	183	P	Lf31-a petric

Annual rainfall calculated as 30-year mean

P Palaeozoic (Voltaian sandstone)

pC pre-Cambrian (Granite or Birrimian formations)

Figure 3.1. Study locations selected for *V. paradoxa* assessment in Ghana

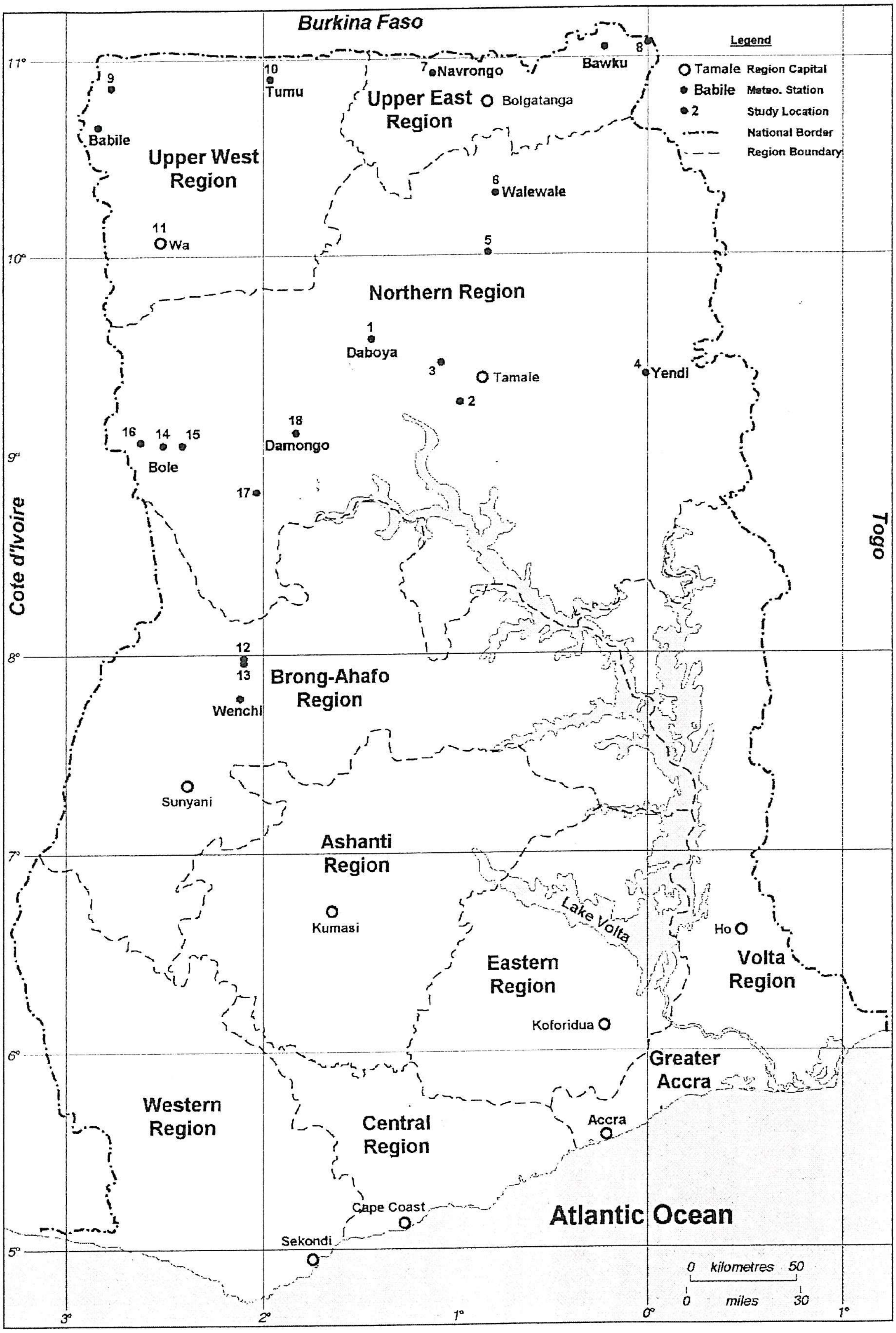
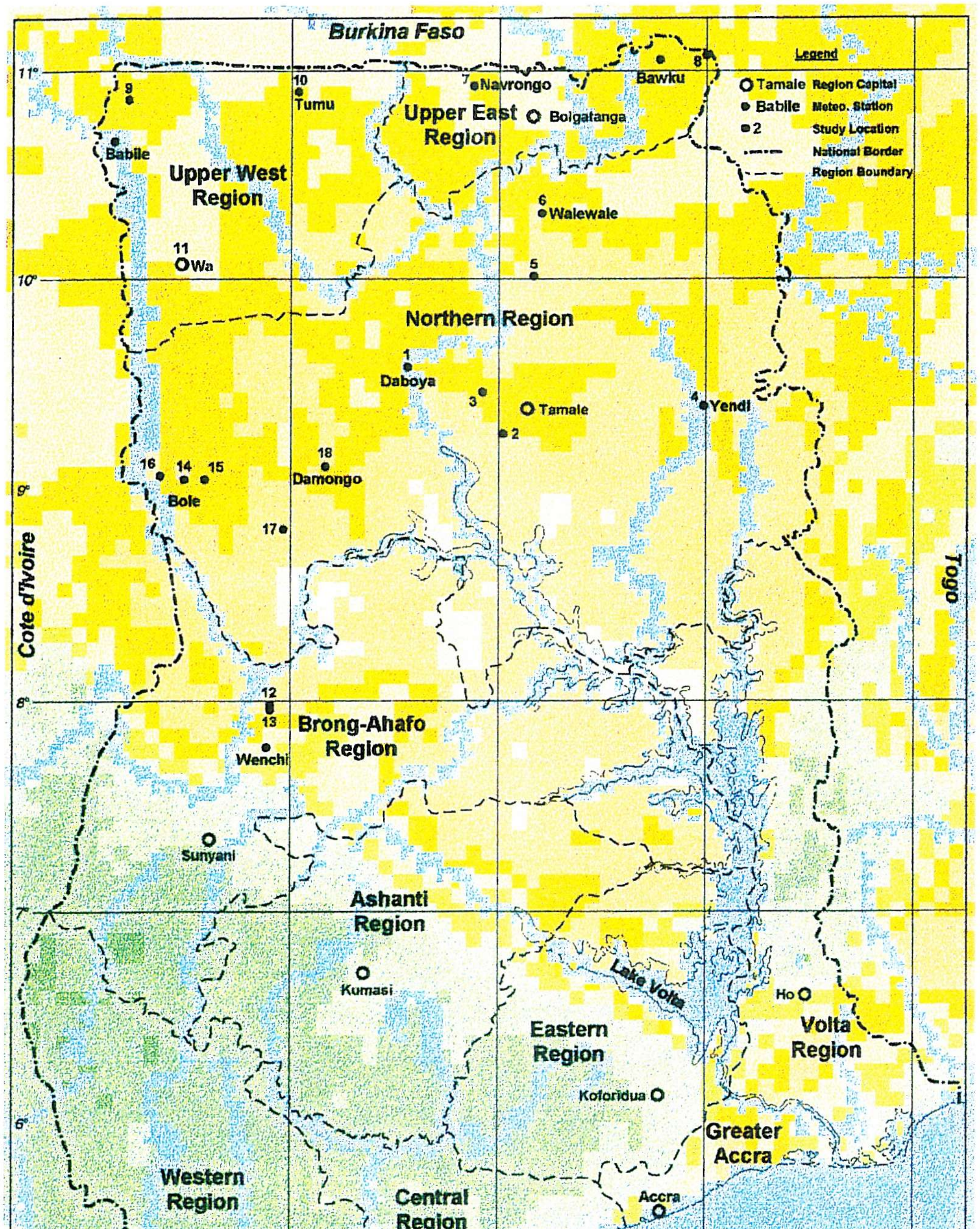


Figure 3.2. Study locations map overlaid January NVDI of Ghana (dekad 2)



Green areas indicate evergreen forest, orange / yellow areas indicate savanna woodland or grassland

Positions only approximate due to combination of different map scales / projections

3.2. Assessment of land and tree management

3.2.1. *Ethnobotanical survey*

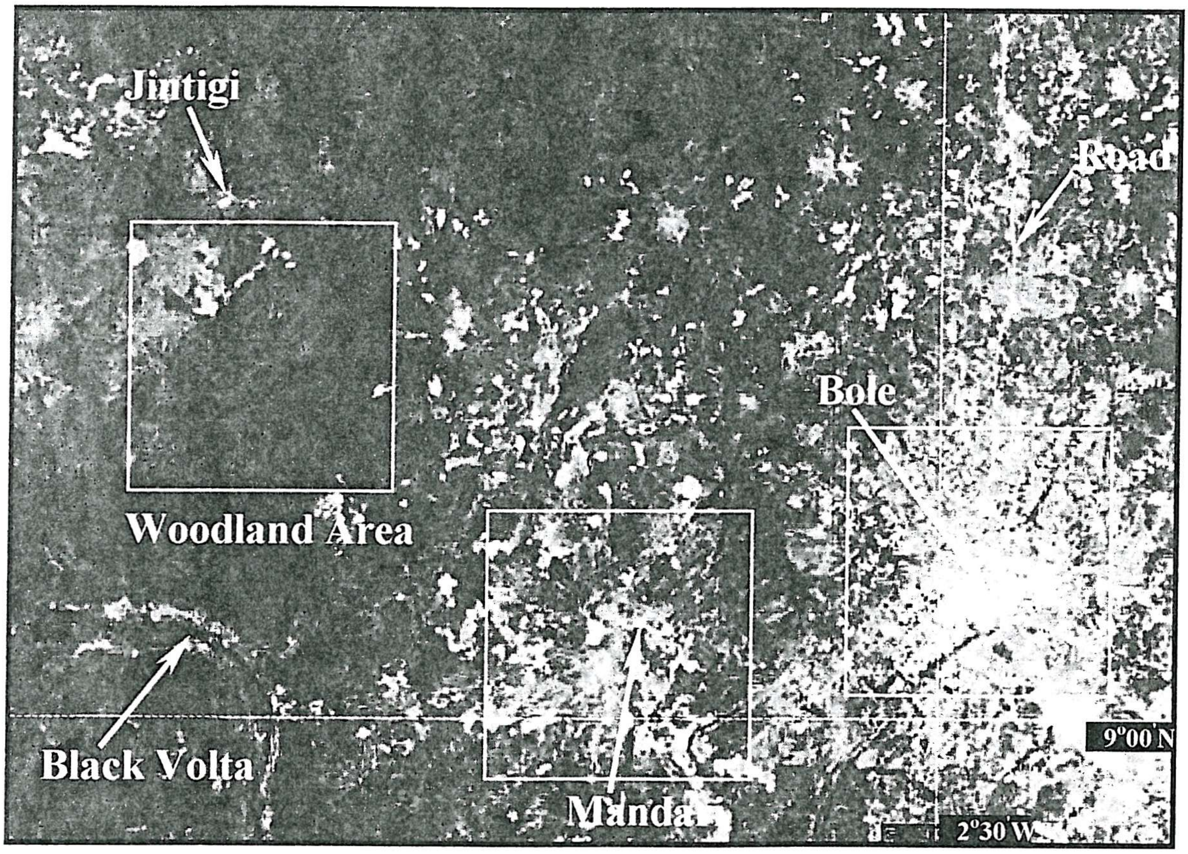
With the aim of assessing species composition and in order to determine which woody species were locally utilised or actively protected. Uniquely numbered herbarium samples were collected from each woody species encountered, dried and taken to the Botany Department herbarium, University of Legon. These were identified with the kind assistance of the senior curator, Daniel Abbiw. Where possible these samples were also used, through participatory discussion with local farmers, to ascertain information on vernacular names, local cultural significance, utilisation and management.

3.2.2. *Woody species inventory*

With the aim of comparing *V. paradoxa* populations in different landuses, a Landsat thematic image (January 1986) was used to select an area encompassing different landuse intensities in the West Gonja District, Northern Region, Ghana (Figure 3.2.). Following discussions with long-term residents, it was established that few changes had occurred in the previous ten years (apart from the introduction of mechanised ploughing and chemical fertilisers near Bole). The intensities of landuse were defined as: low - unmanaged woodland south of the hamlet of Jintigi, medium - scattered farmland around the small village of Mandari and high - intensively farmed land around the more densely populated district capital of Bole. Using soil maps (FAO-UNESCO, 1977; FAO, 1968), 1:50,000 maps (Survey of Ghana, 1966) and information from a local geological survey (Meadows-Smith S., pers. comm., 1997) it was shown that all locations had similar soil types (FAO soil unit Lf31-a, petric phase), altitude (ca. 300 m above sea level) and geology (pre-Cambrian granites). Using data from Meteorological Services Department, Legon, (Yelifari N.B., pers. comm., 1996), climatic similarity was assumed as locations were all less than 25 km from Bole (1092 ± 72.9 mm year^{-1}, 33-21°C).

Square 1 ha plots were surveyed on randomly selected farms, between May and September 1997, after obtaining permission from local landowners on medium intensity farmland (around Mandari village) and on high intensity farmland (near Bole town). To prevent potential confusion with farmed land that had been left fallow and areas of unknown management history, plots were located only on land currently being cultivated. Due to high tree density and transport limitations, smaller 0.25 ha plots were located in the low intensity area (south of Jintigi hamlet, in woodland about twenty miles west of Bole) using random positions on a geological survey grid. With the aim of quantifying long-lived woody biomass, e.g. palms, bamboos, shrubs and trees. All perennial stems inside these plots (≥7cm diameter at breast height, dbh) were measured and identified by species. This definition also prevented any possible confusion regarding different perceptions over 'what is a tree?' (Schulman et al, 1998).

Figure 3.3. Landsat Thematic Image of survey sites in Bole area (18th January 1986)



Lighter areas indicate vegetation clearance (cloud cover minimal)

A one-degree latitudinal transect was performed by repeating this protocol on intensively cultivated land near Wa and Nandom (two sites at each location). Sites were located in areas with similar edaphic conditions along a climatic gradient. No unmanaged woodland was found in the northern areas, as farming was more widespread. Logistics (high fire risk) prevented a similar inventory at the southern location Wenchi.

3.2.3. Participatory survey

A participatory approach was used with the aim of gaining an insight on the farming practices in the area and to identify potential anthropic influences on *V. paradoxa* populations. The methodology used was based on the 'semi-structured interview' technique that had proven to be successful in the Upper West Region (Young, 1997). This method of interviewing has been defined by Petty et al. (1995 in: Young, 1997) as 'guided conversations in which only the topics are predetermined and new questions or insights arise as a result of the discussion and visualised analyses'. Discussions, either with individuals or groups of community members, were held with a total 130 people during 39 interview sessions conducted at villages near Wenchi, Bole, Wa and Nandom in Ghana (Table 3.2.). Since gender has been identified as an important factor with regard to sheanut related activities, women were asked to express their views. Women made comments at 59 % of interviews although they were the sole or main respondents at only 15 %.

Table 3.2. Numbers of people attending participatory interviews

Region	**Location**	**Villages**	**Interviews**	**Men**	**Women**	**Total**
Brong Ahafo	**Wenchi**	3	10	18	17	35
Northern	**Bole**	5	21	41	31	72
Upper West	**Nandom/Wa**	8	8	14	9	23

A number of main areas were addressed that aimed to obtain information on the characteristics of the location, the community members and on the farm and tree management systems employed in the area. These were divided into smaller 'topics' for which it was hoped an answer could be obtained following discussion on that subject (Table 3.3.). The most notable point arising from these interviews was the need to ask the 'right' question. For example asking the question "Do you ever cut sheanut trees?" usually brought the answer "No, it is taboo", however by asking "Which sheanut trees do you keep when clearing the land?" often brought more informative answers such as "We cut the trees smaller than waist height - 'He that is low has no pride' - and keep the healthy mature ones". The following hypothetical question was also asked "What would you do if there were too many mature sheanut trees on your farm", this brought answers such as "I would farm somewhere else" - a negative answer to mature tree cutting - or "If they were in a group I would remove those that are old, unproductive or causing too much shade to my crops" - an affirmative answer to cutting mature trees.

Care was taken to ask questions without suggestion and to crosscheck answers through reliable translators who were asked to give as direct a translation of the question/answer as possible and to always highlight areas where translation problems were encountered. Whenever possible, individual farms were visited and complex topics discussed more fully with visual examples available, e.g. when describing soil types one translator often used the word 'gravel' which was originally thought to describe river-bed gravels but after visiting a number of farms it became clear that respondents were referring to the coarse upland laterites. Since an outsiders preconceived ideas can easily lead to misinterpretation of results, it is recommended that future studies should develop a visually referenced key in advance that should include vernacular names for as many environmental indicators as possible, e.g. soils, geological features, crops, plants, farming practices, etc.

During the initial interviews, particularly around Bole, it also became clear that affiliation to CRIG often led to a misunderstanding on the purpose of the interview especially with reference to cutting of *V. paradoxa*. Provision of accurate answers, however, was later aided by local knowledge and respect gained during two years of field research in the area.

Table 3.3. Questionnaire for participatory survey in northern Ghana

---

Record date & name of interviewer plus any other information of interest

1. Location: region, district, village, describe tree cover, soil type, etc.

2. Personal details: name, sex, age, tribe, time here, job, education, status, etc.

3. Farm details

- How much land did you cultivate this year? Is this more or less than usual?
- How did you decide which land to farm? (Clan, fertility, other plants, etc.?)
- When you started farming, was the land a) already cultivated, b) fallow, or, c) bush?
- How many miles from village/town is your farmland?
- How was the land prepared? i.e. hand clearing, ploughing & fertilising methods.
- What crops are planted, in what sequence & for how many years is the land farmed?
- How long is land usually left fallow (maximum & minimum number of years)?
- Describe land ownership, (right to use/own, can you sell, can someone else cultivate?)
- Describe a recent conflict over a land tenure issue & how it was resolved?

4. General tree details

- Are there trees on the farm? How many of each species, especially sheanut trees?
- What are the reasons for leaving the trees and what effect do they have on the crops?

5. Farm Management of sheanut trees

- Which sheanut trees are kept on the farm (which seedlings, saplings or mature trees)?
- Would you like more or less sheanut trees on your farm, what is the best No. per acre?
- What would you do if too many on your farm, would you remove extra trees?
- What would influence your decision to remove a sheanut tree?
    e.g. age, shade, growth, yield, quality, fruit sweetness, nut size, tree health, etc.
- Do you prune sheanut (or other trees) for any reason?
    e.g. for firewood, remove mistletoe, disease, reduce shade, promote fruiting, etc.
- Do you know if other people (or tribes) cut sheanut trees in the area? For what reason?

6. Identifying superior trees

- Who in your family harvests/processes sheanuts & from where (farm/bush)?
- Where do the healthiest trees grow in terms of location (valley, on hills) & soil types?
- Do you know which are the best trees on your farm? Describe better characters.
- Are best sheanuts reserved for home consumption or higher price charged at market?
- Describe processing stages and effect on final oil yield/quality (plant extracts used?).
- Which trees yield sheanuts that produce better quality or higher yield of oil? Describe.

7. Tree tenure/usufruct

- Have you (or anyone else) ever planted sheanuts or other tree species (wild & exotic)?
- If yes, describe planting method, where planted & how seeds were chosen
- Describe tree ownership for each species - who can harvest, cut for firewood, etc.?
- Describe a recent conflict over tree ownership or use, & how it was resolved?
- What would encourage you to plant more wild tree species especially sheanut?
    e.g. changes to land/tree ownership needed or is improved stock required?

8. Future improvements

- Describe any other personal ideas or thoughts for sheanut tree development.

3.3. *In situ* assessment and germplasm collection of *V. paradoxa*

3.3.1. Morphoagronomic assessment of diversity

With the aim of assessing *in situ* diversity, observations were made on the morphology of sheanut trees maintained in actively farmed fields during the 1994 field season. A large number of characters were seen to show variation: tree height; diameter at breast height; number of stems; leaf and petiole size; fruit size, shape, weight and taste; seed size and colour; canopy size; bark colour and texture; leaf and petiole colour; and flower size. A selection of those characteristics that could easily be collected and quantitatively compared, were then chosen to assess the morphological variation of trees (Table 3.4). The biggest constraint to this work was the need to collect enough viable germplasm for planting, while travelling throughout northern Ghana by public transport. Using market buses, arrival time at a 'site' was usually at about 8 am, followed immediately by one hour of discussion with local elders to explain the reason for the research. In most instances a local guide was then assigned to take us to village *V. paradoxa* trees and it was aimed to assess a minimum of ten trees, which were at least 100 m apart, per site. Walking to each tree, complete characterisation (*in situ* measurements, collection of fruit, de-pulping, etc.) took about 20-30 minutes; therefore a total of ca. 5 hours was usually spent in the field. This left enough time to thank our hosts before taking the evening market bus back to a study base where the fresh leaves and seeds could be measured in the evening. During the 1995 harvest season, between two to three sites were visited each week before returning to Bole or Accra to plant or send viable samples to the UK. Since the earliest harvest started at the southern sites (near Wenchi) in April and the latest harvest finished in late August in the northeast (near Bawku) it was possible to visit all locations during one season of field work with enough time to set vegetative propagation experiments on leafy cuttings *V. paradoxa* at the CRIG sub-station at Bole. Results are presented from a total of 294 randomly selected productive trees that were characterised on farm land sites (Table 3.5.) and a limited assessment was also made on leaf morphology for trees in unmanaged woodland, though after extensive exploration, only one tree was found with fruit. With the aim of assessing the accuracy of site means, trees were characterised at two or more sites along the 'latitudinal transect (Wenchi, Bole, Wa and Nandom). Sites with less than 5 trees characterised per site have not been included in any analysis.

Table 2.4. Methods and abbreviations used during morphological assessment

Characteristic	**Abbreviation**	**Method used**	**Sample size per tree**
Diameter at Breast height	dbh	Dbh tape to nearest whole centimetre	Each tree stem ≥ 7cm at breast height (1.3m above root collar)
Basal Area	BA	BA (m^2) = $\pi(dbh/200)^2$	Each tree
Height	h	'Stick method' to nearest *half metre*	Each trees highest point
Canopy diameter	cd	30m tape to nearest tenth of a metre	Each trees north-south and west-north canopy
Number of stems	Stems	Count	Number stems ≥ 7cm dbh per tree
a) Leaf lamina length	LfL	30cm ruler to nearest millimetre	Twenty (20) random fresh leaves per tree taken from most accessible south-facing branch.
b) Petiole	LfP		
c) Lamina width	LfW		
a) Seed length	NtL	Veneer callipers to nearest tenth of a millimetre	Twenty (20) freshly de-pulped seeds per tree randomly selected from total sample
b) Seed breadth	NtW1		
c) Seed depth	NtW2		

Table 2.5. Trees sampled at each site

Location	**Site No.**	**Trees Sampled**
Bole	Bol1	12
	Bol2	18
	Bol3	40
	Bol4	24
Bomburi	Bom1	11
Branam	Bra1	10
	Bra2	10
Daboya	Dab1	10
Damongo	Dam1	10
Disiga	Dis1	10
Kiape	Kia1	5
Mandari	Man1	10
Nandom	Nan1	12
	Nan2	12
Navrongo	Nav1	10
Polmakom	Pol1	10
Sankpala	San1	10
Subinso	Sub1	10
Tolon	Tol1	10
Tumu	Tum1	5
Wa	Wa1	11
	Wa2	12
Walewale	Wal1	12
Yendi	Yen1	10
18 locations	**24 sites**	**294 trees**

5.3.2. Germplasm collection and maintenance

With the aim of growing *V. paradoxa* seedlings for progeny trials by CRIG, to produce young leaves for isozyme analysis and explants for *in vitro* propagation studies, a total of 40-60 ripe fruit, were collected from each tree, de-pulped, stored in cloth bags and sent to CRIG research stations or Southampton University, for planting. In the UK, fresh seeds were surface planted, hilum side down, in trays containing a 5 cm layer of a 50:50 mix of sharp sand and 'Levington's No1®' compost, irrigating every 3 days. These were maintained in sunny positions on a 16 hr day, supplemented with mercury vapour lamps, in glasshouses at 15/27 ± 2 °C (night/day). On germination (when the pseudo-radicle emerged), the seeds were transferred to 17.5 cm diameter pots containing the same soil mix and the true shoots observed after a further 4-6 weeks.

5.4. Fat analysis

The fat content of each tree was analysed with the aim of determining morphological diversity in this character, to identify any high fat-yielding varieties and to ascertain whether variation in other morphological characters was correlated with fat content. Fresh *V. paradoxa* seeds were weighed on arrival at Southampton and those not planted were immediately placed in desiccation ovens at 60 °C. After three weeks, the samples were re-weighed before all loose material and shells were removed from four kernels per sample. The dry kernels from these nuts were re-weighed and thoroughly ground using a coffee grinder. Samples stored for long periods were kept in sealed containers at room temperature and re-desiccated for two weeks with silica gel immediately before re-weighing and extraction. A modification of the method used by Kershaw and Hardwick (1981) enabled determination of fat content of dried samples after washing with 60:80 petroleum ether for 6 hours in a Soxhlet extractor. Duplicate samples (four kernels combined from each tree per sample), from which fat had been extracted, were desiccated for five days at 60 °C and re-weighed. The total fat content was calculated as the weight difference resulting from extraction and expressed as a percentage of unextracted desiccated kernel weight. As the fat content of duplicate samples never differed by more than 0.5 %, results are given as a mean fat percentage per tree.

5.5. Isozyme analysis

An isozyme analysis protocol was developed for use with leaves from *V. paradoxa* seedlings in order to determine seedling genotype to calculate heterozygosity, estimates for geneflow and genetic distance between populations. The first stage in developing this protocol was to determine a means to produce a thoroughly macerated sample without reducing enzyme activity. Due to the recalcitrant nature of *V. paradoxa* seeds, relatively few seedlings from each site were established in the UK and in order to maximise the survival chances, it was decided that sections of new leaves would be taken as samples instead of other possible material, e.g. roots or germinating embryos. Unfortunately, even the very young leaves of *V. paradoxa* taken from three-month-old seedlings, were found to be very resistant to grinding when fresh and it was found that the only effective way to macerate was to freeze with liquid nitrogen prior to grinding in a pre-chilled mortar with a few grains of quartz sand. To prevent de-activation of enzymes by poly-phenolics (Loomis, 1974; Gegenheimer, 1990), 30 mg of polyclar insoluble polyvenylpyrvolidone (PVP) was also added for every 100 mg of young leaf used.

Before grinding was complete, a pre-chilled extraction buffer was added. Since initial attempts at extraction using simple buffers had led to very low levels of enzyme activity. The best buffer (at pH 7.5) was determined empirically following advice given in the literature (Kelley & Adams, 1977; Soltis et al., 1983; Wendel & Weeden, 1989; Kephart, 1990; Liengsiri et al., 1990). This was achieved using a wide range (Table 3.6.) of extraction buffers added at a ratio of 1 : 5 (sample weight : buffer volume), i.e. 500 μl was added if 100 mg of leaf was used). Results showed that mercaptoethanol together with PVP-40 were always required to achieve any reasonable levels of isozyme extraction and the addition of BSA (0.1 – 1 %) improved resolution but was also thought to interfere with gel running due to overloading with protein and was therefore used at only 0.2 %. The addition of either DIECA or ascorbic acid apparently made little or no difference to isozyme extraction and bands were weaker with a small reduction in yellow background staining for Esterase. The 'final' extraction buffer (prepared using Sodium phosphate instead of Tris-HCl due to chemical availability) was found to be the most effective except that Alcohol Dehydrogenase bands were notably weaker than with other buffers and reasonable staining was only obtained after staining overnight at 4^0C.

Table 3.6. Buffers used for empirical determination extraction of isozymes

Buffer No.	Constituents	Concentration
1.	Tris-HCl buffer pH 7.5	100mM
	Sucrose (w/v)	7%
	Mercaptoethanol	140mM
2.	Tris-HCl buffer pH 7.5	100mM
	Sucrose (w/v)	7%
	Mercaptoethanol	140mM
	PVP 40 (w/v)	5%
3.	Tris-HCl buffer pH 7.5	100mM
	Sucrose (w/v)	7%
	PVP 40 (w/v)	5%
4.	Tris-HCl buffer pH 7.5	100mM
	Sucrose (w/v)	7%
	Mercaptoethanol	14mM
	PVP 40 (w/v)	5%
	Bovine serum albumin (BSA)	0.1%
5.	Tris-HCl buffer pH 7.5	100mM
	Sucrose (w/v)	7%
	Mercaptoethanol	14mM
	PVP 40 (w/v)	5%
	Bovine serum albumin (BSA)	0.1%
	Ascorbic acid, Na-salt	100mM
	Diethyldithiocarbamate (DIECA)	20mM
6.	Tris-HCl buffer pH 7.5	100mM
	Sucrose (w/v)	7%
	Mercaptoethanol	140mM
	PVP 40 (w/v)	5%
	Bovine serum albumin (BSA)	0.1%
7.	Tris-HCl buffer pH 7.5	100mM
	Sucrose (w/v)	7%
	Mercaptoethanol	140mM
	PVP 40 (w/v)	5%
	Bovine serum albumin (BSA)	1%
8.	Tris-HCl buffer pH 7.5	100mM
	Sucrose (w/v)	7%
	Mercaptoethanol	14mM
	PVP 40 (w/v)	5%
	Bovine serum albumin (BSA)	1%
	Diethyldithiocarbamate (DIECA)	20mM
Final	Na-phosphate buffer pH 7.5	200mM
	Sucrose (w/v)	7%
	PVP 40 (w/v)	7%
	Mercaptoethanol	140mM
	Bovine serum albumin (BSA)	0.2%

After extraction all samples were removed from the mortar with a chilled spatula, placed in eppendorfs, maintained on ice and then centrifuged at 3,000 rpm for 10 min. Polyacrylamide (10 %) gels were used, instead of starch gels, for electrophoresis of the supernatant due to previous experience obtaining high resolution banding of proteins and DNA samples in other laboratories. 15 µl of supernatant per gel was pipetted into each pre-formed wells with 15 µl loading buffer (20% sucrose, 0.05% w/v bromo blue). The gel electrophoresis apparatus was kept on ice and run at 50mA constant current for about 3.5 hours. Gel buffer (0.019 M boric acid, 0.004 M lithium hydroxide, 0.047 M tris, 0.007 M citric acid, pH 8.3), electrode buffer (0.192 M boric acid, lithium hydroxide 0.038 M, pH 8.3) were determined empirically from the literature mentioned above. The following enzyme staining buffers were prepared with minor modifications, according to Wendel and Weeden (1989) and used to produce visible zymograms:

Esterase (EST). E.C. 3.1.1.-

100mM Na-phosphate buffer (pH 6.0)	50ml
α-Napthyl acetate	25mg
β-Napthyl acetate	25mg
fast garnet	50mg

Substrates were dissolved in acetone (25mg/ml) before combining with other chemicals, poured onto gel and incubated (shaken at room temperature) until red-brown bands appeared. The gel was then rinsed and fixed in 50% (v/v) glycerol.

Acid phosphatase (ACP) I.U.B.: Orthophosphoric-monoester phoshophydrolase. E.C. 3.1.3.2

50mM Na-acetate buffer, pH 5.0	50ml
Na-α-napthyl acid phosphate	50mg
$MgCl_2$	50mg
Fast Garnet GBG salt	50mg

Dissolve reagents in buffer and pour onto gel. Incubate in the dark until the required staining intensity has occurred. Rinse and store in water or fix.

Glutamate oxaloacetate transaminase (GOT) = Aspartate amino transferase (AAT). I.U.B.: L-Aspartate:2-oxoglutarate aminotransferase. E.C. 2.6.1.1

AAT substrate solution	50ml
Fast Blue BB salt	50mg (1ml)

AAT substrate solution (pH 7.4)

H_2O	800ml
α-ketoglutaric acid	292mg
L-aspartic acid	1.07g
PVP-40	4.00g
EDTA, Na_2 salt	400mg
Sodium phosphate, dibasic	11.36g

Add fast blue to substrate solution and incubate gel at room temperature in the dark, until blue bands appear. Rinse and fix. Substrate solution can be refrigerated for up to 3 weeks with no loss activity.

Alcohol dehydrogenase (ADH). I.U.B.: Alcohol; NAD+ oxidoreducterase. E.C. 1.1.1.1

50mM Tris-HCl, pH 8.0	50ml
NAD	10mg
Ethanol	200µl
MTT	10mg
PMS	2mg

Combine ingredients and pour over gel. Incubate until bands are optimally developed. Rinse and fix in 50% glycerol.

Glucose-6-phosphate isomerase (GPI) [=Phoshoglucoisomerase (PGI)]. I.U.B.: D-Glucose-6-phosphate ketol-isomerase. E.C. 5.3.1.9

50mM Tris HCl, pH 8.0	50ml
NAD	10mg
Fructose-6-phosphate, Na_2-salt	20mg
Glucose-6-phosphate dehydrogenase	20 units
MTT	10mg
PMS	2mg

Combine ingredients and pour over gel. Incubate until blue bands optimally developed. Rinse and fix.

Malate dehydrogenase (MDH). I.U.B.: (S)-Malate:NAD+ oxidoreductase. E.C. 1.1.1.37

50mM Tris-HCl, pH 8.5	50ml
NAD	10mg
DL-Malic acid	150mg
MTT	10mg
PMS	2mg

Malic acid added as a neutralised (with NaOH) solution. Ingredients combined and poured over gel. Incubated until blue bands optimally resolved.

Peroxidase (PRX). I.U.B.: Donor:hydrogen peroxide oxidoreducterase. E.C. 1.11.1.7

50mM Na-acetate buffer, pH 5.0	50ml
$CaCl_2$	50mg
Hydrogen peroxide, 3%	250µl
3-Amino-9-ethylcarbazole	25mg
N,N-Dimethylformamide	2ml

Dissolve 3-amino-9-ethylcarbazole in the N,N-Dimethylformamide. Add along with remaining ingredients to buffer and pour over gel. Incubate at room temperature until red bands appear.

3.4. *In vitro* propagation

With the aim of developing a protocol for *in vitro* propagation of *V. paradoxa* seedling shoot tips, a four stage experimental protocol was used:

1. Develop procedure for production of healthy and sterile explant material
2. Determine appropriate media for maintenance of live explants
3. Develop a shoot regeneration media for proliferation of shoots
4. Develop a rooting media to encourage the formation of adventitious roots

Stage 1.

Due to the lack of live germplasm, and before any experiments on culture media or use of plant growth regulators could be tried, a consistent means to produce enough sterile explant material was required. All attempts to germinate de-corticated seeds *in vitro* met with failure as the large size of the kernel, and inability to excise the embryo, prevented adequate sterilisation. Even after 2 minutes in 70 % ethanol followed by 30 minutes in 100 % Domestos (Lever Brothers), all samples were found to be contaminated with a fungal infection after only 3 days. The lack of live seedlings to produce new shoots tips was therefore the biggest initial constraint to developing an *in vitro* propagation protocol for this species. It was found, however, that after trimming the initial bud, an new, adventitious shoot tip usually formed between 4-6 weeks later, with some seedlings producing 2 or more new shoots after each round of explant collection. It was therefore possible, with time, to obtain an adequate experimental number of explants from a selection (albeit random) of *V. paradoxa* seedlings. Successful sterilisation of live explants was only found to be possible when an 'ethanol-washing' step was included in the protocol. After empirical determination of concentration and washing time, the following sterilisation method consistently gave between 80 – 100 % success rates for production of sterile explants that were still alive after 7 days in culture:

Seedling shoot tips, ca. 10-15 mm long, were washed three times in distilled water before being surface sterilised by washing in 70% v:v ethanol for 1½ min followed by immersion in 8% v:v Domestos for 25 min and then washed four times in sterile distilled water. The tips were then trimmed to remove 2-3 mm of stem and all remaining leaves or petioles, before being aseptically placed into culture media.

Stage 2.

In order to develop a culture media for the maintenance of live explants, two media were used containing 30 g l^{-1} sucrose (Fisons), 8 g l^{-1} Gum Agar (Sigma) with MS (Murashige & Skoog, 1962) macro- and micronutrients at either full, or half-strength (50% w:v).

All media were adjusted to pH 5.7 with 0.1M NaOH and autoclaved at 120 ^{0}C for 15 minutes. Cultures were maintained in a culture room at 26 ±1 ^{0}C under a photoperiod of 16 h daylight provided by cool white fluorescent lights at 18.5μE$m^{-2}s^{-1}$ and transferred to fresh media every 21 days.

Stage 3.

Due to the lack of material, trials to determine a suitable culture media were also combined with experiments to establish an effective shoot regeneration media and combinations of the plant growth regulators, 6-benzylaminopurine (BAP: 0 to 22.2 µM) and α-napthaleneacetic acid (NAA: 0 to 5.4 µM) (Sigma), were added to the culture media described above, with the aim of stimulating shoot induction. Results were recorded after 42 days of culture prior to transfer to rooting media.

Stage 4.

Regenerated shoots were transferred to rooting media consisting of 30 g l^{-1} sucrose (Fisons), 8 g l^{-1} Gum Agar (Sigma) with MS macro- and micronutrients at either half (50% w:v), or quarter-strength (25% w:v). The plant growth regulators, indolebutyric acid (IBA) or NAA (0 to 16 µM) (Sigma) were added with the aim of stimulating root induction. Results were recorded after 70 days of culture.

The lack of time, and therefore large enough quantities of explant material, prevented trials with other types of culture media, plant growth regulators or specific clones.

3.5. Data analysis

3.5.1. Woody species inventory

To compare species diversity from inventory data, Simpson's diversity index was calculated (Equation 3.1.). This index provides an integrated value for the two components of α-diversity, variety and evenness (Hamilton & Perrott, 1981).

Equation 3.1. Simpson's diversity index

$$\mathbf{D} = \sum_{i=1}^{s}(n_i/N)^2$$

[s = number of species, n_i = density of each species ha^{-1}, N = density ha^{-1} of all species]

The results are presented in the form 1-D per plot, where values approaching 1 indicate areas with many species, each at low density, and a value of 0 indicates an area with only one species. For equal number of species, higher values indicate evenness.

V. paradoxa population structure was examined by plotting the mean number of stems occurring at each location in each 7 cm dbh size class. Using SPSS for Windows ver. 8.0, ANOVA was used to determine significant differences between quantitative site variables and post-hoc pairwise multiple comparisons were performed using Tukey's honestly significant difference test. The contribution to woody biomass was calculated as the basal area per hectare (Equation 3.2.) of each species divided by the total per hectare of all species. All means are presented together with 95% confidence intervals.

Equation 3.2. Basal area (BA) per hectare

$$\mathbf{BA\ [m^2ha^{-1}] = \pi(dbh\ in\ cm/200)^2 \times 10{,}000/plot\ size\ in\ m^2}$$

In order to make a visual comparision of biomass/species variation between plots, a similarity matrix of the basal area per hectare for all species by plot was produced with Hierarchical Cluster Analysis, using Ward's method and squared Euclidean distance (HCA) and two dimensional coordinates were produced from this matrix using multidimensional scaling (MDS).

3.5.2. *V. paradoxa diversity assessment*

Histograms were plotted, together with normal curves superimposed, for all samples and Skewness / Kurtosis values were calculated in order to determine whether samples taken for individual tree, site, location and area means were normally distributed and could therefore be used to produce reliable estimates for a mean. These analyses can be defined as follows (SPSS for Windows ver. 9.0):

> **Skewness** – A measure of the asymmetry of a distribution. The normal distribution is symmetric, and has a skewness value of zero. A distribution with a significant positive skewness has a long right tail. A distribution with a significant negative skewness has a long left tail. A skewness value greater than 1 generally indicates a distribution that differs significantly from a normal distribution. The ratio of skewness to its standard error can be used as a test of normality (that is, you can reject normality if the ratio is less than -2 or greater than +2). A large positive value for skewness indicates a long right tail; an extreme negative value, a long left tail.
>
> **Kurtosis** – A measure of the extent to which observations cluster around a central point. For a normal distribution, the value of the kurtosis statistic is 0. Positive kurtosis indicates that the observations cluster more and have longer tails than those in the normal distribution and negative kurtosis indicates the observations cluster less and have shorter tails. The ratio of kurtosis to its standard error can be used as a test of normality (that is, you can reject normality if the ratio is less than -2 or greater than +2). A large positive value for kurtosis indicates that the tails of the distribution are longer than those of a normal distribution; a negative value for kurtosis indicates shorter tails (becoming like those of a box-shaped uniform distribution).

Using SPSS for Windows ver. 8.0, Pearson bivariate correlations was used to determine any obvious trends between quantitative and location variables. These trends were further examined using partial correlations, with two tailed significance tests, to control for any covariance and to identify any genuine relationships before performing linear regression analysis. ANOVA, together with post-hoc pairwise multiple comparisons performed using Tukey's honestly significant difference test, was used to determine homogeneous subsets and significant differences between tree / site means. To improve variance comparisons for different sized population samples, the corrected coefficient of variance was calculated (Equation 3.3. and 3.4.) (Sokal & Braumann, 1980).

Equation 3.3. Coefficient of variance (expressed as a percentage)

$$V = 100s/\hat{Y}$$

[s is sample standard deviation and $\hat{Y}$ is sample mean]

Equation 3.4. Corrected coefficient of variance

$$V^* = (1+1/4n)V$$

[n is sample size]

Principle Components Analysis (PCA) and Hierarchical Cluster Analysis, using Ward's method and squared Euclidean distance (HCA), was performed on all phenotype descriptors, including a set of seven ratios that were not significantly correlated with any location parameter.

Estimates for heterozygosity, F-statistics, polymorphic loci and genetic distances were obtained from isozyme genotypes using TFPGA ver. 1.3 (tools for population genetic analyses) (Miller, 1997). Geneflow was estimated using F-statistics to determine the effective gene replacement rate (Equation 3.5.) (Wright, 1965). An UPGMA dendrogram (unweighted pair group arithmetic average clustering method) was generated using Nei's (1972) minimum distances where populations were defined by geographic areas. This was compared with the separation from HCA, for the same individuals and population structure, using the new phenotypic descriptors as above.

Equation 3.5. F-statistics and effective gene replacement rate

$$Fst = 1/(4Nm + 1)$$

[N = population size and m = gene replacement rate]

3.5.3. Shoot regeneration data collection and analysis

Due to the recalcitrant nature of *V. paradoxa* seeds, relatively few live seedlings were germinated in Southampton and until the sterilisation procedure had been optimised many explants were infected with a fungal growth soon after establishment. Therefore, possible contamination sources were reduced by estimating and recording callus size and growth without opening culture containers, except during transfer to fresh media, using the following scale. Callus width (scaled 0-6 respectively): none; <1; 1-5; 6-10; 10-15; 15-20; and >20mm. Growth (scaled 0-6 respectively): dead; alive but no growth signs; only leaf bud growth; leaf growth / stem elongation (<10mm); leaf growth / stem elongation (10-30mm); leaf growth / stem elongation (30-50mm); and leaf growth / stem elongation (>50mm). One-way ANOVA was used to compare the number of axillary shoots induced, growth and callus size after 42 days in culture. For shoot proliferation experiments, a minimum of 20 explants were used per treatment and for rooting experiments, at least 24 regenerated shoots were used per treatment (seedlings randomly selected). Post-hoc pairwise multiple comparisons were performed to determine treatments that were significantly different at 95% or 99% level using the least significant differences test (SPSS for Windows ver. 8.0).

Chapter 4. Anthropic influences on vegetation

4.1. Woody species composition of woodland and farmland

A total of 70 woody perennial species from 33 families, on farmland and woodland sites between Bole and Nandom, were identified to at least genus level (Table 4.1.). In addition to these, 16 samples were collected (considered to be different species) but not identified due to the lack of fertile material. All uniquely numbered herbarium samples (No. series: PL 700-795) have been presented to the herbarium at the Botany Department, Legon University, Ghana.

Table 4.1. Woody species identified in northern Ghana

Family	Genus	Species	Authority	Six-letter code
Anarcardiaceae	*Anacardium*	*occidentale*	L.	anaocc
Anarcardiaceae	*Haematostaphis*	*barteri*	Hook.f.	haebar
Anarcardiaceae	*Lannea*	*acida*	A.Rich.	lanaci
Anarcardiaceae	*Mangifera*	*indica*	L.	manind
Annonaceae	*Annona*	*senegalensis*	Pers.	annsen
Apocynaceae	*Saba*	*senegalensis*	(DC.) Pich.	sabsen
Araliaceae	*Cussonia*	*barteri*	Sm.	cusbar
Balanitaceae	*Balanites*	*aegyptiaca*	(L.) Del.	balaeg
Bombacaceae	*Adansonia*	*digitata*	L.	adadig
Bombacaceae	*Bombax*	*costatum*	Pell. & Vuill.	bomcos
Bombacaceae	*Ceiba*	*pentandra*	(L.) Gaertn.	ceipen
Boraginaceae	*Cordia*	*myxa*	L.	cormyx
Boraginaceae	*Ehretia*	*cymosa*	Thonn.	ehrcym
Burseraceae	*Commiphora*	*dalziella*	Hutch.	comdal
Caesalpinioideae	*Afzelia*	*africana*	Pers.	afzafr
Caesalpinioideae	*Berlinia*	*grandiflora*	(Vahl.) Hutch. & Dalz.	bergra
Caesalpinioideae	*Burkea*	*africana*	Hook.	burafr
Caesalpinioideae	*Daniellia*	*oliveri*	(Rolfe) Hutch. & Dalz.	danoli
Caesalpinioideae	*Detarium*	*macrocarpum*	Guill. & Perr.	detmac
Caesalpinioideae	*Erythrophleum*	*africanum*	(Welw.) Harms.	eryafr
Caesalpinioideae	*Isoberlinia*	*doka*	Craib. & Stapf.	isodok
Caesalpinioideae	*Piliostigma*	*thonningii*	(Schum.) Milne-Redh.	piltho
Caesalpinioideae	*Tamarindus*	*indica*	L.	tamind
Chrysobalanaceae	*Parinari*	*curatellifolia*	Benth.	parcur
Combretaceae	*Anogeissus*	*leiocarpus*	(DC.) Guill. & Perr.	anolei
Combretaceae	*Combretum*	*collinum*	Fresen.	comcol
Combretaceae	*Combretum*	*glutinosum*	Perr.	comglu
Combretaceae	*Combretum*	*molle*	G.Don	commol
Combretaceae	*Terminalia*	*avicennioides*	Guill. & Perr.	teravi
Combretaceae	*Terminalia*	*mollis*	Laws.	termol
Ebenaceae	*Diospyros*	*mespiliformis*	A.DC.	diomes
Euphorbiaceae	*Bridelia*	*ferruginea*	Benth.	bridfer

Table 4.1. contd.

Family	**Genus**	**Species**	**Authority**	**Six-letter code**
Loganiaceae	*Strychnos*	*spinosa*	Lam.	strspi
Lythraceae	*Lawsonia*	*inermis*	L.	lawine
Meliaceae	*Azadirachta*	*indica*	A.Juss.	azaind
Meliaceae	*Khaya*	*senegalensis*	(Desr.) A.Juss.	khasen
Mimosoideae	*Acacia*	*sp1*	-	acasp1
Mimosoideae	*Acacia*	*sp2*	-	acasp2
Mimosoideae	*Acacia*	*sp3*	-	acasp3
Mimosoideae	*Entada*	*africana*	Guill. & Perr.	entafr
Mimosoideae	*Faiderherbia*	*albida*	(Del.) A.Chev.	faialb
Mimosoideae	*Parkia*	*biglobosa*	(Jacq.) G.Don	parbig
Moraceae	*Ficus*	*anomani*	-	ficano
Moraceae	*Ficus*	*asperifolia*	Miq.	ficasp
Moraceae	*Ficus*	*sp1*	-	ficsp2
Moraceae	*Ficus*	*sp2*	-	ficsp2
Moraceae	*Ficus*	*sp3*	-	ficsp3
Myrtaceae	*Psidium*	*guajava*	L.	psigua
Myrtaceae	*Syzygium*	*guineense*	(Willd.) DC.	syzgui
Ochnacaeae	*Lophira*	*lanceolata*	Keay	loplan
Olacaceae	*Ximenia*	*americana*	L.	ximame
Opiliaceae	*Opilia*	*celtidifolia*	(Guill. & Perr.) Walp.	opicel
Palmae	*Borassus*	*aethiopum*	Mart.	boraet
Palmae	*Elaeis*	*guineensis*	Jacq.	elagui
Papilionoideae	*Erythrina*	*senegalensis*	-	erysen
Papilionoideae	*Pericopsis*	*laxiflora*	(Benth.) Van Meeuwen	perlax
Papilionoideae	*Pterocarpus*	*erinaceus*	Poir.	pteeri
Papilionoideae	*Xeroderris*	*stuhlmannii*	(Taub.) Mend & Sousa	xerstu
Polygalaceae	*Securidaca*	*longipedunculata*	Fresen.	seclon
Rubiaceae	*Crossopteryx*	*febrifuga*	(Afzel.) Benth.	crofug
Rubiaceae	*Gardenia*	*ternifolia*	Schum. & Thonn.	garter
Rubiaceae	*Mitragyna*	*inermis*	(Willd.) O.Ktze	mitine
Rubiaceae	*Nauclea*	*latifolia*	Sm.	naulat
Rutaceae	*Citrus*	*sinensis*	(L.) Osbeck	citsin
Sapindaceae	*Blighia*	*sapida*	Koenig	blisap
Sapotaceae	*Vitellaria*	*paradoxa*	Gaertn.f.	vitpar
Simaroubaceae	*Hannoa*	*undulata*	Planch.	hanund
Sterculiaceae	*Sterculia*	*setigera*	Del.	steset
Tiliaceae	*Grewia*	*cissoides*	Hutch. & Dalz.	grecis
Verbenaceae	*Vitex*	*doniana*	Sweet	vitdon

All unknown species coded as unkspe $^{\#}$(1-16)

A total of 1755 trees >7cm dbh (883 individuals of *V. paradoxa*), were sampled in 39 plots at five sites and a significant variation in the total number, and abundance of each tree species was noted between sites. In particular, species commonly utilised for their edible or other non-timber products, were found in greater numbers on farmland. *Parkia biglobosa* (Don) was not found in any woodland plots and large individuals of *Diospyros mespiliformis* (Hochsetter ex A. de Condelle) were common near high intensity farmland (Table 4.2.).

Table 4.2. Representation of the top ten woody species at sites in north Ghana

Bole (high intensity farmland)			**Mandari (med. intensity farmland)**			**Unmanaged Woodland**			**Wa (high intensity farmland)**			**Nandom (high intensity farmland)**		
Species[1]	**Use**[2]	**%**[3]	**Species**	**Use**	**%**	**Species**	**Use**	**%**	**Species**	**Use**	**%**	**Species**	**Use**	**%**
vitpar	EBFM	79.7	vitpar	EBFM	84.2	bergra	BF	32.2	vitpar	EBFM	42.9	vitpar	EBFM	61.0
parbig	EF	7.1	hanund	FMC	3.6	unkspe #15	-	12.2	parbig	EF	36.4	parbig	EF	10.4
diomes	EFM	4.4	steset	M	2.4	vitpar	EBFM	10.2	diomes	EFM	7.3	diomes	EFM	7.9
azaind	M	3.3	parbig	EF	2.0	perlax	F	10.1	khasen	M	4.0	faialb	ES	6.9
danoli	SM	1.6	danoli	SM	1.8	isodok	BF	8.0	ficsp1	E	3.4	ficsp1	E	4.7
hanund	FMC	0.7	diomes	EFM	1.6	lanaci	EF	6.3	bomcos	C	1.3	azaind	M	4.4
perlax	F	0.6	vitdon	E	0.9	afzafr	BM	4.4	hanund	FMC	1.0	ficsp3	EM	2.3
steset	M	0.5	lanaci	EF	0.7	commol	F	2.8	ficano	E	0.8	lanaci	EF	2.3
lanaci	EF	0.4	bomcos	C	0.7	teravi	F	2.5	steset	M	0.8	acasp2	-	0.1
unkspe #12	-	0.3	detmac	EFM	0.5	danoli	SM	2.5	balaeg	E	0.7	erysen	M	0.03

[1] Please refer to Table 4.1. for full species names

[2] Local utilisation of products (E – Edible, including animal fodder, B - Building, F - Fuel, S - Soil improver, M - Medicine, C - carving)

[3] Average percentage contribution to average basal area at each site

The number of individual trees, total woody biomass and species number were significantly lower in farmed areas (Table 4.3.). Biodiversity in woodland was also significantly higher than that found on cultivated sites except for the far northern site of Nandom. In addition, the lack of any sign of tree cutting in the woodland study area supports the purported lack of anthropic influences at this site. Tree pruning, usually for firewood, was notably more abundant in the Upper West Region.

Table 4.3. Comparison of biomass at sites in north Ghana, all woody species ≥7cm dbh

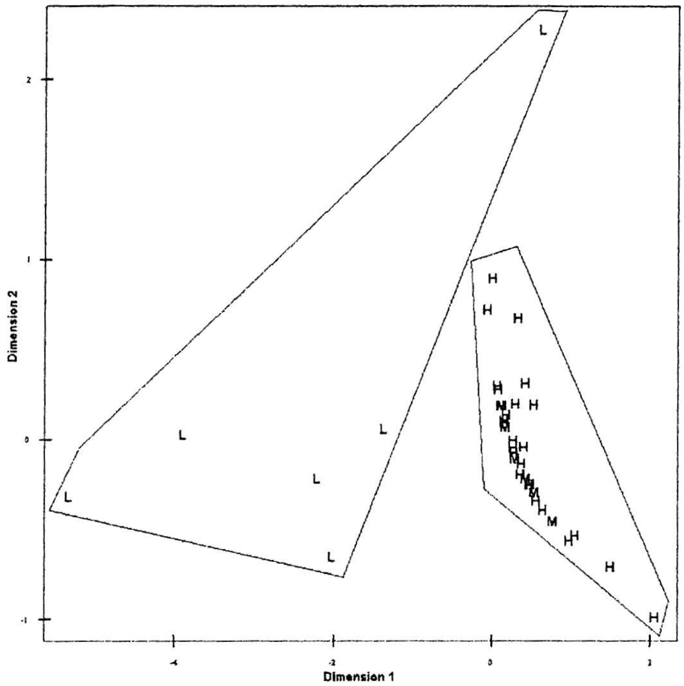
Site (intensity)	No. of plots	Trees ha^{-1}	Total BA m^{2}ha^{-1}	Species ha^{-1}	1-D	Trees pruned ha^{-1}
Bole (high)	15	29 ± 13^{a}	2.7 ± 0.7^{a}	4 ± 2^{a}	0.30 ± 0.13^{a}	0.5 ± 0.8^{a}
Mandari (med.)	6	61 ± 24^{a}	2.6 ± 0.6^{a}	8 ± 3^{ab}	0.37 ± 0.19^{a}	0.5 ± 1.0^{a}
Woodland (low)	6	353 ± 61^{b}	9.6 ± 2.0^{b}	$13^{1} \pm 2^{b}$	0.81 ± 0.03^{b}	n/a
Wa (high)	6	39 ± 11^{a}	3.3 ± 1.3^{a}	5 ± 1^{a}	0.43 ± 0.11^{a}	1.7 ± 1.9^{ab}
Nandom (high)	6	31 ± 13^{a}	3.3 ± 0.7^{a}	5 ± 2^{a}	0.54 ± 0.21^{ab}	5.8 ± 4.8^{b}

Means by column without same superscript letters - Tukey's honestly significant difference ($p < 0.05$)
[1] In woodland species numbers are per 0.25 hectare plot

These differences are clearly visible as a distinct separation between farmland and unmanaged areas following multivariate comparisons of all species biomass by plot (Figure 4.1.). Although the similarity between individual woodland plots is low, indicative of a wide variation in species composition and woody biomass. The tight cluster of all farmland plots indicates their relative similarity to each other, in terms of these variables, as compared to the unmanaged areas.

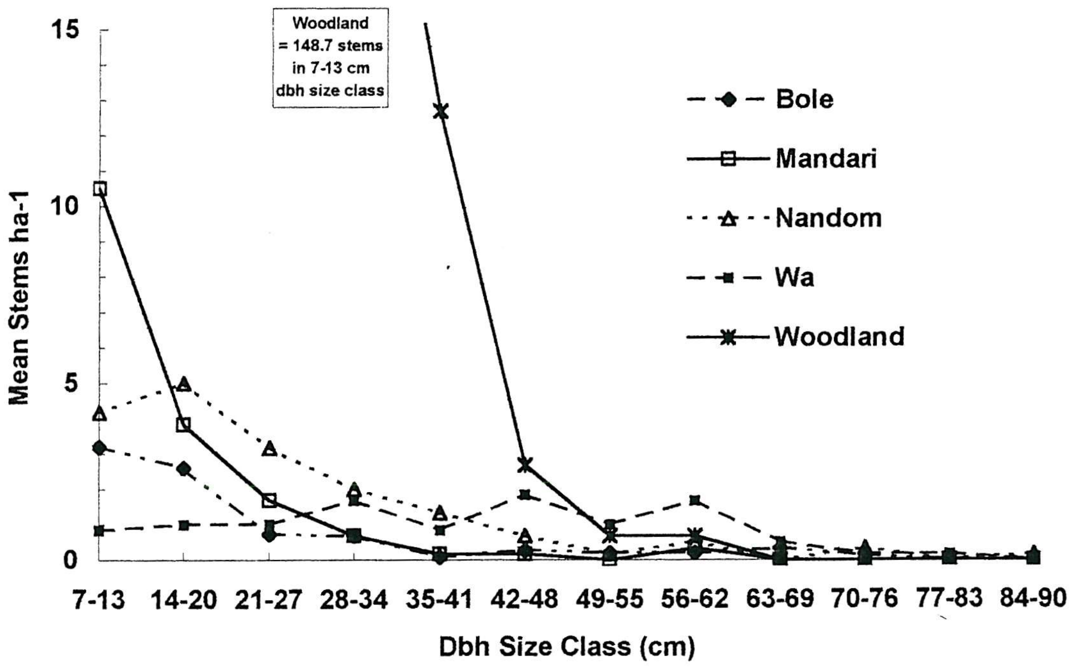
In woodland the population structure of all woody species showed many similarities to that expected in other 'natural' systems, with abundant small, probably immature individuals, and few large trees (Figure 4.2.). Although at much lower densities (148.7 stems ha^{-1} in woodland vs 10.5 stems ha^{-1} on Mandari farmland in the 7-13cm dbh size class), this trend in the population structure of woody species (except *V. paradoxa*) is also apparent to some degree at all farmland sites except at Wa.

Figure 4.1. Multivariate comparison of plot similarity using species biomass estimates

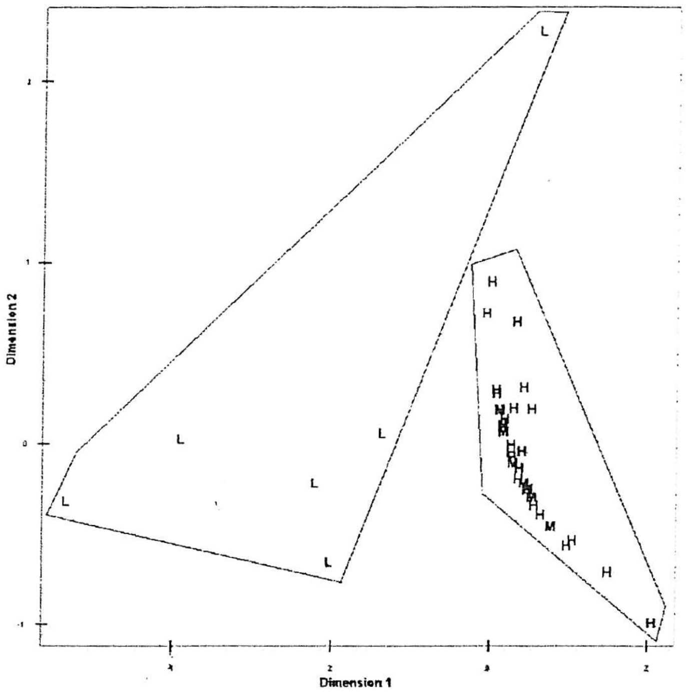


Similarity matrix produced with HCA using BA ha^{-1} of each and every species
Two-dimensional coordinates produced from this matrix using MDS
H = High intensity farmland; **M** = Medium intensity farmland; **L** = Unmanaged woodland plots

Figure 4.2. Size class distribution of all woody species except *V. paradoxa*

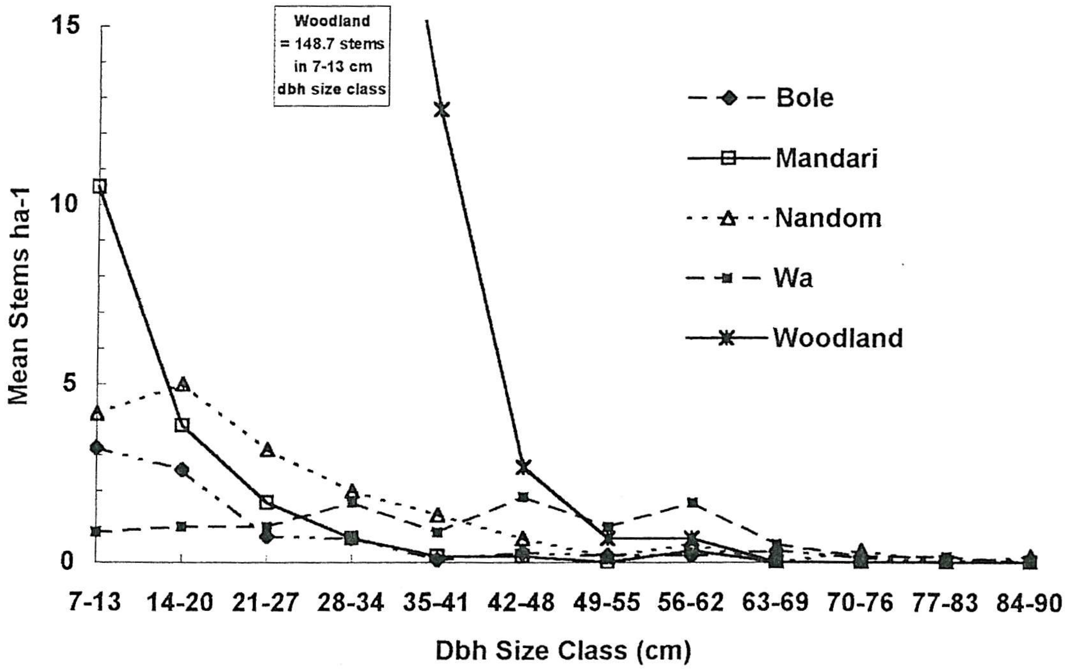


1. Multivariate comparison of plot similarity using species biomass estimates



Similarity matrix produced with HCA using BA ha^{-1} of each and every species
Two-dimensional coordinates produced from this matrix using MDS
H = High intensity farmland; **M** = Medium intensity farmland; **L** = Unmanaged woodland plots

2. Size class distribution of all woody species except *V. paradoxa*



7.2. *V. paradoxa* populations in woodland and on farmland

Results for *V. paradoxa* only, show no significant variation in numbers of individual trees (dbh ≥7cm) except between Nandom and Mandari (Table 4.4.). The mean number of large individuals (dbh > 20cm), however, was always higher on farmland though only significantly greater than woodland near Bole and Nandom. 82.6% of all sheanut stems sampled in woodland were small trees (dbh < 20cm) although some plots contained only trees greater than this size class. It was evident that only a few small (dbh < 13cm) individuals of *V. paradoxa* were maintained (i.e. regeneration not cut) on land cleared for cultivation, particularly in farms mechanically ploughed, and ring barking was observed as a method used to kill occasional mature trees.

Table 7.4. Comparison of biomass at sites in north Ghana, *V. paradoxa* only

Site (intensity)	No. of plots	Trees ha^{-1}	Stems ha^{-1}	% Stems >20cm dbh	BA m^{2}ha^{-1}	% contribution to total BA	BA Tree^{-1}
Bole (high)	15	21 ± 6^{ab}	24 ± 7^{ab}	72.0 ± 9.4^{ab}	2.19 ± 0.64^{a}	79.7 ± 7.2^{a}	0.11 ± 0.02^{a}
Mandari (med.)	6	44 ± 11^{a}	48 ± 12^{a}	53.1 ± 15.1^{a}	2.16 ± 0.57^{ab}	84.2 ± 10.0^{a}	0.05 ± 0.01^{b}
Woodland (low)	6	41 ± 28^{ab}	46 ± 30^{a}	40.2 ± 32.9^{a}	0.92 ± 0.23^{b}	10.2 ± 3.3^{b}	0.05 ± 0.04^{b}
Wa (high)	6	28 ± 11^{ab}	31 ± 12^{ab}	47.5 ± 21.8^{a}	1.39 ± 0.60^{ab}	42.9 ± 13.3^{c}	0.05 ± 0.03^{b}
Nandom (high)	6	13 ± 2^{b}	14 ± 3^{b}	93.8 ± 5.5^{b}	1.96 ± 0.31^{ab}	61.0 ± 9.8^{c}	0.16 ± 0.03^{a}

Means by column without same superscript letters - Tukey's honestly significant difference ($p < 0.05$)

A supposed 'natural' *V. paradoxa* population structure, similar to that seen for all other woody species, was identified in woodland. In contrast, nearby farmed sites had few small sheanut trees but a much larger proportion of large, mature trees (Figure 4.3.). This observation differs to that observed for the structure of other woody species on farmland where the proportion of all stems (not including *V. paradoxa*) sampled >20cm dbh, was less than 50% of the total population except at Wa (Table 4.5.).

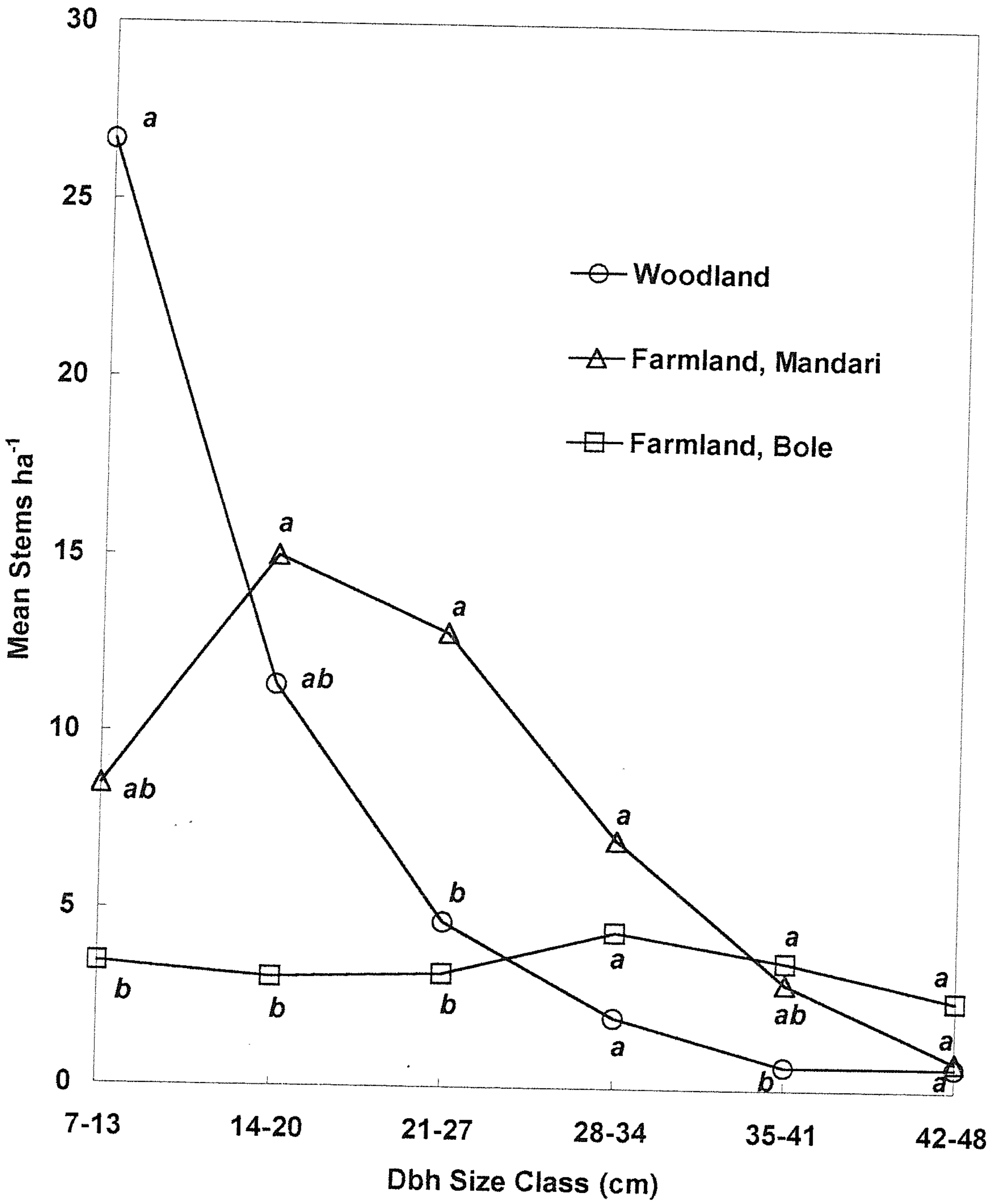
Table 7.5. Proportion of other woody species stems >20cm dbh

Site (intensity)	% of all stems sampled >20cm dbh (except *V. paradoxa)*
Bole (high)	**31.5%**
Mandari (med.)	**17.3%**
Woodland (low)	**27.8%**
Wa (high)	**83.3%**
Nandom (high)	**48.6%**

At Nandom, sheanut tree population structure showed many similarities to those recorded on farmland near Bole but with less regeneration apparent (Figure 4.4.). Near Wa, the sheanut tree population has apparent similarities to that found in woodland, however, sampling at a smaller size class would have revealed that the overall population profile was more comparable to that seen at Mandari. The random sample of mature trees used for germplasm collection near Wa was also smaller (age unknown) than those at sites either to the north or the south indicating the possibility that either environmental differences are 'stunting' tree growth, or that genetically determined 'dwarf' varieties exist which mature at a smaller size.

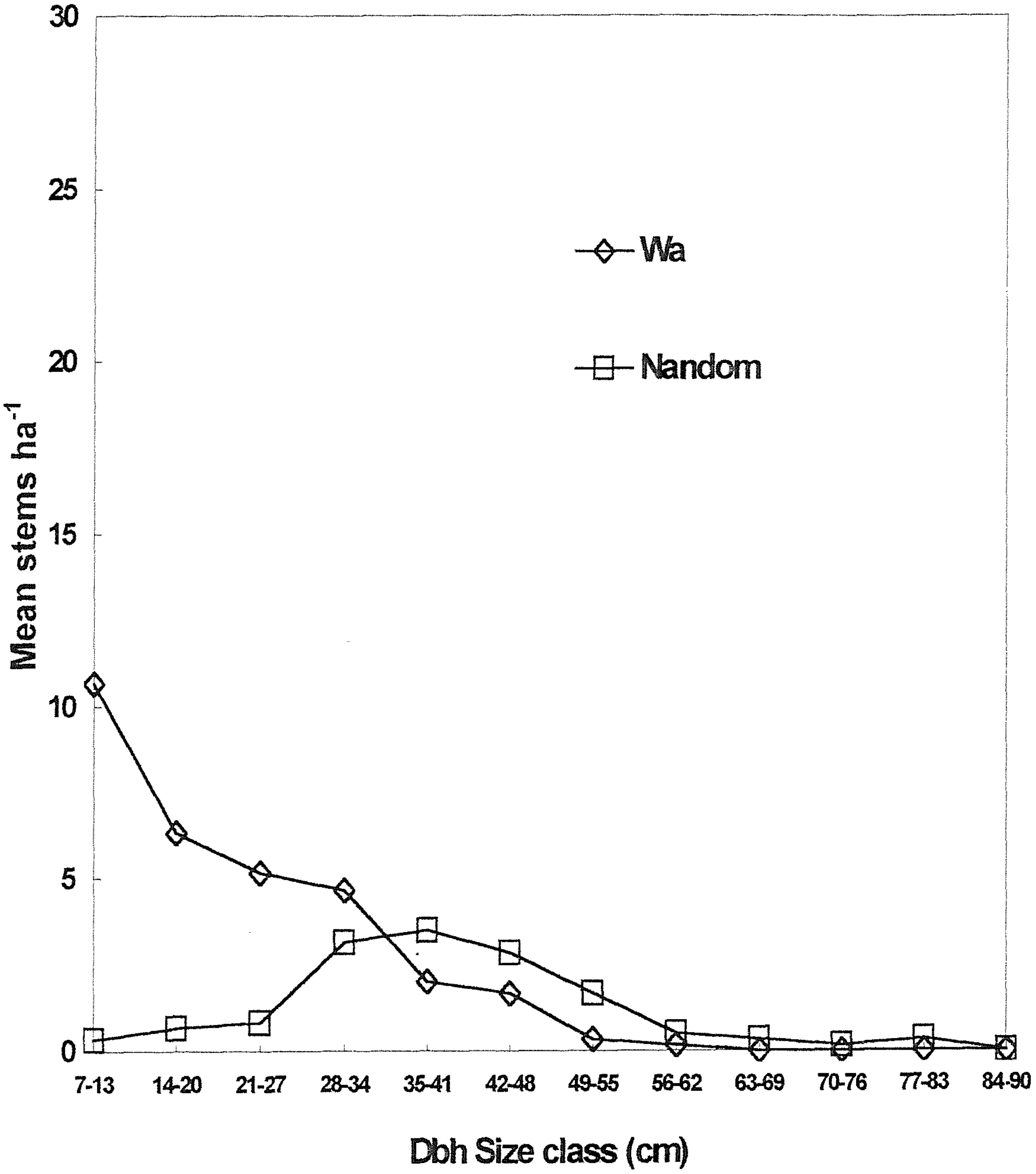
All mature trees in farmland, greater than 20cm dbh, were seen to bear at least some fruit during the harvest season. In woodland, even after extensive searching of the whole area during the same period, only one individual with fruit was found (dbh = 18cm). Mature fruit collection from this individual required vigorous shaking and viability was still high four weeks after collection. On farmed land, fruit fell to the ground when mature (April-June) but all collections lost viability after three weeks.

Figure 4.3. Size Class distribution of *V. paradoxa*, West Gonja District, Ghana

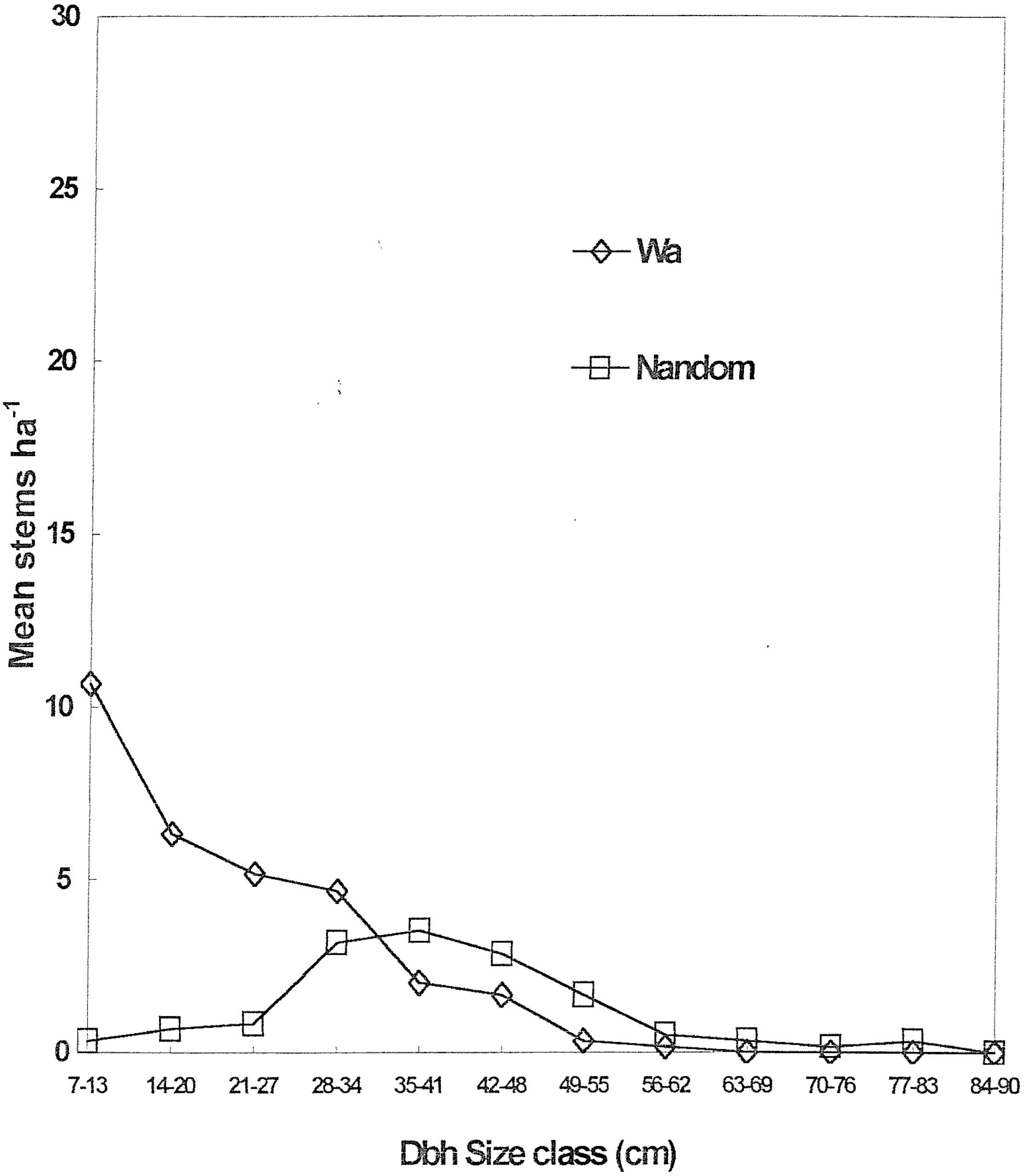


Points without similar lower case letters, by size class, indicate Tukey's honestly significant difference ($p < 0.05$)

Figure 4.4. Size Class distribution of *V. paradoxa*, Upper West Region, Ghana



4. Size Class distribution of *V. paradoxa*, Upper West Region, Ghana



7.3. Local utilisation of woody species

The quantity and quality of information available on local uses, spiritual beliefs and vernacular names of locally found species clearly demonstrates that farmers have access to an extensive technical indigenous knowledge base. All species commonly found on farmland were well known and utilised for a wide range of products, e.g. soil improvement, firewood, charcoal, building poles, food and traditional medicines (Please refer to Table 4.2. above).

During vegetation clearance for cultivation (details below), traditional farmers also described the conscious preservation and management of many woody species (mature trees or regenerated shoots not cut), particularly those providing edible non-timber products. Beside *V. paradoxa*, specific cultural beliefs were noted for a number of highly valued species for which special permission had to be obtained before cutting.

In West Gonja, *Afzelia africana*, *Diospyros mespiliformis* and *Haematostaphis barteri* were taboo to cut due to the belief that they were inhabited by powerful spirits (often called 'dwarves') despite the fact that they provide good quality hardwood. These species were also used to make traditional medicines and the latter two produce edible fruit. Silk cotton trees (*Ceiba pentandra*) though rarely seen in farmland, were common close to habitations. Individuals of this species are said to be 'owned' or planted by local Chiefs and are traditionally only cut for the funeral of that particular person. *Gardenia ternifolia* was rarely cut as the Gonja described it as being a ‘powerful’ tree, and although local farmers did not give exact details, D. Abbiw (pers. comm., 1998) described that many ethnic groups ascribed associations with lightening to this species. Around the Bole area, only those ethnic groups described as 'landowners' (e.g., the Vagla) were given rights to harvest the fruit of the Locust bean or Dawa dawa trees (*Parkia biglobosa*) although portion is often given as a ‘tax’ to the Gonja. This species is utilised throughout West Africa for the protein rich seeds and in other ‘non-Gonja’ study areas it was seen to be much more widely protected on cleared land. In the Upper West Region, permission to cut trees was usually obtained from the landowner (Tandaana) and it was notable that in addition to the above trees, two species were also more widely valued – *Faiderherbia albida* for animal fodder or to increase soil fertility on farmed land, and *Balanites aegyptiaca* for fruit or oil.

7.4. Indigenous agroforestry systems

Farming systems appeared to be generally similar throughout northern Ghana and almost pure stands of sheanut trees were frequently seen in the typical 'agroforestry parklands' where cultivated fields were planted with yams, millet, sorghum, maize, cassava or legumes. It was notable that the frequency of indigenous cereals increased towards the northern (drier) locations in contrast to the more resource demanding crops (Table 4.5.). Although observed in other regions, rice was only described as an important crop in the Upper West Region where walled gardens were used to control water levels at seasonally flooded sites – *V, paradoxa* not recorded in these areas.

Table 7.6. Carbohydrate crops planted in northern Ghana

Region	**Carbohydrate crops**					
	Yams	**Sorghum**	**Millet**	**Maize**	**Rice**	**Cassava**
Brong Ahafo	90	10	-	80	-	100
Northern	93	60	73	53	-	80
Upper West	63	63	88	50	38	0

Percentage of respondents who described planting each crop during 1997

In West Gonja, the typical crop rotation started in the first year by clearing woodland or fallow land, leaving specific individual trees of certain species (Table 4.7.) on the farm after the controlled burning of cut vegetation at the base of unwanted trees (Plate 4.1.). Clearing land was described as 'the work of men' and no women mentioned any species of tree that they had been involved in protecting. Of the respondents (55% of total) who described tree-crop interactions, 17% said there was no effect, 17% said trees improved soil fertility and 67% stated that canopy shade reduced crop yield.

After clearing, the land was then hand mounded prior to planting 'seed' yams that were harvested in the second year of cultivation. After levelling the field, the next crop was usually one or more of the cereals - sorghum, millet or maize (the latter often cultivated using mechanised ploughing and fertiliser). These crops were harvested in the same year and a legume planted the following spring (groundnuts, bambara beans, pigeon pea, cowpea, etc) (Table 4.8.). After harvesting later in the season, cassava was often planted and the land left uncultivated for between three and ten years before the cycle was repeated. The cassava was then collected when required, usually after about a year. Vegetables (cabbages, carrots, tomatoes, etc) were usually maintained in compounds or dry season gardens and rarely grown in farm fields. Most crops were planted, tended and harvested by women, although men were not excluded and often involved in any work that required digging (cassava) or harvesting from tall crops (sorghum). The exception to this general rule was that men exclusively cultivated yams and women were only involved in carrying seed yams for planting or back home after harvesting.

Table 7.7. Woody species described as protected by male farmers in northern Ghana

Species	**Described (% respondents)**	**Recorded as top ten farmland species**[1]	**Reason given for protection during land clearance**
Vitellaria paradoxa	100%	****	Economic & food
Parkia biglobosa	91%	****	Food
Diospyros mespiliformis	25%	****	Fruit
Daniellia oliveri	22%	**	Soil fertility
Vitex doniana	19%	*	Fruit
Afzelia africana	13%		Timber
Faiderherbia albida	13%	*	Soil fertility & fodder
Species unknown	13%		Timber
Haematostaphis barteri	9%		Fruit
Ceiba pentandra	6%		Leaves for food
Lannea acida	6%	***	Fruit
Mangifera indica	6%		Fruit
Adansonia digitata	3%		Leaves for food
Anacardium occidentale	3%		Economic
Anogeissus leiocarpus	3%		Timber
Azadirachta indica	3%	**	Fodder
Balanites aegyptiaca	3%	*	Fruit
Borassus aethiopum	3%		Fruit
Burkea africana	3%		Other
Khaya senegalensis	3%	*	Timber
Piliostigma thonningii	3%		Leaves for food
Pterocarpus erinaceus	3%		Timber

[1] Asterisks show number of records in farmland top ten lists from inventory data (refer to Table 4.2.)

Table 4.8. Other crops described as being planted in northern Ghana

Region	**Legumes**						**Vegetables**
	Groundnuts	Bambara beans	Black beans	Cowpea	Pigeon pea	Soya bean	various
Brong Ahafo	80	10	-	10	20	-	30
Northern	33	-	7	-	-	-	7
Upper West	88	63	13	38	-	13	-

Percentage of respondents who described planting each crop during 1997
n.b. first round of interviews in the Northern Region did not focus on non-carbohydrate crops

Most farmers still practised the traditional method of hand ploughing but deep mechanised ploughing was noted as being more frequent near larger habitation centres in intensively farmed areas. Animal traction was, however, the preferred power source in the Upper West Region of Ghana (Table 4.9.).

Table 4.4. Land preparation methods in northern Ghana

Region	**Ploughing method**		
	Hand	**Tractor**	**Bullock**
Brong Ahafo	90	10	-
Northern	94	6	-
Upper West	62	-	38

Percentage of respondents describing preferred method of land preparation

Other quantitative variables revealed that farms were generally similar at all interview locations, although the mean distance from the home to farm in West Gonja was significantly greater than at other locations (p<0.01). This probably indicates a lower intensity of landuse (more free land suitable for farming) as compared to other study locations since most farmers stated that they prefer farmland away from foraging village animals (goats, sheep, pigs, chickens, guinea fowl and cows). The other notable difference was that farms in the Upper West Region were generally larger and fallow periods were shorter (p = 0.07 - 0.10).

Table 4.5. Farm descriptors

Region	**Farm size** acres	**House to farm** miles	**Time farmed** yrs	**Time fallow** yrs
Brong Ahafo	**5.1**±2.1	**2.0**±0.5	**2.9**±0.7	**8.1**±2.7
Northern	**5.3**±1.4	**3.6**±0.7	**3.0**±0.3	**7.2**±2.0
Upper West	**6.4**±1.9	**1.5**±0.6	**3.9**±0.7	**4.3**±1.7

The majority of male farmers interviewed (91%), had planted trees on farmland or at home, though the lowest response (79%) was recorded in West Gonja and no women mentioned tree planting. Apart from a few farmers planting *V. paradoxa* and a couple of other indigenous species, most trees were exotics (Table 4.11.).

Table 4.6. Top five tree species planted by farmers in northern Ghana

Species planted	Described (% respondents)	Location usually planted
Mangifera indica	69%	Compound
Anacardium occidentale	31%	Farmland
Blighia sapida	21%	Compound
Tectona grandis	14%	Farmland
Elaeis guineensis	10%	Compound / Valleys

Other species included Guava, *Citrus* spp., *Cassia* sp., and *Annona* sp.

Plate 4.1. *V. paradoxa* on newly cleared farmland surrounded by dead, unwanted trees

4.2. Traditional sheanut tree management

As described above (see Table 4.7.) all male farmers interviewed stated that they would protect *V. paradoxa* when clearing land for cultivation although only two-thirds of farmers would answer questions on whether they would perform some form of selective management (consciously choose which individuals to keep or which they would remove) and how many they actually had on their farms, (Table 4.12.). No women answered any questions on sheanut tree selection, stating they were not involved in land clearance.

Table 4.7. Male farmers response to selective management of *V. paradoxa*

Region	**Described selection of sheanut trees (% respondents)**	**Number on farm ha^{-1}**
Brong Ahafo	**80**	**25**
Northern	**36**	**28**
Upper West	**100**	**25**
Mean	**72±37**	**28±5**

Most farmers (68%) said that they would prefer not to clear dense stands of productive *V. paradoxa*, but would rather cultivate elsewhere. The majority of farmers (92%) also said that they wanted more on their land, offering preferred densities of between 20-50 and 400 sheanut trees ha^{-1} depending on whether they planned to farm the land or maintain as an orchard without other cultivation. Some of the 'selective' farmers did, however, admit to having cut mature trees (Table 4.13.).

Table 4.8. 'Selective' male farmers admitting to having ever cut mature sheanut trees

Region	**Cut mature sheanut trees? (% respondents)**
Brong Ahafo	**22**
Northern	**14**
Upper West	**75**
Mean	**37±37**

The decision to keep a sheanut tree on cleared land was not based a single factor but on a complex, situation based range of criteria determined through long-term observations on location, size, growth, health, age and yield characteristics (Table 4.14.).

Table 4.9. Selection criteria of sheanut trees during clearance for cultivation

Sheanut trees kept if criteria met	Reason described (% answers)
Mature	**77**
Immature but >1-2m	**36**
Not old and unproductive	**23**
Healthy and high yield	**23**
Not affecting crops (shade)	**19**

n.b. Respondents often gave more than one reason for keeping a sheanut tree

In answer to another set of questions, only 60% of all farmers said they would keep young sheanut trees on farmland (Table 4.15.). Of these farmers, 36% gave reasons, explaining that a regenerating sapling would be protected if it were taller than 1m at land clearance, healthy, fast growing and well spaced in relation to other trees on the farm. The main reasons given for not wanting to keep immature saplings, was that they take a long time to mature (15-20 years often suggested) or that they were not present.

Table 4.10. Protection of immature sheanut trees on cleared farmland

Region	Described young sheanut tree protection? (%)
Brong Ahafo	**80**
Northern	**57**
Upper West	**38**
Mean	**58±24**

This confirms field observations made during on-farm inventory, since many male farmers said that although cutting of sheanut trees was locally taboo, they still removed both unwanted immature and mature sheanut trees from the land they wished to cultivate. The low response rate at some sites, and high confidence limits for the whole study area, are probably indicative of farmers being afraid of admitting to a stranger that they ever cut this valuable economic species since it was apparent that traditional agroforestry practices conflicted with the national forestry laws (enforced ban on cutting any sheanut tree). It is therefore likely that many of these practices are much more widespread than the results of this study demonstrate and any differences between sites, farming systems or ethnic groups are currently impossible to accurately evaluate.

Cutting of sheanut trees was also said to occur in remote areas of woodland/farmland, particularly by ethnic groups not processing shea butter, for use in production of charcoal or other wood products. It is unknown how widespread this practise was, but if caught, traditional penalties can be very high e.g., the provision of goats or a cow for land pacification rites.

As demonstrated from results of the on-farm inventory, pruning of many species of trees was widespread although it was most noticeable on *V. paradoxa* in the Upper West Region. This incidence of 'non-destructive' tree management was confirmed by the numbers of male farmers who stated that they prune this species (Table 4.16.).

Table 4.11. Male farmers pruning sheanut trees in northern Ghana

Region	**Prune sheanut trees? (% of respondents)**
Brong Ahafo	40
Northern	54
Upper West	100
Mean	65±36

The final end-use of the wood was most probably as fuelwood, particularly for local beer (*pito*) brewing, although relatively few respondents suggested this as the primary motive for pruning and many other reasons for this practice were offered (Table 4.17.). It is also of interest to note that the parasitic mistletoe (probably *Tapinanthus* spp.) was widely believed to reduce fruit yield, though it was still commonly maintained (particularly in Upper West) to provide medicinal herbs or even special firewood.

Table 4.12. Reasons given for pruning sheanut trees

Reason for pruning trees	Described (% of answers)
Decrease crop shade	**61%**
Removal of mistletoe	**44%**
Fuelwood	**28%**
Increase shea fruit yield	**22%**
Building poles	**6%**
Remove dead wood	**6%**

n.b. Respondents often gave more than one reason for pruning

Apart from the selection criteria given above, male farmers had a wide range of preferences when describing the 'best' trees and often gave more than one character. Good health, low competitive effects on crop yield, large sweet fruit, fast growth and resistance to mistletoe were all mentioned as preferred traits (Table 4.18.). Trees with sweet fruit and high total fruit yield were equally the most popular traits although some confusion existed between male farmers as to which seed size (whole sheanut) was the best for high oil yield. Farmers in the Upper West Region also described a 'Black' variety of sheanut tree, which supposedly had high yields and dark buds. This variety was said to have less of an effect on crop yield as compared to trees with lighter buds.

Table 4.13. Preferred characteristics given by male farmers

Characteristic	Described (% of respondents)
Sweet fruit	45%
High yield	45%
Small nuts	38%
Big nuts	28%
Healthy	17%
'Black' buds	7%
Big fruit	3%
Fast growth	3%
Less mistletoe	3%

n.b. Respondents often gave more than preferred character

All women, apart from one interviewee, said that small seeds would yield more oil than the same volume of large seeds and no other preferred characteristics were offered.. The only woman, who preferred big seeds, said that she was a lazy market trader, and as sheanuts were sold by volume, it was easier to fill a basin with large kernels.

Local knowledge on the sheanut tree was extensive and 88% of respondents said they knew where the 'best' – healthiest and most productive – trees grew (Table 4.19.).

Table 4.14. Best sites for sheanut trees

Character	Described (% respondents)	'Best' site characteristics
Topographic location	40	High land
Vegetation type	28	Cleared or cultivated land
Soil type	28	Stony and loamy soils good
Fire	4	No burning

Topographic situation was the most important influence described and the best trees were always said to be on higher land (relative to nearby watercourses). The most productive trees were always said to be on well-drained fertile soils that were usually farmed. Few farmers mentioned that fire had any detrimental effect on sheanut trees, stating that if vegetation was burnt during the flowering season (November-January) the fruit yield would be reduced.

Throughout the study area, a wide range of products were utilised from *V. paradoxa*. The delicious fruit pulp was eaten by everyone encountered and often sold by children to passengers of passing vehicles. Compound animals were also seen eating the pulp from seeds left to dry and in Tumu, whole fruit was given to confined pigs before boiling the de-pulped seeds. Apart from being commonly stated as being good for soil fertility, leaves were rarely used except occasionally as animal fodder in urban areas with few trees, e.g. near Tamale. Dead wood was collected for firewood and whole trees were sometimes cut for making charcoal. Along with other hardwoods, carefully selected poles ('Y-shaped') were used in construction and large, high quality boles were hollowed to make mortars. It is also of note that the pestles for use with these were often poles from the so-called 'male' sheanut tree (*Lophira lanceolata* Van Tiegh. ex Keay), with which *V. paradoxa* is sometimes confused. The flowers were said to be an important food for bees, from which an excellent honey was harvested, and large defoliating caterpillars were known to be edible. The destructive effects of which were only seen once during fieldwork in an area south of Nandom where virtually every sheanut tree, in a 30km radius, was leafless. Many medicines were made from most parts of the tree, e.g., roots, butter and bark. Shea butter was often used as an external ointment against sprains or respiratory complaints and an extract made using *V. paradoxa* bark was extensively known as a cure for stomach disorders. The latex was also used as glue or to seal a broken calabash. Even the liquid waste products from shea butter extraction were utilised as a waterproof coating to mud-houses and leftover husks were put onto paths to prevent erosion.

Women and children were the main collectors of mature sheanut fruits and usually given priority rights to harvest on land currently being farmed by family members or from trees around the compound. Open access rights are often granted in fallow or unmanaged areas, although women often described that it was easier and less hazardous to collect fallen fruit from cleared land. During germplasm collections it was noted that few viable sheanuts remained on cleared land as women gather everything they find in the early morning (Plate 4.2.). Harvested seeds are de-pulped and immediately boiled before sun drying. This processing stage is said to improve yields and oil quality by preventing germination and fungal infection of seeds.

Despite this extensive knowledge and intense local competition for sheanuts, a traditional two-week ban on all harvesting was strictly enforced once fruit abscission had commenced. Local residents explained the reasons behind this and a number of alternatives offered. One viewpoint suggested that it freed up human resources during a busy cultivation period. Another explanation was that the ban allowed land pacification ceremonies to take place and ensured good rainfall. The last reason, described by an influential member of the community, was that the strictly enforced fines allowed all women to have an equal share of the harvest when the ban was lifted.

Plate 4.2. Women with harvested fruit (protected sheanut tree regeneration behind)

As described above evidence for tree planting was widespread but few farmers (five in Upper West Region and one in Northern Region) had ever planted sheanut trees. Numerous traditional taboos against planting this species were said to exist in northern Ghana and local people often held the belief that "you will die the year the tree fruits". Most farmers who had described either planting sheanut trees themselves, or had heard of someone else who had, were linked to agroforestry extension projects where foreign development workers had promoted the importance of planting sheanut trees, particularly in areas lacking regeneration, i.e. northern areas of the Upper West Region. One farmer near Nandom, who described choosing sheanuts from the most productive trees and planting them in the best positions on his farm, explained that his grandfather had shown him what to do when he was a small boy. He went on to show which trees had fruited in seven years on good land, and those, which had taken twelve years on poor land. Another farmer from a village near Wa described sheanut trees that were now bearing fruit, after planting seedlings five years previously during an agroforestry project.

After completing the participatory interview, farmers were invited to offer suggestions (Table 4.20.) as what would encourage them to plant or how the sheanut industry could be improved. Information from this survey highlighted the fact that farmers in northern Ghana are keen to be involved in developing *V. paradoxa* and should be encouraged to participate in client-orientated research and development.

Table 4.15. Suggestions offered by sheanut tree farmers in northern Ghana

Described (% respondents)	**Comments offered**
58	Management needs improving
42	Farmers need agricultural extension
29	Strengthen land/tree tenure
26	Provide improved planting stock
23	Enhance market structure
13	Value of locally utilised shea products
13	Research needed
6	Importance of fruit during cultivation
6	Better processing technology required
3	Intention to plant not yet started
3	Important to diversify risk/food security

The main suggestion voiced was the need to improve the management of this species throughout northern Ghana (protection of trees from cutting, increased planting or early burning) and better provision of information through better agricultural extension. Farmers said that they would be more interested in planting sheanut trees if land and tree tenure/usufruct was strengthened, stating that customary land ownership and traditional tenure of indigenous species discouraged investment in anything other than exotic tree species. In addition, the Vagla in West Gonja said that they used to plant *Parkia biglobosa*, but due to changes imposed by the Gonja, few were now interested in even protecting naturally occurring individuals of this species. The demand for improved planting stock of *V. paradoxa* – fast maturing varieties with higher/consistent yields – was widespread and offered as motivation to encourage planting by both women and men.

The need for better processing technology was only mentioned by women although the importance of market factors in the promotion of the sheanut industry was described by both sexes. Women said that they would continue to encourage the protection or planting of sheanut trees because the products (oil, soap and traditional cosmetics) were very important in the household since they did not need to spend money on purchasing these items. A few male farmers also offered a similar viewpoint stating that the fruit was good to eat when farming or that the long-lived trees ensured an economic return from the land even when annual crops failed.

Although given as a major comment, the stakeholder demand for research into *V. paradoxa* is clearly demonstrated by the lack of scientific knowledge in many areas of the sheanut industry ranging from agronomy to improving market structure. The farmers who showed an interest in research, emphasized a desire to learn more about increasing yields through improved management and information whether better varieties existed in other parts of Ghana or Africa.

Chapter 5. Diversity of the Sheanut tree in Ghana

5.1. Morphological diversity

As the summary below indicates (Table 5.1.), *V. paradoxa* demonstrates a wide range of phenotypic variation throughout northern Ghana.

Table 5.1. Summary of morphological characteristics from productive trees

Characteristic	**Number of trees**	**Min.**	**Max.**	**Sample Mean**	**% Coefficient of variance**
Dbh[1] (cm)	291	7.0[2]	104.0	36.8	47.5%
Height (m)	285	3.0	30.0	11.3	46.7%
Canopy diameter (m)	55	3.8	17.0	8.5	38.8%
Number of stems	291	1	4[2]	1.2	41.9%
Leaf lamina length (cm)	289	9.2	22.5	14.9	15.0%
Leaf petiole length (cm)	289	3.4	13.1	7.7	19.6%
Leaf lamina width (cm)	289	2.8	8.2	4.9	17.6%
Seed length (cm)	217	1.74	3.74	2.81	11.7%
Seed breadth (cm)	217	1.66	2.73	2.16	9.3%
Seed depth (cm)	217	1.55	2.67	2.08	8.7%
Fat content (% dry kernel)	119	29.1	61.9	51.6	9.4%

[1] Equivalent dbh calculated from basal area irrespective of stem number
[2] Only stems with a dbh of ≥ 7 cm were recorded

This chapter aims to demonstrate the reliability of these means for comparing inter-tree, site, location and geographic area variation. Possible environmental causes of some of the inter-site variation are identified and an effort made to reduce these effects which have confounded attempts to demonstrate genetic variation. Lastly, population similarity is assessed using phenotypic descriptors not correlated with environmental effects and the results compared with those obtained from isozyme analysis.

5.1.1. Tree form

Short 'dwarf' varieties (Plate 5.1.), often multi-stemmed, were commonly found on petroferric phase soils underlain with Voltaian sandstones in the east of the region, notably at Sankpala, Disiga and Yendi, with a site mean height of between 5-6m. Larger trees (site mean 7-16m), usually with a single short bole and spreading canopy (often 'round' (Plate 5.2.) though also 'erect' or 'conical') were commonly seen on the granite uplands that form a crescent to the north and west of Ghana. Tall, long boled 'timber' trees (site mean 17-22m) with a compact high canopy were noted in the southwest of the study site, particularly in association with watercourses, unmanaged woodland or in high rainfall areas. Linear regression of 255 sampled trees showed that height and basal area were significantly related: $BA = 0.016h - 0.054$, $r = 0.693$, $p < 0.001$ (Figure 5.1.).

Plate 5.1. Mature 'dwarf' variety of *V. paradoxa* near Tolon

Figure 5.1. Basal Area against Tree Height

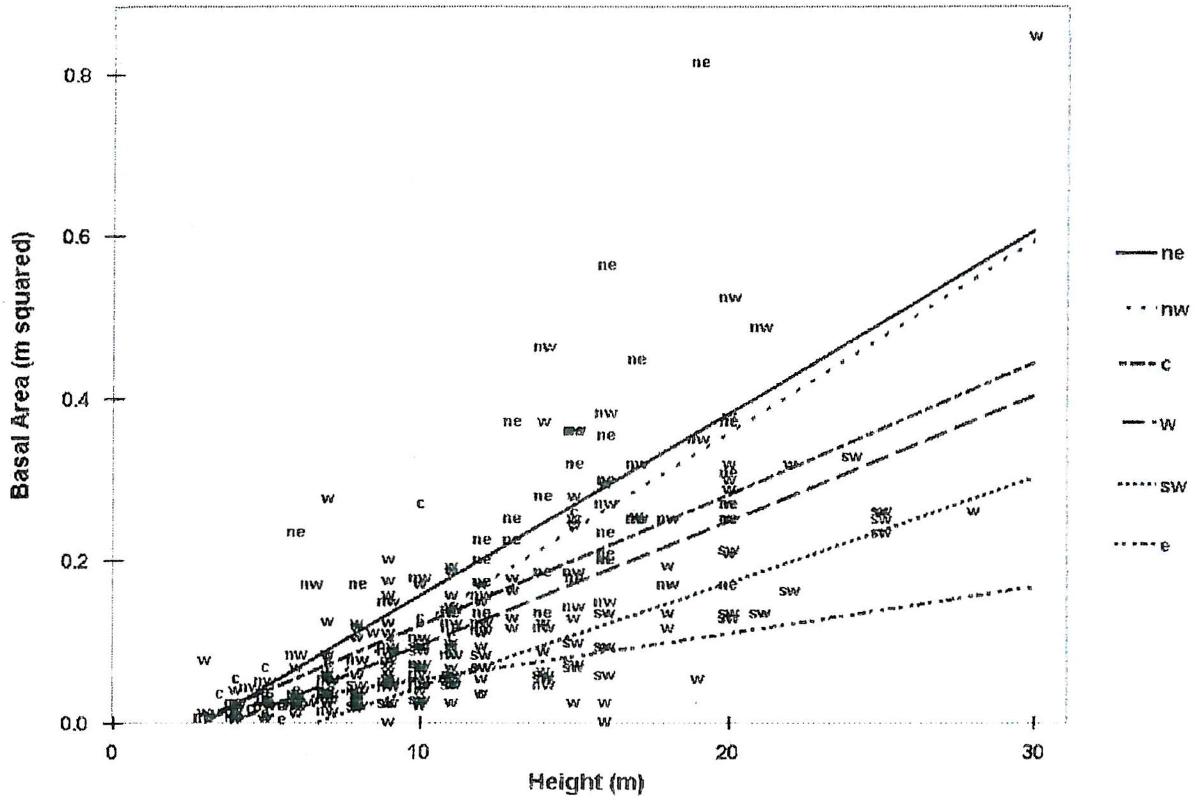
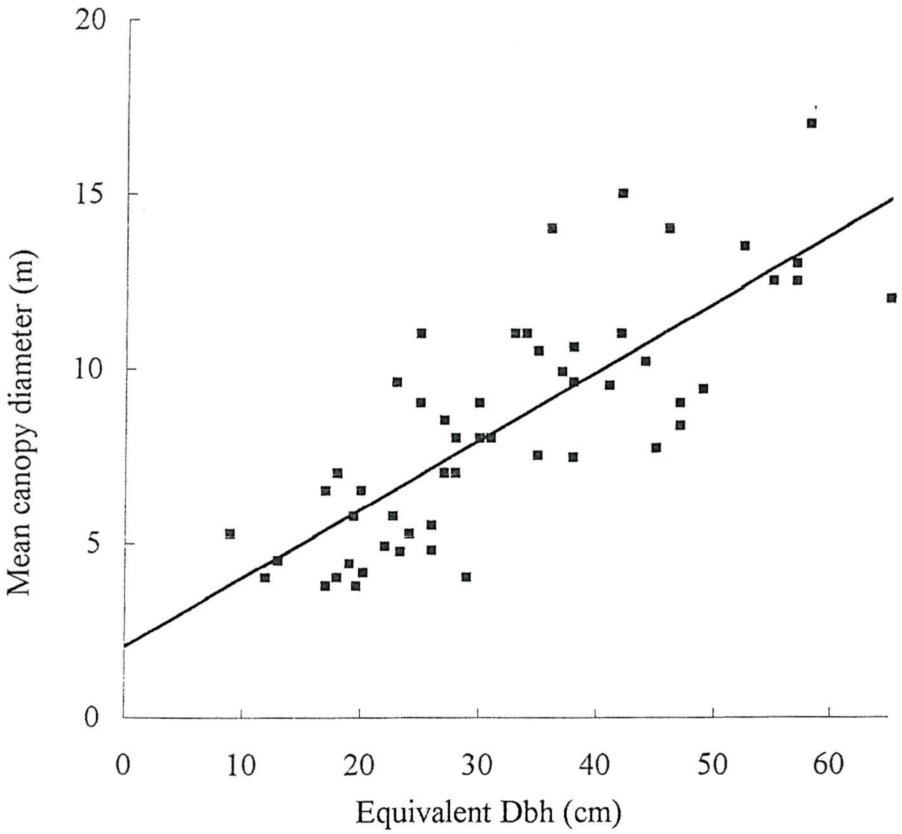


Plate 5.2. Mature 'round' variety of *V. paradoxa* near Bole

The regression lines shown for BA against height for each geographic area confirm the observations stated above but also highlight the potential role of genetics / environment in determining tree form due to the presence of stronger relationships when geographical groups are analysed seperately. Higher BA to height ratios are common in extensively farmed parklands in the lower rainfall areas to the north whereas low BA to height ratios are more common at high rainfall areas in the south-west and east. Since tree form can be affected by both plant genetics and by many environmental factors, e.g. water and light availability during early growth, this observation is most interesting because the lowest mean BA to height ratio was recorded at a high rainfall site (Yendi) despite the fact that these trees were also thought to be of the 'dwarf' variety. This geographic area, however, is only represented by one site. A small but random sample of 55 trees surveyed throughout the study area (small sample size prevented analysis by geographic area), showed that diameter at breast height and crown diameter were also significantly related: $cd = 0.197dbh + 2.027$, $r = 0.793$, $p < 0.001$ (Figure 5.2.).

Figure 5.2. Mean canopy diameter against Dbh



Accurate interpretation of the influence of either genetics or environment on tree form, is still limited due to lack of data on age and past history/management for individual trees.

5.1.2. Leaf

An analysis of 'skewness' was performed in order to determine whether the sampled characteristics had normal distributions, and could be used with confidence as representative mean values for each tree. Less than 9% (scattered around study area) of all leaf lamina, petiole and width means by tree (n = 289), had a skewness value greater than –1 or less than +1 and a ratio of skewness to its standard error, of greater than +2 or less than –2. Since accurate assessment, of whether those samples had a distribution that truly differed significantly from normality, would need much larger numbers of leaves per tree to be sampled (Sokal & Rohlf, 1997) and taking into account field logistics (availability of time). This study will include all individual tree means following the assumption that this analysis illustrates that a reasonable sampling strategy had been employed and that the greater proportion of trees showed normal, continuous variation in these characters.

In order to determine how representative the random tree sampling technique had been for a population, ANOVA was used to compare differences between the means of trees sampled at two sites at each location (less than 5 km apart) along the latitudinal transect (Branam, Bole, Wa and Nandom). Results from this analysis (Table 5.2.) clearly demonstrate that many significant differences existed between sampled trees, as shown by the example from Branam (Table 5.3.), and that the null hypothesis – tree leaf means are equal in a population – must be rejected. This evidence suggests that a representative proportion of variation in leaf characteristics has been sampled at each location and that these individual tree means offer a reliable way to compare inter-tree variation.

The lack of any significant differences between sites at each location indicates that the null hypothesis – 'site' samples of population leaf means are equal within a 5km 'location' – must be accepted. This evidence suggests that sampling one site per location will provide a representative sample for a given population. The notable differences (p = 0.07 to 0.09) between the petiole lengths at Wa and lamina lengths at Nandom are thought to be due to environmental differences, i.e. the southern site at Wa was on drier sandy soil near an escarpment whereas the northern site was on loamy soil near a small seasonal stream. Though variation is not significant it does highlight the fact that while these trees were not likely to be genetically isolated, identification of the cause of these differences is currently confounded without more detailed data on micro-environmental variation. Without additional data this study therefore assumes that environmental effects on leaf characters at a location is usually negligible, but can be noticeable between certain sites.

Table 5.2. Variation between tree and site leaf means along a 1° latitudinal gradient

Location	Site	Trees sampeld	Leaf lamina				Leaf petiole				Leaf width			
					Significance level between:				Significance level between:				Significance level between:	
			Mean	Std dev.	Trees	Sites	Mean	Std dev.	Trees	Sites	Mean	Std dev.	Trees	Sites
Branam	E	10	14.8	*2.9*	0.000	0.182	8.0	*1.3*	0.000	0.785	4.6	*0.7*	0.000	0.211
	W	9	16.6	*2.8*			7.8	*1.4*			5.1	*1.0*		
Bole	E	12	14.8	*2.2*	0.000	0.656	8.6	*1.3*	0.000	0.840	4.9	*0.6*	0.000	0.480
	N	18	15.2	*2.8*			8.6	*1.1*			4.7	*0.9*		
Wa	N	11	15.1	*2.1*	0.000	0.166	7.4	*0.8*	0.000	0.078	5.2	*0.9*	0.000	0.382
	S	12	13.9	*1.9*			6.6	*1.2*			4.9	*0.8*		
Nandom	N	12	14.2	*1.9*	0.000	0.085	7.8	*1.1*	0.000	0.635	4.7	*0.8*	0.000	0.315
	S	12	15.4	*1.5*			7.6	*1.0*			5.1	*0.7*		

Table 5.3. Leaf lamina variation between sheanut trees at Branam sites in Brong Ahafo

Tree	Mean	N	Std. Dev.	W1	W2	W3	W4	W5	W7	W8	W9	W10	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
W1	18.9	20	*3.1*	-	**			*	**	**	**	*	**		**	**	**	**	**	**	**	**
W2	14.7	20	*1.9*	**	-	**	**	**						*					**	**	*	
W3	18.0	18	*1.8*		**	-		**	*	**	**		**		**	**	**	**	**	**	**	*
W4	18.6	20	*1.9*		**		-	**	**	**	**		**		**	**	**	**	**	**	**	**
W5	21.3	20	*3.1*	*	**	**	**	-	**	**	**	**	**	**	**	**	**	**		**	**	**
W7	15.6	20	*2.4*	**		*	**	**	-	*	**						*		**	**	**	
W8	13.1	19	*1.8*	**		**	**	**	*	-		**		**					**			*
W9	12.9	18	*1.5*	**		**	**	**	**		-	**		**					**			**
W10	16.5	20	*2.5*	*				**		**	**	-			**		**	**	**	**	**	
E1	15.0	20	*1.7*	**		**	**	**					-						**	**	**	
E2	17.0	17	*1.7*		*			**		**	**			-	**	*	**	**	**	**	**	
E3	13.8	20	*1.5*	**		**	**	**				**		**	-				**	*		
E4	14.4	18	*2.3*	**		**	**	**						*		-			**	**		
E5	13.1	20	*1.8*	**		**	**	**	*			**		**			-		**			**
E6	13.5	18	*1.7*	**		**	**	**				**		**				-	**			
E7	21.6	20	*2.3*	**	**	**	**		**	**	**	**	**	**	**	**	**	**	-	**	**	**
E8	11.4	19	*1.3*	**	**	**	**	**	**			**	**	**	*	**			**	-		**
E9	12.4	20	*1.3*	**	*	**	**	**	**			**	**	**					**		-	**
E10	15.7	19	*1.4*	**		*	**	**		*	**						**		**	**	**	-
Total	**15.7**	**366**	*3.5*																			

blank cell indicates no significant difference between tree means

*   indicates significance level p<0.05

** indicates significance level p<0.01

When all study site means were compared using ANOVA analysis, a significance level p<0.001 was obtained for all leaf characters and the null hypothesis – leaf site means are equal throughout the study area – must be rejected. There was, however, a wide overlap between homogeneous ANOVA determined subsets for leaf site means (Table 5.4.) and relatively few significant differences between sites across the whole study area (Table 5.5.). Although there were more differences between sites located in dissimilar geographical areas, there is no firm evidence for spatial isolation due to the lack of significant differences between very widely separated sites. There is, however, evidence for differences due to environmental variation, i.e. water availability influencing leaf size. For example, petiole site means are commonly significantly different between high and low rainfall sites, this influence of water availability is highlighted by the fact that no significant differences exist between petiole means from NW, W and SW sites except for WaS, a site previously identified as environmentally marginal.

Table 5.4. ANOVA determined homogeneous subsets for leaf petiole site means

Site	**Trees**	**Subset for alpha = 0.05**							
		1	2	3	4	5	6	7	8
Polmakom	10	5.72							
Disiga	10	5.90	5.90						
Navrongo	10	5.94	5.94	5.94					
Daboya	10	6.40	6.40	6.40	6.40				
WaS	12	6.56	6.56	6.56	6.56	6.56			
Sankpala	10	6.74	6.74	6.74	6.74	6.74	6.74		
Walewale	12	6.90	6.90	6.90	6.90	6.90	6.90	6.90	
Tolon	10	7.19	7.19	7.19	7.19	7.19	7.19	7.19	7.19
Damongo	10	7.20	7.20	7.20	7.20	7.20	7.20	7.20	7.20
Tumu	5	7.25	7.25	7.25	7.25	7.25	7.25	7.25	7.25
WaN	11	7.38	7.38	7.38	7.38	7.38	7.38	7.38	7.38
NandomS	12	7.56	7.56	7.56	7.56	7.56	7.56	7.56	7.56
NandomN	12		7.77	7.77	7.77	7.77	7.77	7.77	7.77
BranamW	9		7.78	7.78	7.78	7.78	7.78	7.78	7.78
Bomburi	11			7.87	7.87	7.87	7.87	7.87	7.87
BranamE	10				7.96	7.96	7.96	7.96	7.96
Yendi	10				8.14	8.14	8.14	8.14	8.14
BoleW	39					8.47	8.47	8.47	8.47
BoleN	18						8.56	8.56	8.56
BoleE	12						8.65	8.65	8.65
BoleC	24							8.76	8.76
Mandari	10							8.76	8.76
Subinso	8								8.88
Kiape	4								9.14
Sig.		0.10	0.08	0.06	0.17	0.07	0.07	0.09	0.06

Post hoc tests using Tukey Honestly Significant Difference tests[a, b]
Means for groups in homogeneous subsets are displayed.

[a] Uses Harmonic Mean Sample Size = 9.969.

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

Table 5.5. Sheanut tree leaf variation between sites in geographic areas of Ghana

	Site	Daboya	Sankpala	Tolon	Yendi	Disiga	Navrongo	Polmakom	Walewale	NandomN	NandomS	Tumu	WaN	WaS	BranamE	BranamW	Subinso	BoleE	BoleN	BoleW	BoleC	Bomburi	Damongo	Kiape	Mandari
Petiole	Daboya																**	**	**	**	**			*	**
	Sankpala		C														*	*	*	*	**				*
	Tolon																								
	Yendi				E	**	*	**																	
	Disiga				**										*		**	**	**	**	**	*		**	**
	Navrongo				*		NE								*		**	**	**	**	**	*		**	**
	Polmakom				**					*					**	*	**	**	**	**	**	**		**	**
	Walewale																		*	*	**				
	NandomN							*																	
	NandomS																								
	Tumu											NW													
	WaN																								
	WaS																**	**	**	**	**			*	**
	BranamE					*	*	**																	
	BranamW							*								SW									
	Subinso	**	*			**	**	**						**											
	BoleE	**	*			**	**	**						**											
	BoleN	**	*			**	**	**	*					**											
	BoleW	**	*			**	**	**	*					**											
	BoleC	**	**			**	**	**	**					**							W				
	Bomburi					*	*	**																	
	Damongo																								
	Kiape	*				**	**	**						*											
	Mandari	**	*			**	**	**						**											
Lamina	Daboya															*									
	Sankpala		C		*											**				**	**	*		*	**
	Tolon																								
	Yendi		*		E	**																			
	Disiga				**											**				**	**	*		*	**
	Navrongo						NE																		
	Polmakom																								
	Walewale																								
	NandomN																								
	NandomS																								
	Tumu											NW													
	WaN																								
	WaS																								
	BranamE																								
	BranamW	*	**			**										SW									
	Subinso																								
	BoleE																								
	BoleN																								
	BoleW		**			**																			
	BoleC		**			**															W				
	Bomburi		*			*																			
	Damongo																								
	Kiape		*			*																			
	Mandari		**			**																			
Width	Daboya								**		*		**							**	**	**		**	**
	Sankpala		C																			**			
	Tolon																								
	Yendi				E																				
	Disiga								**				*							**	*	**		**	**
	Navrongo						NE															*			
	Polmakom																								
	Walewale	**				**																			
	NandomN																								
	NandomS	*																							
	Tumu											NW													
	WaN	**				*																			
	WaS																								
	BranamE																					*			
	BranamW															SW									
	Subinso																								
	BoleE																								
	BoleN																					*			
	BoleW	**				**																			
	BoleC	**				*															W				
	Bomburi	**	**			**	*								*				*				**		
	Damongo																					**			
	Kiape	**				**																			
	Mandari	**				**																			

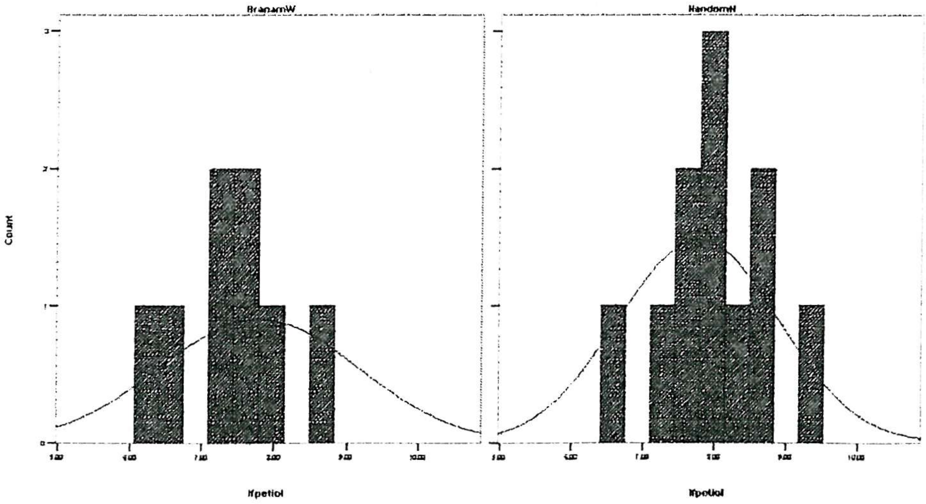
All leaf characters were seen to demonstrate continuous variation as analyses performed indicated a 'normal distribution' for the majority of sites (Table 5.6.). Histograms of the sites which were identified as differering significantly from normality (Figure 5.3.) suggest these results are probably due to low sample numbers, coupled with the chance selection of some outliers, as a normal distribution is visually apparent.

Table 5.6. Skewness and kurtosis of the distribution of mean petiole by tree at all sites

Site	Kurtosis	S.E. of Kurtosis	Ratio K/SEK	Skewness	S.E. of Skewness	Ratio S/SES
BoleE	-1.32	1.23	-1.08	-0.44	0.64	-0.68
BoleN	0.77	1.04	0.74	0.39	0.54	0.73
BoleW	-0.21	0.74	-0.29	0.00	0.38	0.00
BoleC	-0.32	0.92	-0.35	-0.79	0.47	-1.68
Bomburi	-1.16	1.28	-0.91	0.49	0.66	0.74
BranamE	-1.57	1.33	-1.17	0.22	0.69	0.32
BranamW	**2.81**	**1.40**	**2.00**	**1.48**	**0.72**	**2.07**
Daboya	0.37	1.33	0.27	0.26	0.69	0.38
Damongo	2.57	1.33	1.92	1.26	0.69	1.84
Disiga	0.11	1.33	0.08	0.36	0.69	0.52
Kiape	1.11	2.62	0.42	-0.07	1.01	-0.07
Mandari	1.25	1.33	0.94	0.88	0.69	1.27
NandomN	**2.88**	**1.23**	**2.34**	**-1.43**	**0.64**	**-2.25**
NandomS	-0.27	1.23	-0.22	0.01	0.64	0.02
Navrongo	-1.24	1.33	-0.93	-0.46	0.69	-0.67
Polmakom	0.61	1.33	0.45	-0.28	0.69	-0.41
Sankpala	-0.17	1.33	-0.13	-0.14	0.69	-0.20
Subinso	1.82	1.48	1.23	1.11	0.75	1.48
Tolon	-0.51	1.33	-0.38	0.56	0.69	0.82
Tumu	2.10	2.00	1.05	-0.74	0.91	-0.82
Walewale	0.11	1.23	0.09	0.48	0.64	0.75
WaN	-1.27	1.28	-1.00	0.29	0.66	0.43
WaS	-0.12	1.23	-0.10	0.47	0.64	0.73
Yendi	2.17	1.33	1.63	1.32	0.69	1.92

Analysis of sites in bold suggest a distribution which differs significantly from normality

Figure 5.3. Histograms of petiole distribution at sites lacking 'normality'



This normal distribution and continuous variation of all leaf characteristics is further confirmed using all tree means sampled (Petiole: Kurtosis = 0.24, Skewness = 0.33) as shown below in Figure 5.4. The positive skew is probably due to the fact that the sample size (number of trees) was larger in western areas from trees with longer petioles. Since very few significant differences exist between sites in similar geographic regions, tree means from these sites were combined (eastern area omitted due to small sample size) to illustrate the range of variation in petiole characters across the study area (Figure 5.5.).

Figure 5.4. Histogram showing normal distribution of all petiole means by tree

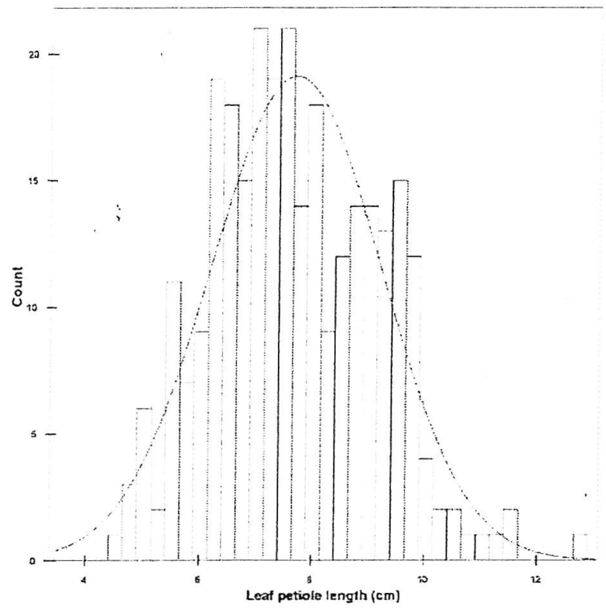
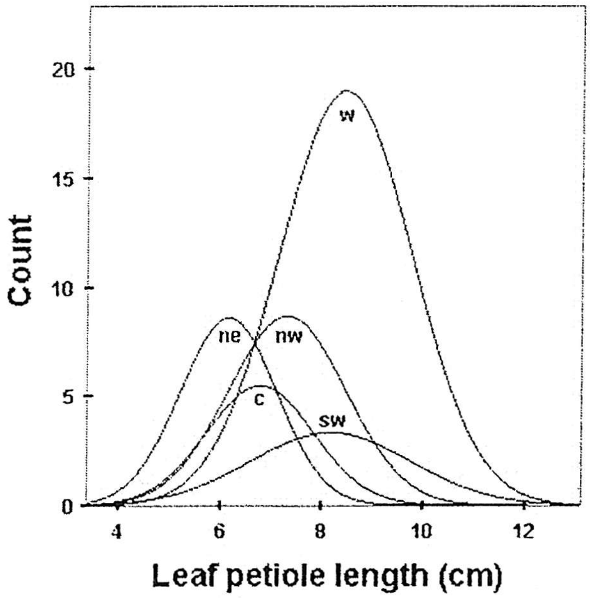
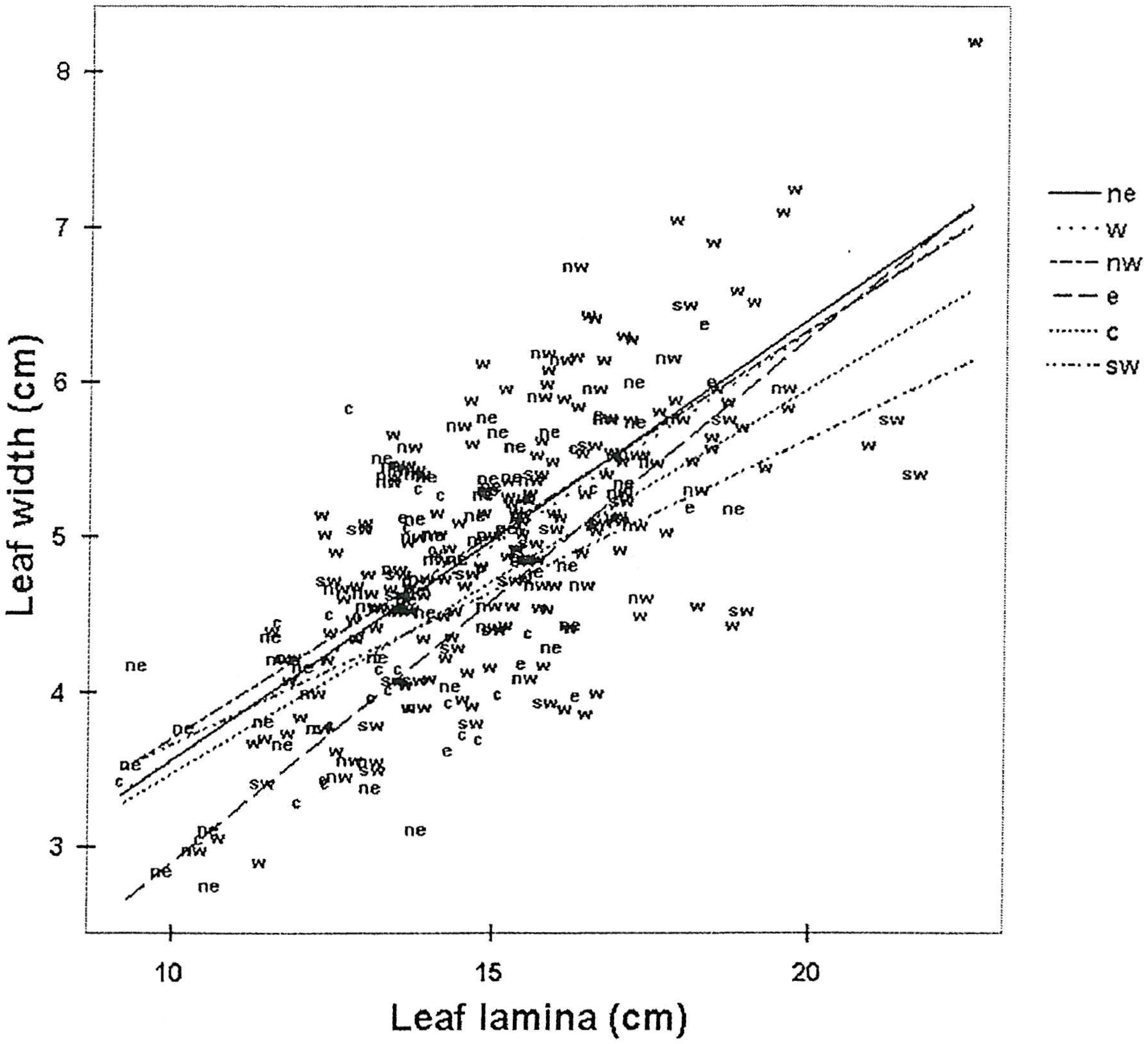


Figure 5.5. Distribution of petiole means by numbers of trees in each geographic area



The smallest leafed trees were recorded at the low altitude sites - Disiga, Sankpala and Daboya - although trees with the shortest petioles were noted on granite uplands near Polmakom, the only site surveyed in the Sudan ecozone. The largest leaves were recorded from individuals in the south-western and western areas, most notably at Kiape and Mandari. All leaf characters were positively and significantly related with each other, e.g. LfW = 0.266LfL + 0.920, $r = 0.693$, $p < 0.001$ (Figure 5.6.). Regression analysis by geographic location also gave very similar relationships except for the eastern site which had the fewest number of samples. No tree and leaf size variables were significantly related ($r < \pm0.07$, $p > 0.25$). It was also observed that the lamina and petioles usually turned red when the plant was stressed, e.g. after cutting the outer bark during air-layering experiments.

Figure 5.6. <u>Leaf Width against Leaf Lamina Length</u>



Each data point represents a mean of twenty fresh leaves from each tree (number of trees = 289)

5.1.3. Seed

Round and long seeds appeared to be two visually distinct seed shape types (Plate 5.3.), however, after tests for normality (cf. section 5.2.2.) no evidence was found for this morphological separation, as continuous variation was evident for this character in the majority of individual trees. Less than 11% of all seed length, breadth and depth means by tree (n = 217), had a skewness value greater than −1 or less than +1 and a ratio of skewness to its standard error, of greater than +2 or less than −2. Since accurate assessment, of whether those samples had a distribution that truly differed significantly from normality, would need much larger numbers of seeds per tree to be sampled (Sokal & Rohlf, 1997) and taking into account field logistics (availability of time). This study will include all individual tree seed means following the assumption that this analysis illustrates that a reasonable sampling strategy had been employed and that the greater proportion of trees showed normal, continuous variation in these characters.

There is, however, the possibility that some individuals could be exhibiting seed dimorphism. Unfortunately analyses of potential candidates for this trait were inconclusive due to the small sample size per tree ($n \leq 20$). For example, multivariate analysis of all individual seed charateristics of sheanut tree #78 from BoleE (Kurtosis = 7.6, Skewness = 2.4), with HCA using Wards method, gave two clusters of seeds (Figure 5.7.) whose means showed significant differences between quantitative variables but not between their ratios (Table 5.7.). Dimorphism may therefore be occurring but still difficult to identify with certaintly as the ratios indicated that all seeds were of similar 'shape'.

Figure 5.7. Cluster identity for possible seed dimorphism in sheanut tree #78 from BoleE

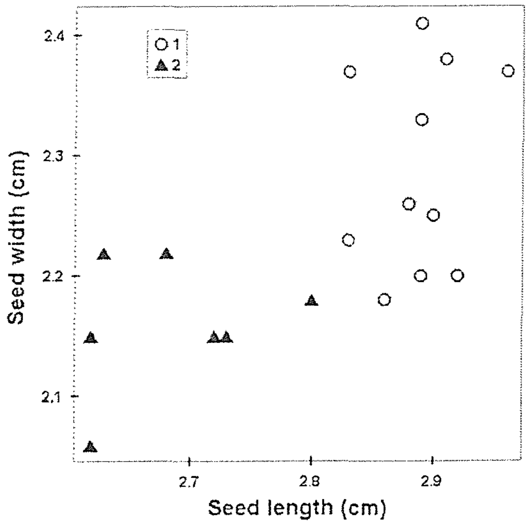


Table 5.7. ANOVA of two clusters generated using HCA from seeds of sheanut tree #78

Seed character		Sum of Squares	df	Mean Square	F	Sig.
NtL	Between Groups	0.180	1	0.180	72.182	0.000
	Within Groups	0.042	17	0.002		
	Total	0.222	18			
NtW1	Between Groups	0.064	1	0.064	11.265	0.004
	Within Groups	0.096	17	0.006		
	Total	0.160	18			
NtW2	Between Groups	0.064	1	0.064	36.318	0.000
	Within Groups	0.030	17	0.002		
	Total	0.093	18			
NtL/NtW1	Between Groups	0.003	1	0.003	1.323	0.266
	Within Groups	0.032	17	0.002		
	Total	0.035	18			
NtL/NtW2	Between Groups	0.002	1	0.002	1.321	0.266
	Within Groups	0.022	17	0.001		
	Total	0.023	18			
NtW1/NtW2	Between Groups	0.000	1	0.000	0.069	0.797
	Within Groups	0.023	17	0.001		
	Total	0.024	18			

ANOVA was also used to compare differences between the means of trees sampled at sites along the latitudinal transect. Results from this analysis (Table 5.8.) clearly demonstrate that many significant differences existed between sampled trees at all sites, as shown by the example from Wa (Table 5.9.), and that the null hypothesis – tree seed means are equal in a population – must be rejected. This suggests that a representative proportion of variation in seed characteristics has been sampled at each location and that these individual tree means offer a reliable way to compare inter-tree variation.

The lack of significant differences between 8 out 12 of the site/character combinations at each location indicates that the null hypothesis – 'site' samples of population seed means are equal within a 5km 'location' – should be accepted if the assumption below holds true. This evidence suggests that sampling one site per location will usually provide a representative sample for a given population. The significant differences ($p < 0.04$) between the site means at Wa and Bole are considered to be due to local environmental variation. Without additional data this study will assume that environmental effects on seed size at a location are usually negligible providing that inter-site variation is low. Seed characters appear more susceptible to environmental variation than leaves, as significant effects can be identified between sites. The Wa example, also highlights that different levels of seed variation are also found at different sites at the same location and that analysis of site variation of all characters may offer a useful way to compare site means.

Table 5.8. Variation between tree and site seed means along a 1° latitudinal gradient

Location	Site	Trees sampeld	Seed Length				Seed Breadth				Seed Depth			
					Significance level between:				Significance level between:				Significance level between:	
			Mean	Std dev.	Trees	Sites	Mean	Std dev.	Trees	Sites	Mean	Std dev.	Trees	Sites
Branam	E	10	2.86	*0.22*	0.000	0.444	2.10	*0.09*	0.000	0.592	2.02	*0.07*	0.000	0.595
	W	9	2.78	*0.22*			2.13	*0.12*			2.05	*0.12*		
Bole	E	10	3.19	*0.27*	0.000	0.495	2.42	*0.14*	0.000	0.034	2.28	*0.12*	0.000	0.038
	W	14	3.12	*0.24*			2.30	*0.12*			2.19	*0.10*		
Wa	N	11	2.86	*0.12*	0.000	0.003	2.25	*0.16*	0.000	0.099	2.20	*0.14*	0.000	0.039
	S	12	2.63	*0.19*			2.14	*0.15*			2.09	*0.10*		
Nandom	N	12	2.74	*0.16*	0.000	0.745	2.14	*0.14*	0.000	0.664	2.08	*0.12*	0.000	0.788
	S	12	2.77	*0.22*			2.17	*0.14*			2.10	*0.13*		

Table 5.9. Seed length variation between sheanut trees at Wa sites in Upper West

Tree	Mean	N	Std Dev.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11
S1	2.66	10	0.10	-						**				**			**				**				**	
S2	2.70	9	0.23		-					**				**			*				**				**	
S3	2.88	10	0.18			-			**	**	*			**												
S4	2.83	10	0.18				-		**	**				**											*	
S5	2.66	10	0.17					-		**				**			**				**				**	
S6	2.43	9	0.26			**	**		-						**	**	**	**	**	*	**	**	**	**	**	**
S7	2.34	8	0.10	**	**	**	**	**		-		*	*		**	**	**	**	**	**	**	**	**	**	**	**
S8	2.61	9	0.09			*					-			*	*		**				**				**	
S9	2.63	9	0.12							*		-		*			**				**				**	
S10	2.62	10	0.13							*			-	*	*		**				**				**	
S11	2.32	10	0.16	**	**	**	**	**			*	*	*	-	**	**	**	**	**	**	**	**	**	**	**	**
S12	2.90	10	0.29						**	**	*		*	**	-											
N1	2.84	10	0.15						**	**				**		-										
N2	2.98	10	0.12	**	*			**	**	**	**	**	**	**			-									
N3	2.77	10	0.12						**	**				**				-							**	
N4	2.78	10	0.14						**	**				**					-						**	
N5	2.73	9	0.22						*	**				**						-	*				**	
N6	3.04	10	0.09	**	**			**	**	**	**	**	**	**						*	-		*			
N7	2.86	10	0.20						**	**				**								-				
N8	2.77	10	0.06						**	**				**							*		-		**	
N9	2.82	10	0.13						**	**				**										-	*	
N10	3.10	10	0.15	**	**		*	**	**	**	**	**	**	**				**	**	**			**	*	-	**
N11	2.78	10	0.21						**	**				**											**	-
Total	**2.75**	**223**	**0.25**																							

blank cell indicates no significant difference between tree means

* indicates significance level p<0.05

** indicates significance level p<0.01

Coeff. of Variation: NtL, north = 4.4%, south = 7.4%; NtW1, north = 7.4%, south = 7.0%

When all study sites means were compared using ANOVA analysis, a significance level $p<0.001$ was obtained for almost all seed characters and the null hypothesis – seed site means are equal throughout the study area – must be rejected. There was however, a wide overlap between homogeneous ANOVA determined subsets for seed site means (Table 5.10.) and relatively few significant differences between sites across the whole study area (Table 5.11.) though there were more than for leaf characters. The only exception to this finding was the complete lack of significant differences between any sites means of the NtW1/NtW2 ratio suggesting seeds have similar shape across the study area, although there was significant inter-tree variation in this ratio at locations. As with leaf characters, there were more differences for seed characters between sites located in dissimilar geographical areas, although there is still no firm evidence for spatial isolation due to the lack of significant differences between very widely separated sites. For example, Nandom, Walewale and Subinso were not significantly different from each other in all leaf and seed characteristics but are separated by at least 200km. There is, however, more evidence for differences due to environmental variation. For example Sankpala and Disiga are significantly different, in most seed characters, to other sites. These are most notable for being situated on the Voltaian sandstones, with poor shallow soils, which suggest a susceptibility of seed size to variation in edaphic conditions or altitude as there are few significant differences in rainfall between these sites and those on granite in the northeast.

Table 5.10. ANOVA determined homogeneous subsets for seed depth site means

Site	Trees	Subset for alpha = 0.05					
		1	2	3	4	5	6
Disiga	10	1.78					
Sankpala	10	1.83	1.83				
Tolon	10	1.94	1.94	1.94			
BranamE	10	2.02	2.02	2.02	2.02		
Navrongo	10	2.02	2.02	2.02	2.02		
BranamW	9		2.05	2.05	2.05	2.05	
Daboya	10		2.05	2.05	2.05	2.05	
Bomburi	10		2.07	2.07	2.07	2.07	
Damongo	10		2.08	2.08	2.08	2.08	
NandomN	12			2.08	2.08	2.08	
WaS	12			2.09	2.09	2.09	
NandomS	*12*			*2.10*	*2.10*	*2.10*	
Walewale	12			2.10	2.10	2.10	
Subinso	10			2.11	2.11	2.11	2.11
Polmakom	10			2.12	2.12	2.12	2.12
Mandari	5			2.17	2.17	2.17	2.17
BoleW	14				2.19	2.19	2.19
Tumu	5				2.20	2.20	2.20
WaN	11				2.20	2.20	2.20
BoleE	10					2.28	2.28
Kiape	5						2.35
Sig.		0.05	0.05	0.07	0.51	0.06	0.06

Post hoc tests using Tukey Honestly Significant Difference tests[a, b]

Means for groups in homogeneous subsets are displayed.

[a] Uses Harmonic Mean Sample Size = 9.104

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

Table 5.11. Sheanut tree seed variation between sites in geographic areas of Ghana

	Site	Daboya	Sankpala	Tolon	Disiga	Navrongo	Polmakom	Walewale	NandomN	NandomS	Tumu	WaN	WaS	BranamE	BranamW	Subinso	BoleE	BoleW	Bomburi	Damongo	Kiape	Mandari
Seed length	Daboya		**		**												*					
	Sankpala	**	C				**	**	**	**	**	**		**	**	**	**	**	**	**	**	**
	Tolon				*		**										**	**				
	Disiga	**		*		**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
	Navrongo				**	NE	**										**	**				
	Polmakom		**	**	**	**			*				**									
	Walewale		**		**																	
	NandomN		**		**		*										**	**				
	NandomS		**		**												**	*				
	Tumu		**		**						NW											
	WaN		**		**																	
	WaS				**		**										**	**				
	BranamE		**		**																	
	BranamW		**		**										SW		*					
	Subinso		**		**																	
	BoleE	*	**	**	**	**			**	**			**		*					*		
	BoleW		**	**	**	**			**	*			**									
	Bomburi		**		**														W			
	Damongo		**		**												*					
	Kiape		**		**																	
	Mandari		**		**																	
Seed breadth	Daboya				*												*				**	
	Sankpala		C				**	**	*	**	**	**	*			**	**	**	**	*	**	**
	Tolon																**	**			**	
	Disiga	*					**	**	**	**	**	**	**		*	**	**	**	**	**	**	**
	Navrongo					NE											**				**	
	Polmakom		**		**																	
	Walewale		**		**												*				*	
	NandomN		*		**												**				**	
	NandomS		**		**												*				*	
	Tumu		**		**						NW											
	WaN		**		**																	
	WaS		*		**												**				**	
	BranamE																**				**	
	BranamW				*										SW		*				**	
	Subinso		**		**																	
	BoleE	*	**	**	**	**		*	**	*			**	**	*					*		
	BoleW		**	**	**																	
	Bomburi		**		**														W			
	Damongo		*		**												*				*	
	Kiape	**	**	**	**	**		*	**	*			**	**	**					*		
	Mandari		**		**																	
Seed depth	Daboya				**												*				*	
	Sankpala		C				**	**	*	**	**	**	**			**	**	**	*	*	**	**
	Tolon											**					**	**			**	
	Disiga	**				*	**	**	**	**	**	**	**	*	*	**	**	**	**	**	**	**
	Navrongo				*	NE											*				**	
	Polmakom		**		**																	
	Walewale		**		**																	
	NandomN		*		**																	
	NandomS		**		**																	
	Tumu		**		**						NW											
	WaN		**	**	**																	
	WaS		**		**																	
	BranamE				*												**				**	
	BranamW				*										SW						*	
	Subinso		**		**																	
	BoleE	*	**	**	**	*								**								
	BoleW		**	**	**																	
	Bomburi		*		**														W			
	Damongo		*		**																	
	Kiape	*	**	**	**	**								**	*							
	Mandari		**		**																	

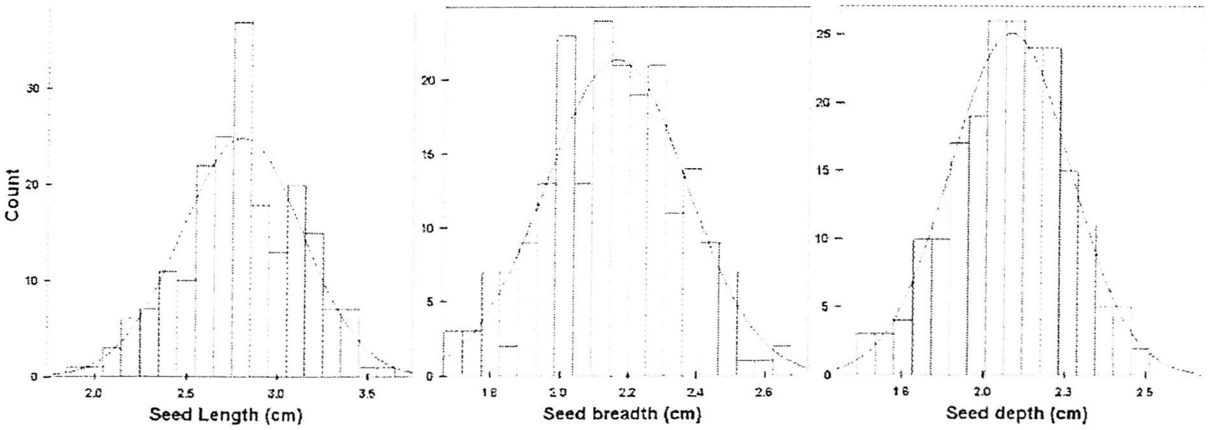
All seed characters were seen to demonstrate continuous variation as analyses performed indicated a 'normal distribution' for the majority of sites. For seed length and depth, only two sites at Nandom and Branam were identified as differing significantly from normality, whereas all sites were found to be normally distributed for seed breadth. Since this is the same result as found for leaf characters, it suggests that slightly sub-optimum sampling at these sites may be to blame as visual interpretation of histograms again indicates normality with either occasional outliers present or a tightly clustered distribution..

This normal distribution and continuous variation of all leaf characteristics is further confirmed using all tree means sampled in the whole study area, as shown below in Table 5.12. and Figure 5.8.

Table 5.12. Kurtosis and Skewness of all tree means for seed charateristics

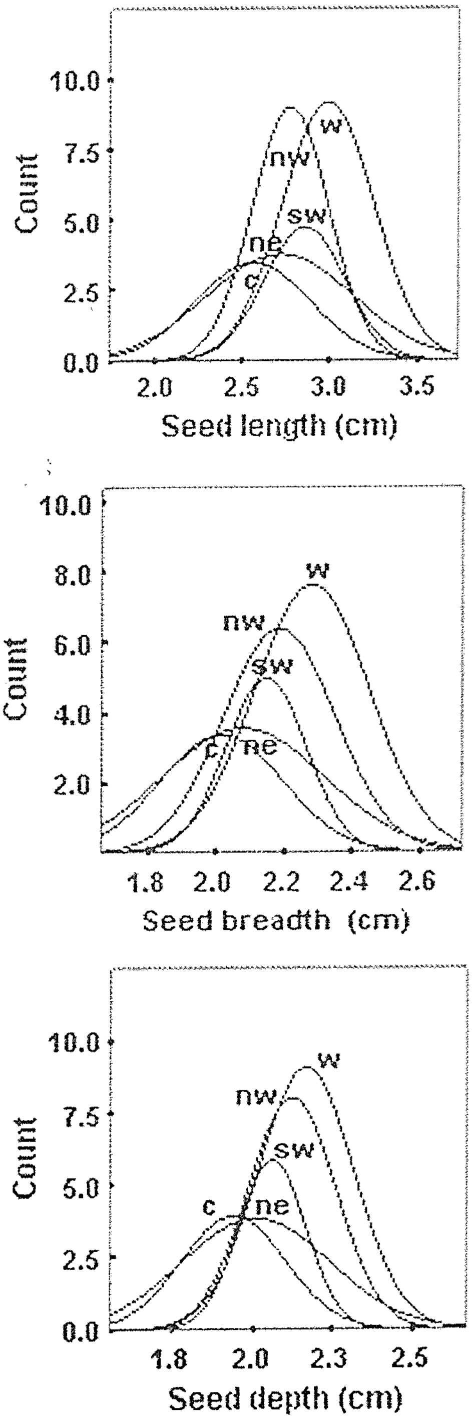
Seed Character	**Kurtosis (K)**	Std Error of Kurtosis (SEK)	Ratio of K/SEK	**Skewness (S)**	Std Error of Skewness (SES)	Ratio of S/SES
NtL	**0.18**	0.34	0.55	**-0.14**	0.17	-0.81
NtW1	**-0.04**	0.34	-0.13	**0.05**	0.17	0.31
NtW2	**0.29**	0.34	0.85	**-0.15**	0.17	-0.91

Figure 5.8. Histograms showing normal distribution of all seed means by numbers of trees



Since relatively few significant differences exist between sites in similar geographic regions, tree seed means from these sites were combined to illustrate the range of variation in petiole characters across the study area (Figure 5.9.).

Figure 5.9. Distribution of seed means by numbers of trees in each geographic area



The smallest seeds were collected from trees at Disiga and Sankpala, whereas the largest seeds were found near Bole, particularly at Kiape. Some trees at Polmakom had notable long but narrow seeds. All seed size variables were found to be positively and significantly related, e.g. NtW1 = 0.457NtL + 0.882, $r = 0.747$, $p < 0.001$ (Figure 5.10.). Regression analysis by geographic location also gave very similar relationships except for the south west (see fit lines on graph) which had an r^2 value < 0.1 as compared to 0.4– 0.7 for other areas. Small but positive significant relationships also existed between tree and seed size ($r = 0.149$ to 0.269, $p < 0.03$ to 0.001).

Other variable seed traits not used due to difficulties with quantification included distinctly 'pointed' seeds and the presence of a 'hood' over the hilum. Pale coloured seeds were common towards the east and north.

Plate 5.3. Variability of *V. paradoxa* seed shape in northern Ghana

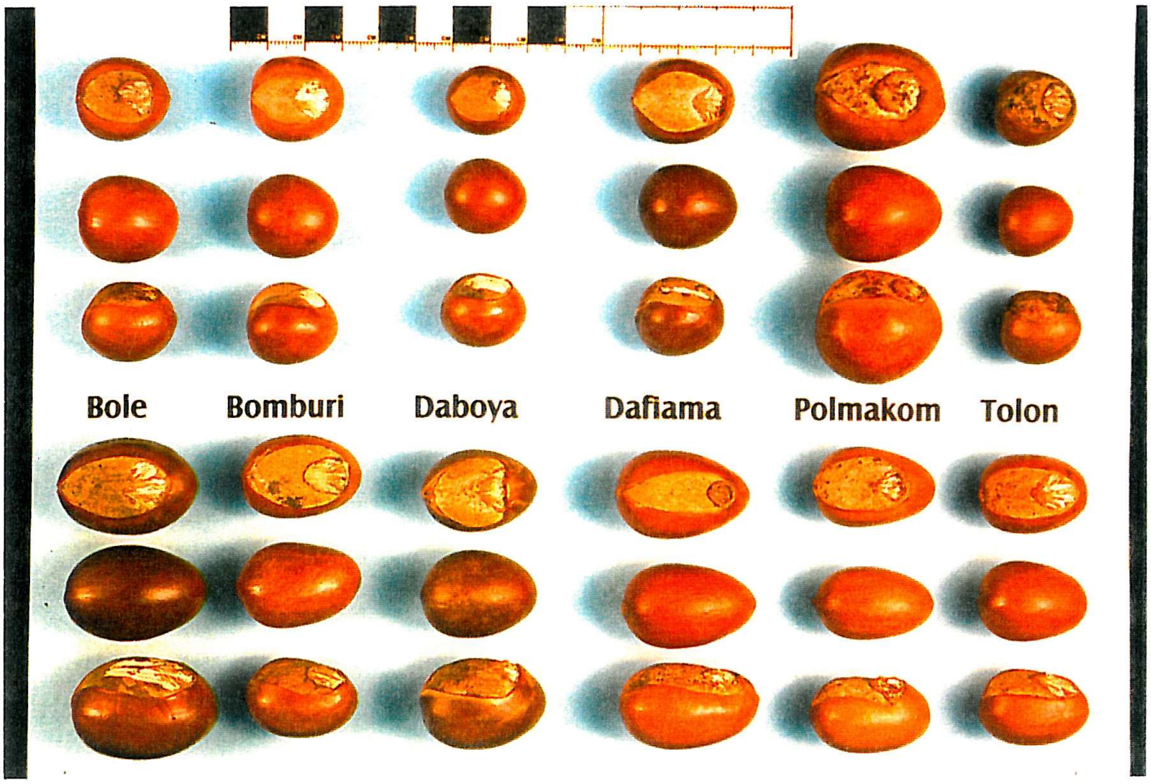
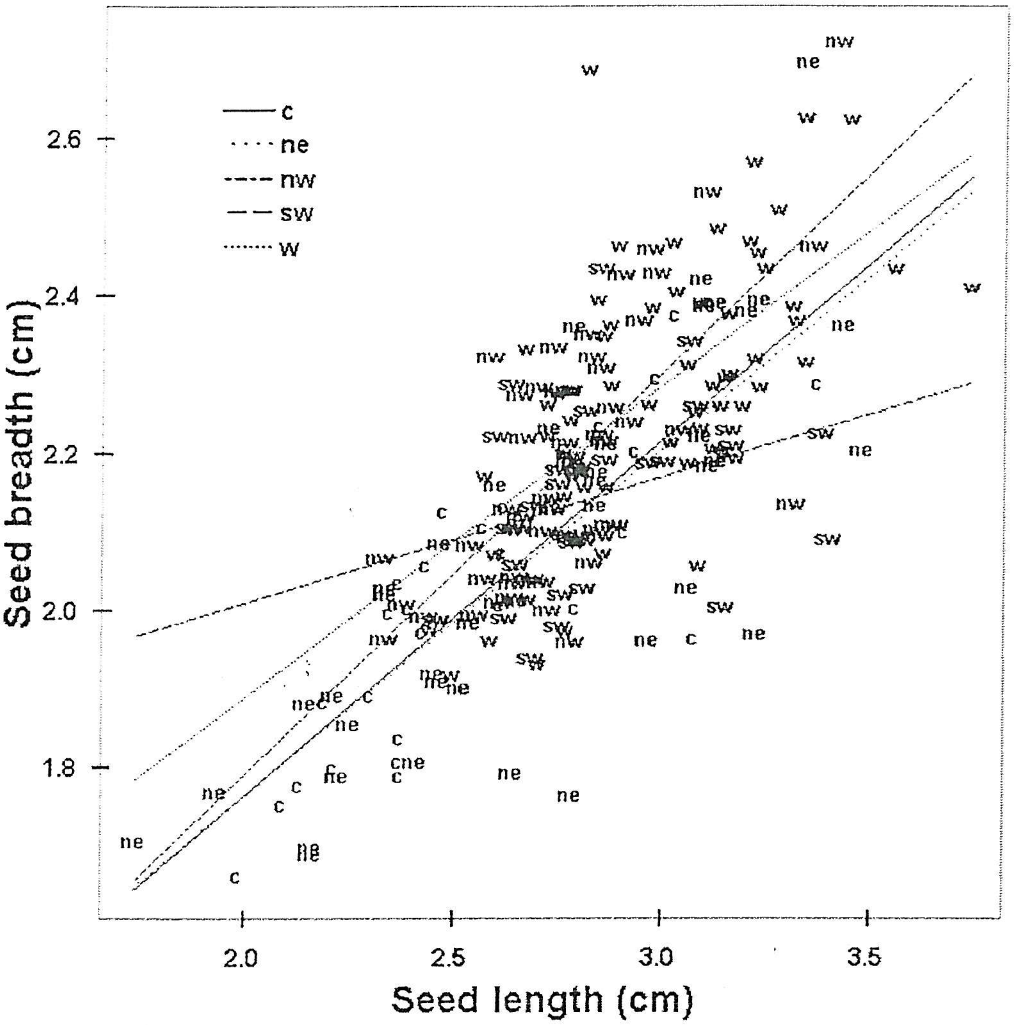


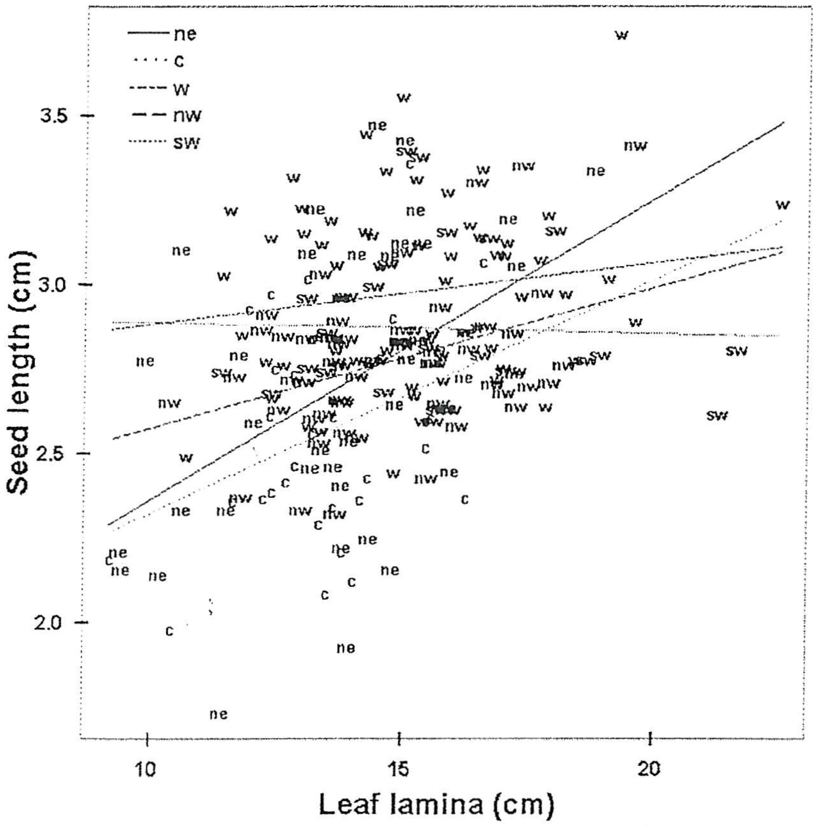
Figure 5.10. Seed Breadth against Seed Length



Each point represents the mean of twenty seeds from each tree (number of trees = 217)

Leaf and seed size were also significantly related, e.g. NtL = 0.054LfL + 2.010, r = 0.369, $p < 0.001$ (Figure 5.11.). Although weak, this relationship does demonstrate potential diversity in the study area with Polmakom, Bole and Daboya notable for the presence of trees with seeds larger than expected for the recorded leaf size. Without additional data on site micro-environment and tree age it is still impossible to predict the cause of this variation although exceptional individuals can be identified through analysis of residuals. Regression analysis by geographic location gave very variable relationships for most areas with a possible trend for increasing strength of this relationship towards the northeast (r^2 : ne = 0.22, nw = 0.14, c = 0.11, w = 0.02, sw = 0.00). Other analysis, however, showed that the LfL/Nt ratio was normally distributed in all areas and that there were few differences between sites except that this ratio was significantly smaller than BranamW at Polmakom, Bole and Daboya, i.e. larger seeds than expected for the given leaf size.

Figure 5.11. Seed Length against Leaf Lamina

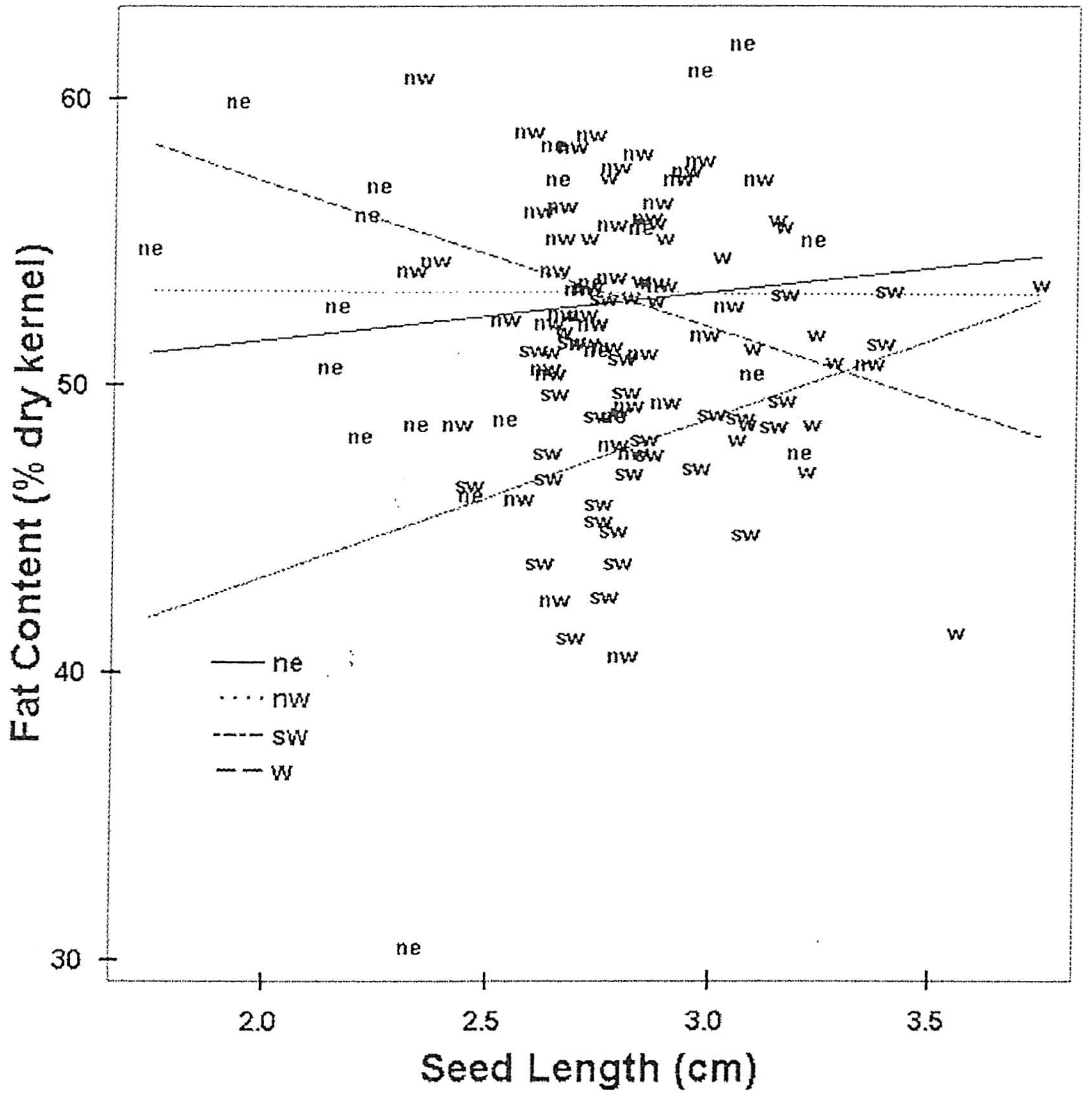


Each data point represents a comparison of means from individual trees (number of trees = 212)

5.2. Fat content

The majority of samples contained between 45 and 60 % fat, although three, individuals (recorded on collection as 'healthy with a good mast') showed exceptionally low fat contents (29.1, 30.5 & 33.0 %). Apart from a small positive association (r = 0.169, p = 0.07) with leaf lamina length and a weak but significant negative association with the shell percentage of whole seeds (r = -0.378, p < 0.01). No significant relationships for fat content and other quantitative morphological variables were found (r = 0.059 to -0.145, p > 0.85 to 0.12), e.g. Fat content = -0.6851NtL + 53.487, r = -0.043, p = 0.640 (Figure 5.6.). Regression analysis by geographic location for correlation of fat content with seed length, gave weak but variable relationships with a possible trend for increasing relationship strength (r^2 : sw = 0.16, w = -0.15, ne = 0.01, nw = 0.00) towards the south west.

Figure 5.12. Fat Content against Seed Length



Each data point represents an individual tree (fat content from two analyses of 4 kernels per tree)

Although fat content was apparently normally distributed from visual interpretation of the histogram for all samples (Figure 5.13.) and by geographic area (Figure 5.14). When analysed for kurtosis and skewness, only samples from the south west area were clearly normally distributed with all other areas (including the total) having a negative skew and positive kurtosis (Table 5.13.). Althought the small sample size per tree prevented any analysis of inter-tree variation, ANOVA revealed that there were significant differences between geographic areas ($p < 0.001$), but not between sites, and that the mean for the south west region was significantly smaller than all other areas ($p < 0.05$) which were statistically similar to each other.

Figure 5.13. Histogram distribution of fat content in *V. paradoxa* kernels in Ghana

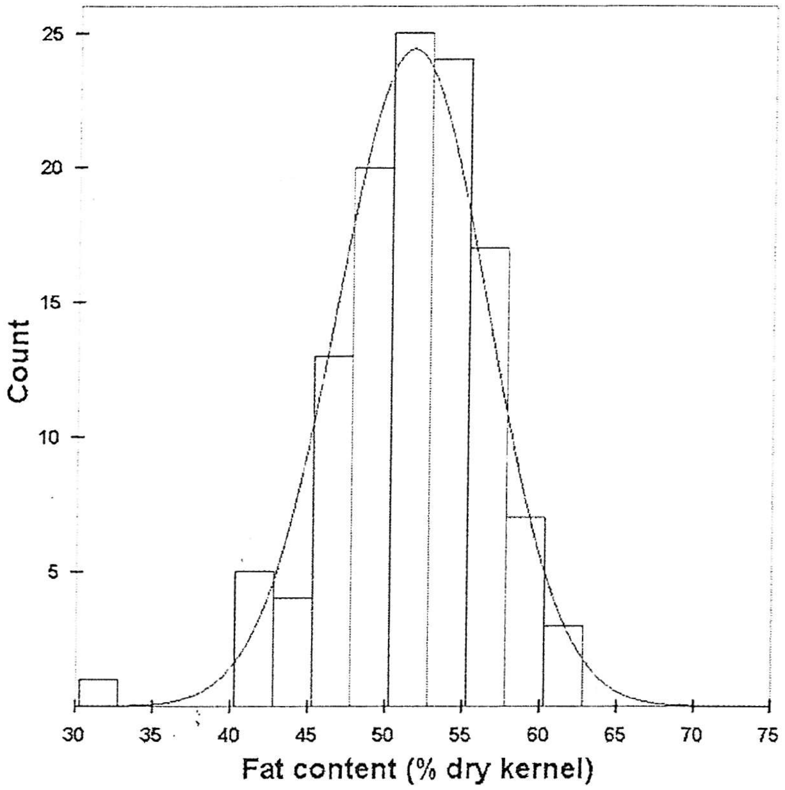


Figure 5.14. Histogram distribution of fat content by geographic area

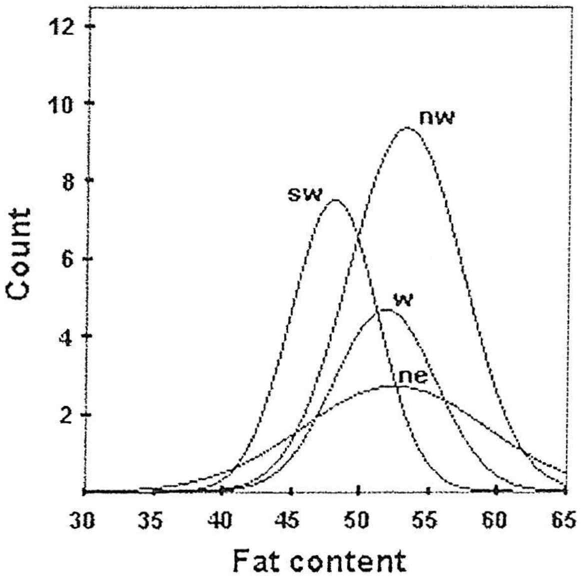


Table 5.13. Kurtosis and Skewness of all fat content means by geographic area

Area	Mean	Number of trees	Kurtosis (K)	Std Error of Kurtosis (SEK)	Ratio of K/SEK	Skewness (S)	Std Error of Skewness (SES)	Ratio of S/SES
nw	53.2	47	1.21	0.68	1.78	-0.83	0.35	-2.40
ne	52.5	22	4.56	0.95	4.78	-1.54	0.49	-3.15
w	51.8	21	1.64	0.97	1.69	-1.03	0.50	-2.05
sw	48.1	29	-0.52	0.85	-0.62	-0.16	0.43	-0.37
Total	51.6	119	2.08	0.44	4.72	-0.74	0.22	-3.34

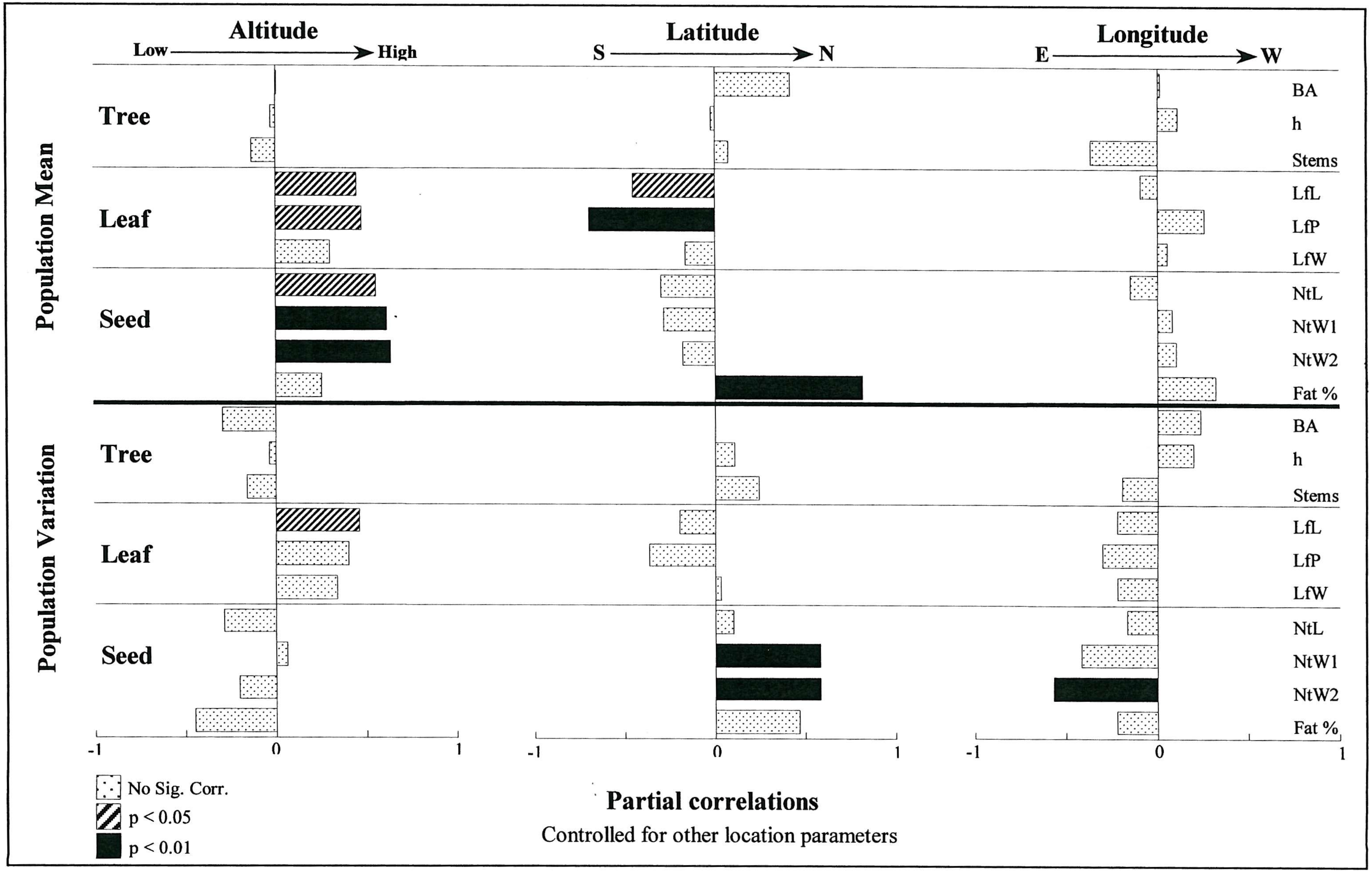
5.3. Environmental influences on morphoagronomic variation

Following analysis of individual tree, site, location and geographic area means of all quantitative characters, it was clear that although significant differences exist, identification of the cause of this variation was confounded by environmental differences between sites. Based on the assumptions given above it was decided to use site means, and their coefficients of variation, as reliable estimates for comparing inter-site variation and use them to identify any possible clinal trends due to environmental variation. If any of the morphological descriptors could be identified as *not* having any significant correlations with environmental variation. It was aimed to use these for multivariate analysis in order to determine if any morphologically defined 'varieties' could be shown to exist. By removing environmental effects, it was hoped that any remaining variation might used to demonstrate potential genetic diversity of *V. paradoxa* in northern Ghana.

Following bivariate correlations, a number of quantitative site means and their coefficients of variation, showed clinal trends and significance relationships with the three location descriptors (altitude, latitude and longitude). A summary of all morphoagronomic variation with location, in the form of a bar chart of significant partial correlations (controlling for the other two parameters) is given in Figure 5.15.

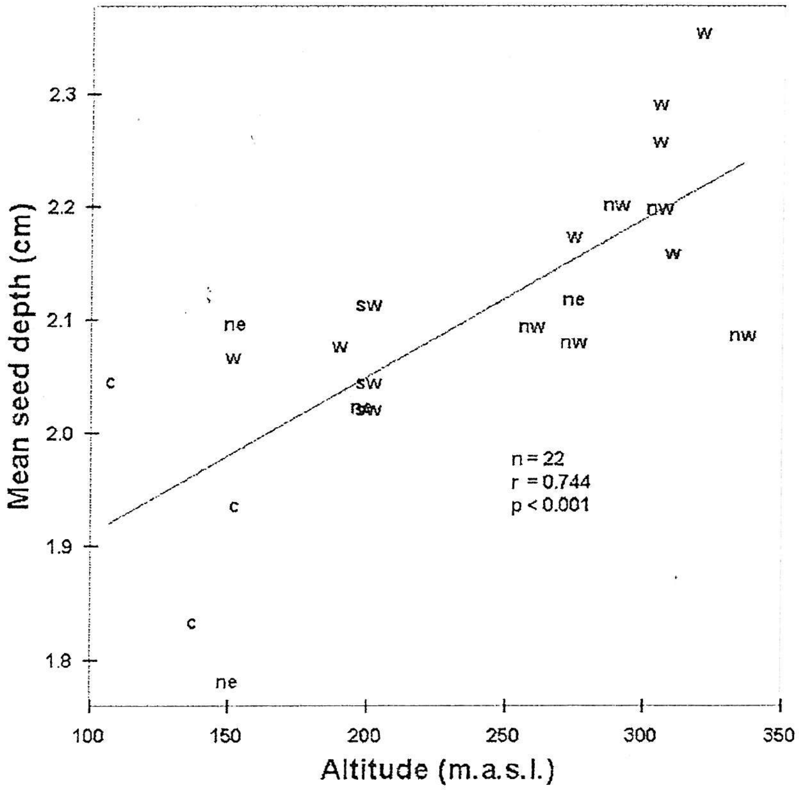
Longitude was not significantly correlated with any morphological characteristic although a possible small positive trend existed towards the west of Ghana for leaf (except lamina length) and seed size/fat content (except seed length). Smaller trees with a higher number of stems were often more abundant in the east.

Figure 5.15. Morphoagronomic variation of *V. paradoxa* populations



Leaf and seed size characteristics, except lamina width, were positively and significantly related to increasing altitude (Figure 5.16.). All high land was underlain with granites or other pre-Cambrian geology, whereas lower land was on Voltaian sandstones. Western high land near Bole and Wa were reported to be among the best Yam growing areas (*Discorea* sp.), a crop usually planted first in a traditional shifting cultivation rotation as it is nutrient demanding. It was therefore assumed that soils on higher ground in the west of Ghana were fertile, particularly when freshly cleared.

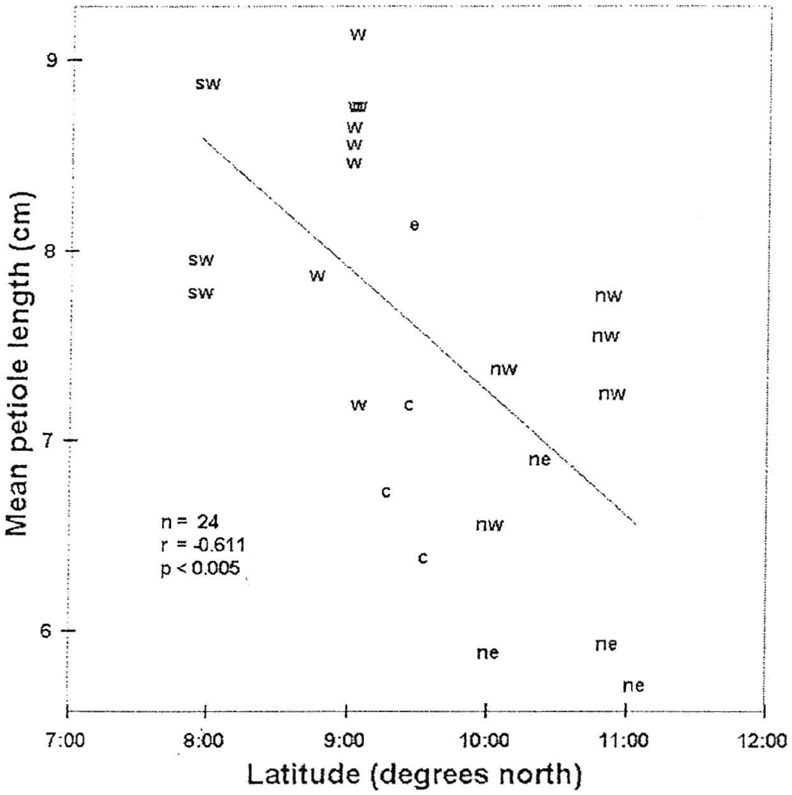
Figure 5.16. Seed Depth against Altitude



Each data point represents the mean value for each site

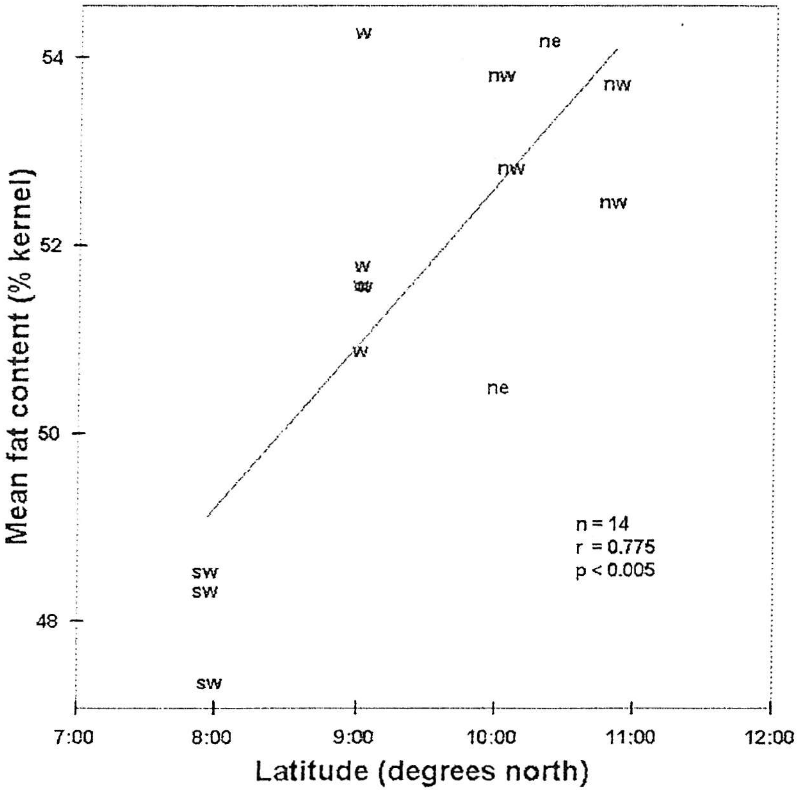
Drier conditions are indicated in the far north, as cereals (Millets and Sorghum) were more widespread. As described earlier, analysis of meteorological data for the semi-arid regions of Ghana revealed that the mean yearly rainfall was significantly and negatively related to increasing latitude. This supported field observations that increasingly arid conditions, with shorter wet seasons, occur towards the north of the study area. Leaf and seed size decreased to the north of the study area. Both leaf lamina and petiole length were significantly and negatively correlated with latitude when partial correlations were calculated, though when covariance was not considered only the latter relationship was significant (Figure 5.17.). Fat content was also significantly but positively correlated with northern latitudes (Figure 5.18), although most samples were obtained in the west.

Figure 5.17. Leaf Petiole against Latitude



Each data point represents the mean value for each site

Figure 5.18. Fat Content against Latitude



Each data point represents the mean value for each site

As suggested above, a number of differences were noted for levels of variation of seed characteristics between sites along the latitudinal transect. Correlation of population coefficients of variation with location was therefore performed and a number of clinal trends were also revealed. Partial correlations showed that leaf variation generally increased at higher altitudes, easterly longitudes and southerly latitudes, although only lamina variation was significantly correlated with increasing altitude (Figure 5.19). Variation of most seed characteristics increased at lower altitudes and to the northeast of the study area. Variation of both seed breadth and depth were significantly and positively related to latitude north (Figure 5.20.), though only variation of seed depth was significantly related to longitude east (Figure 5.21.).

Figure 5.19. Lamina Variation with Altitude

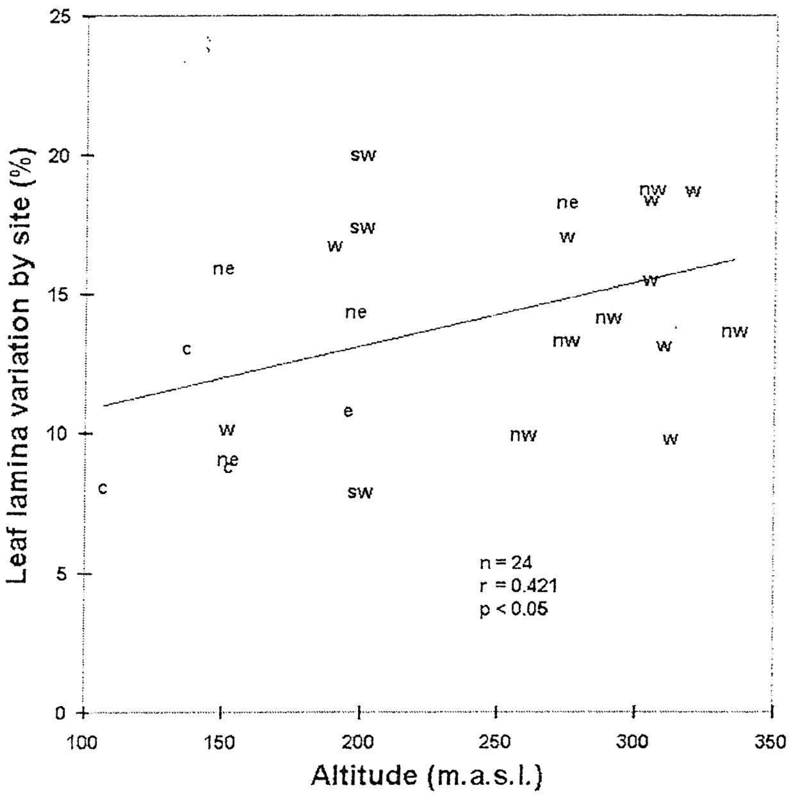


Figure 5.20. Seed Breadth Variation with Latitude

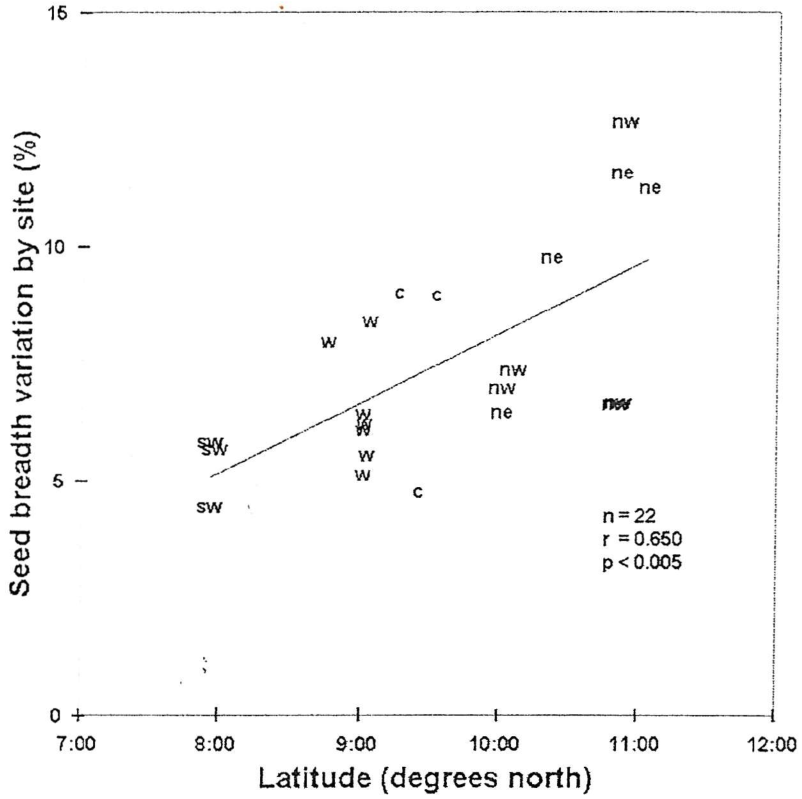
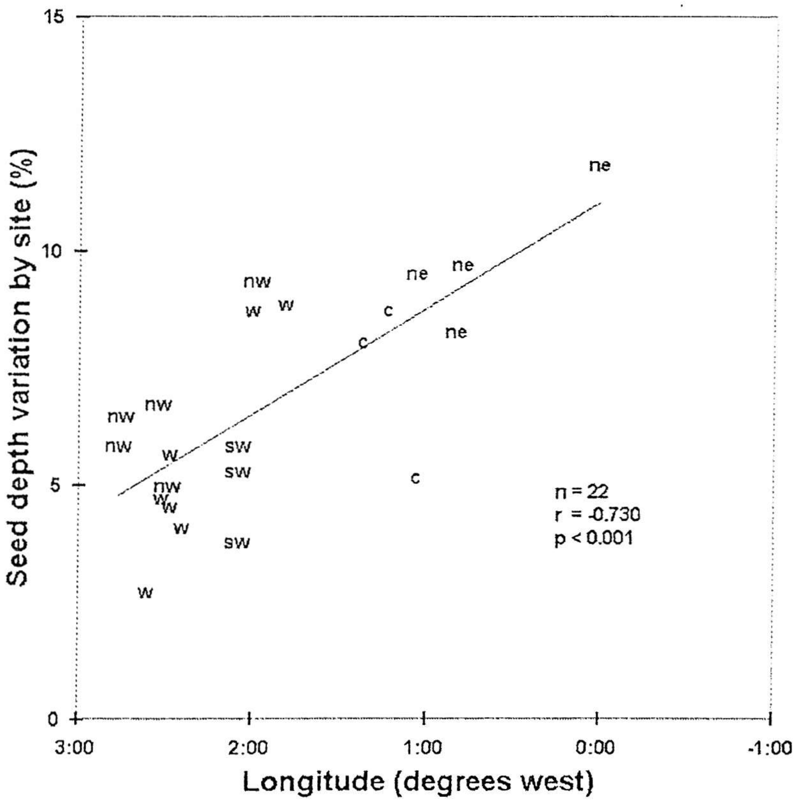


Figure 5.21. Seed Depth Variation with Longitude



5.4. Cluster analysis

As no firm evidence for distinct groups has been demonstrated using individual variables, multivariate analyis was performed. Principal component and hierarchical cluster analysis using all possible quantitative variables from all samples from across the study area, did not reveal the presence of any clearly recognisable groups. Widely distributed but overlapping groups, however, were revealed when only leaf and seed quantitative variables were used. Since it has been demonstrated that many of these quantitative descriptors are significantly correlated with environmental factors, bivariate correlations were used to identify seven morphological ratio descriptors not correlated with the location parameters (Table 5.14.). Unfortunately this type of analysis could not be performed at smaller distances as no evidence was found to show any obvious spatial distribution of morphological variation required to separate distinct 'area' groups for investigation. There is also the need for information on micro-environmental influences in order that non-correlated variables could be selected on an area by area basis..

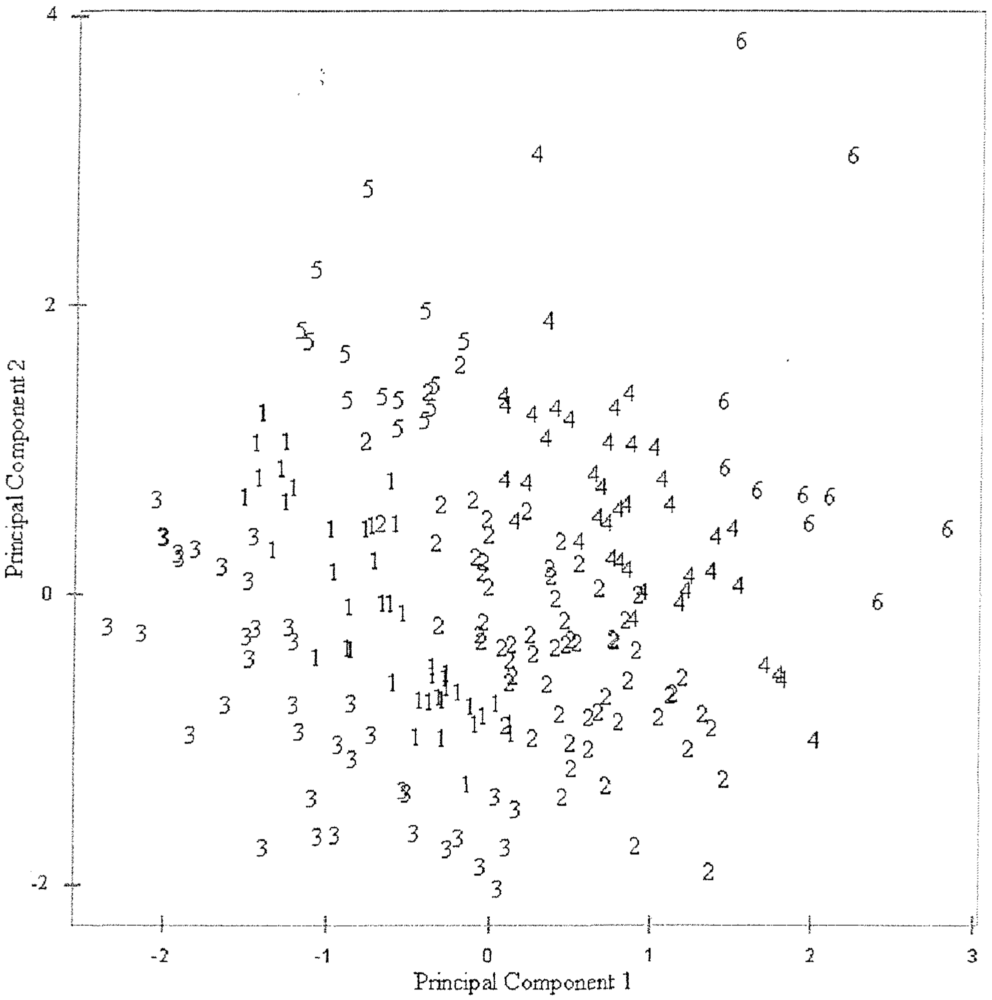
Table 5.14. Selection of morphological ratios not significantly correlated with location

Ratios	**Bivariate correlation with:**		
	Altitude	**Latitude**	**Longitude**
LfL/NtL	-0.13	-0.08	0.03
LfL/NtW1	-0.10	-0.12	0.01
LfL/LfW	-0.05	-0.11	-0.06
LfW/NtL	-0.05	0.03	0.05
LfW/NtW1	-0.03	0.00	0.03
LfW/NtW2	-0.03	-0.03	0.04
NtL/NtW1	0.08	-0.07	-0.07
LfL/NtW2	-0.10	-0.16*	0.02
LfL/LfP	-0.21**	0.34**	-0.41**
LfP/NtL	0.00	-0.29**	0.30**
LfP/NtW1	0.03	-0.34**	0.30**
LfP/NtW2	0.03	-0.37**	0.30**
LfP/LfW	0.15*	-0.34**	0.25**
NtL/NtW2	0.07	-0.14*	-0.05
NtW1/NtW2	-0.02	-0.22**	0.05
h/dbh	-0.04	-0.28*	0.12

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

PCA of these descriptors resulted in three components that explained 59.63 %, 22.43 % and 17.41 % of the variation respectively (cumulative total = 99.47 %). HCA gave between 2 and 8 groups with a squared euclidean coefficient width of greater than 10. Although these groups were not well separated in attribute space, the presence of 6 identifiable groups can be seen on the scatter plot of the first 2 principal components (Figure 5.22.). A number of group means, for leaf and seed size, were also significantly different (Table 5.15.). No clear geographical separation was evident and members of all clusters were distributed throughout the study area (Table 5.16.).

Figure 5.22. Principal components analysis of *V. paradoxa* accessions in Ghana



Clustered using Ward's method and squared Euclidean distance
Data analysis using Leaf and Seed ratios not correlated with location

Table 5.15. Morphological means by cluster membership

Variable		Cluster Number 1	2	3	4	5	6	Total
	Number of trees	44	65	38	41	14	10	212
Equivalent dbh (cm)	Mean	41.1	42.4	43.3	38.7	53.6	39.3	42.3
	±	31.7	25.0	32.0	28.2	56.5	44.3	20.5
Height (m)	Mean	11.5	11.7	11.8	10.2	13.7	11.3	11.5
	±	2.9	2.7	3.2	3.2	8.7	6.1	1.4
Leaf lamina (cm)	Mean	13.7^{23456}	14.8^{13456}	12.1^{12436}	16.5^{1236}	16.1^{1236}	18.5^{12345}	14.7
	±	0.7	0.7	0.9	1.0	1.7	3.7	0.6
Leaf petiole (cm)	Mean	7.0^{4}	7.4^{3}	6.5^{246}	8.1^{13}	7.7	8.1^{3}	7.3
	±	0.7	0.6	0.9	1.0	1.7	1.7	0.4
Leaf width (cm)	Mean	4.4^{246}	5.2^{135}	4.1^{246}	5.3^{1356}	4.2^{246}	6.0^{12345}	4.8
	±	0.3	0.3	0.6	0.4	0.6	1.3	0.2
Seed length (cm)	Mean	2.89	2.78	2.85	2.71	2.86	2.68	2.80
	±	0.20	0.17	0.22	0.20	0.35	0.41	0.09
Seed breadth (cm)	Mean	2.20	2.14	2.23^{46}	2.10^{3}	2.22	2.03^{6}	2.16
	±	0.10	0.09	0.14	0.13	0.25	0.30	0.05
Seed depth (cm)	Mean	2.11^{6}	2.07	2.13^{46}	2.02^{3}	2.15^{6}	1.93^{35}	2.08
	±	0.09	0.08	0.12	0.12	0.27	0.26	0.05
Stems per tree	Mean	1.09	1.14	1.18	1.17	1.29	1.20	1.16
	±	0.18	0.25	0.34	0.24	0.71	0.60	0.12
Canopy diameter (m)	Number of trees	10	11	5	8	2	3	39
	Mean	7.9	10.1	8.5	9.7	10.1	8.7	9.1
	±	4.5	3.4	7.2	4.2	14.0	10.3	1.7
Fat (% dry kernel)	Number of trees	25	38	16	24	5	7	115
	Mean	50.9	52.3	51.1	52.1	53.4	50.4	51.7
	±	3.7	3.1	6.9	4.0	7.5	9.1	1.8

Means ± 95% confidence interval

Different superscript numbers by row, indicate significantly different group means ($p < 0.05$)

Table 5.16. Cluster membership by geographic area

Cluster Number	Number of trees in each geographic area SW	C	NE	NW	W	All areas
1	7	7	6	12	12	44
2	7	7	14	17	20	65
3	2	6	10	8	12	38
4	6	6	7	12	10	41
5	1	3	3	2	5	14
6	3	1	2	1	3	10
All clusters	26	30	42	52	62	**212**

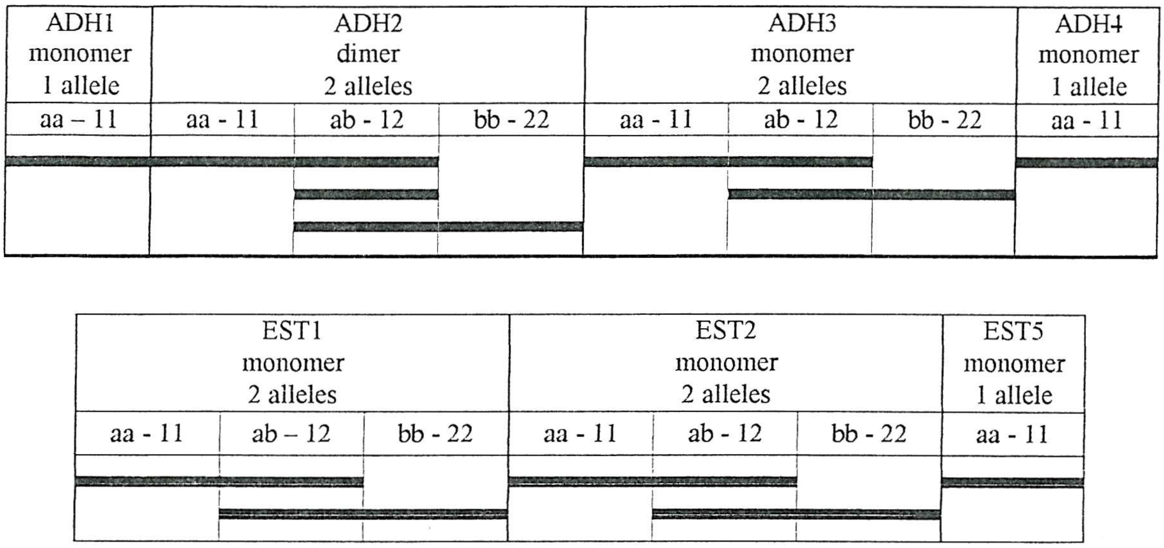
5.5. Isozyme analysis

Apart from the problems described earlier (section 3.5), band smearing was also common place and thought to be due to the presence of polysaccharides in the latex. After staining for over seven enzymes, clearly resolved banding was achieved in only three enzyme systems (EST, ADH and GOT). One monomorphic locus (single band always seen at the same position on all gels) was tentatively identified for GOT, though this was excluded from analysis due to a lack of confidence in results because of band smearing. The putative loci Adh1, Adh4 and Est5 were monomorphic, whereas Adh2, Adh3, Est1 and Est2 were polymorphic (Figure 5.23.). Nine bands were resolved at a fourth region of EST activity which were probably due to polymorphic dimers following random association of four polypeptides coded by two separate loci, Est3 and Est4. Although heterozygosity was indicated, these were also excluded from analysis due to difficulties interpreting genotype. High heterozygosity values were obtained throughout the study area, though F-statistics were low (Table 5.17.).

Similarities were apparent between UPGMA clusters using Nei's (1972) minimum distance on genotypic data and HCA of mean population phenotype (Figure 5.24.). Although dissimilarity distances were small and not proven to be significant, both analyses separated populations found in the west/northwest from those in the northeast/central. Using transformed descriptors, phenotypic plasticity is suggested as the southwest population was more similar to the western one, whereas using genotypic data it was closer to that from the eastern area. When only quantitative phenotype descriptors were used for HCA, the main climatic differences are illustrated, as western/southwestern populations were grouped together (high rainfall), distinct from a cluster formed from northwestern, northeastern and central populations (lower rainfall, hotter or longer dry season).

Despite extensive isozyme analysis of all samples, it is also of interest to note that the second allele of Adh2 was only resolved using samples from the northeast and southwest sites which explains the low value of heterozygosity for this locus and further supports the possible genetic distinction described above.

Figure 5.23. Identifiable isozyme banding patterns (genotype and TFPGA codes)

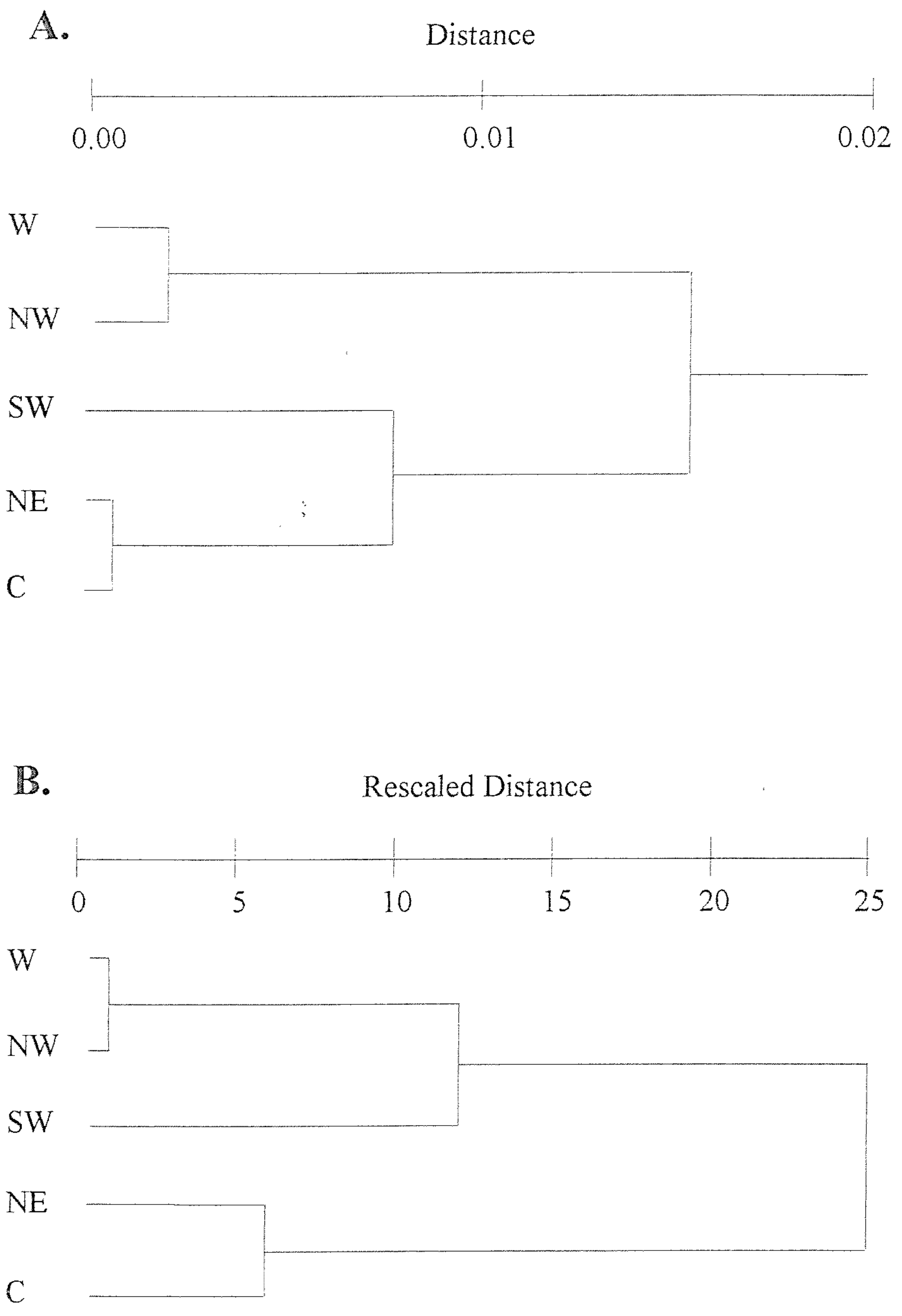


All loci determined according to guidelines in Wendel & Weeden, 1989 and Murphy et al, 1996

Table 5.17. Mean population genetic measures: Heterozygosity and F-statistics

Heterozygosity	**Locus**	Est1	0.4420
		Est2	0.4850
		Est5	0
		Adh1	0
		Adh2	0.0907
		Adh3	0.4669
		Adh4	0
	Direct count		0.2133
	Unbiased estimate (Nei, 1978)		0.2142
	Expected under H-W equilibrium		0.2121
Polymorphic loci (99% criterion)			57.14%
Sample size		**average**	40.7
		actual	70
F-statistics (Wright, 1965)		**Fit**	0.0023
		Fis	0.0247
		Fst	0.0124
		Nm	19.9

Figure 5.24. Dendrograms showing similarity of populations defined by geographic area



A. UPGMA clusters generated with Nei's (1972) minimum distance between populations using isozyme genotypes.
B. Hierarchical clusters generated with Ward's method using mean population phenotype descriptors not correlated with location.

Chapter 6. In vitro propagation of V. paradoxa

6.1. Production of sterile material

As describe earlier (section 3.6.) effective sterilisation of live explants was only found to be possible when an 'ethanol-washing' step was included in the protocol. This suggests that a layer not soluble in water (waxy cuticle) may be present on many surfaces of the young seedlings, possibly as a protective mechanism against desiccation. The use of domestos concentrations >8% resulted in unacceptably low survival rates of explants for any sterilisation period of greater than 10 minutes. Using concentrations <8% reasonable numbers of uncontaminated explants were only produced when sterilised for periods over 40 minutes (Table 6.1.).

Table 6.1. Survival rates of uncontaminated *V. paradoxa* explants after one week of culture

Treatment: (n = 5)	Domestos concentration (v:v)		
	6%	8%	10%
10 mins	0	40	40
25 mins	20	100	20
40 mins	60	80	0

6.2. Survival and growth of explants

During the first year of research it was only possible to use a limited number of explants due to the low survival rates of germplasm collections made during the first field trip. Therefore, having established a suitable sterilisation procedure, initial experiments were attempted using only full strength MS media and a range of concentrations of the plant growth regulator – BAP. Explant survival rates of these early experiments were less than 25% after 21 days and no sign of new shoot formation was observed.

Since many other woody species have been proliferated in media containing a combination of plant growth regulators, this result was initially thought to be due to the use of only BAP. The second round of experiments, using larger numbers of explants from more successful germplasm collections, attempted two different strengths of media and different combinations of two plant growth regulators – BAP and NAA. This clearly showed, however, that *V. paradoxa* explants cultured on shoot regeneration media containing MS macro- and micronutrients at full or half strength showed no significant difference in the mean number of axillary shoots induced (identical range of treatments for plant growth regulator combinations). In shoot regeneration media containing MS macro- and micronutrients at full strength, however, survival and induction of callus or growth was observed to be significantly lower (Table 6.2.) and was therefore considered unsuitable for the maintenance of *V. paradoxa* explant cultures.

Table 6.2. Effect of MS concentration on *V. paradoxa* explants (after 42 days in culture)

MS nutrients (w:v)	Survival[a]	Cultures exhibiting callus[b]	Mean callus size[b]	Mean growth[b]	Mean shoots[b]
100%	**27.4%**	**30.4%**	**0.4[c] ±0.3**	**2.4[d] ±0.5**	**0.2 ±0.3**
50%	**72.0%**	**91.1%**	**1.7[c] ±0.2**	**3.0[d] ±0.2**	**0.2 ±0.2**

[a] As a percentage of original number of explants after 42 days in culture
[b] As mean of all surviving explants (±95% confidence limits)
[c] Significantly different $P<0.01$
[d] Significantly different $P<0.05$

6.3. Shoot proliferation

Proliferation of axillary shoots was most notable when shoot regeneration media, containing MS macro- and micronutrients at half-strength (½MS), was supplemented with various combinations of the plant growth regulators, BAP and NAA. Table 6.3. shows the results for average growth, callus formation and the number of axillary shoots per explant for each treatment. The maximum number of shoots produced from one explant was 16 after 56 days of culture on media containing ½MS, BAP 15.5 µM and NAA 0.5 µM. Adventitious shoots from basal callus were also occasionally induced when *V. paradoxa* explants were cultured on media containing ½MS, and either BAP 4.4 µM and NAA 10.7 µM, or with BAP 15.5 µM, NAA 0.5 µM (see Plate 6.1.).

Plate 6.1. Axillary and adventitious shoot proliferation on half-strength MS media (BAP 15.5 µM and NAA 0.5 µM)

Table 6.3. Effect of BAP and NAA concentration on *V. paradoxa* explants (after 42 days in culture on half-strength MS media)

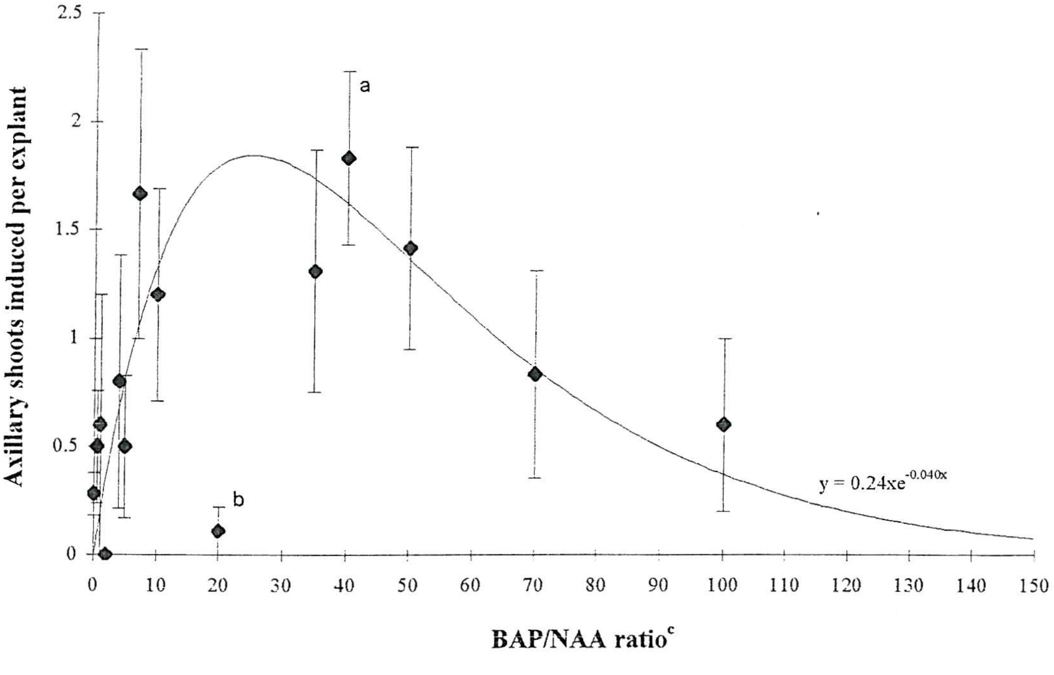
Plant growth regulator (mgl^{-1})		BAP/NAA ratio	Mean growth[a] (scale 0 - 6)	Mean callus size[a] (scale 0 - 6)	Mean new shoots per explant[a]
BAP	NAA				
0	0	0[b]	2.4 ±0.4	0.6 ±0.4	0.3 ±0.7
	0.05	0	-	-	-
	0.1	0	2.8 ±0.5	0.8 ±0.7	0.0 n/a
	0.5	0	-	-	-
	1	0	2.8 ±0.5	1.8 ±0.6	0.3 ±0.7
0.5	0	0[b]	3.3 ±1.4	1.5 ±2.2	0.3 ±1.4
	0.05	10	-	-	-
	0.1	5	-	-	-
	0.5	1	-	-	-
	1	0.5	4.5 ±1.2	2.3 ±5.0	0.0 n/a
1	0	0[b]	3.0 ±1.0	1.3 ±0.8	0.0 n/a
	0.05	20	-	-	-
	0.1	10	2.4 ±1.0	1.4 ±1.0	0.3 ±0.5
	0.5	2	-	-	-
	1	1	3.1 ±0.5	2.1 ±1.0	0.8 ±1.8
2	0	0[b]	3.2 ±0.9	1.5 ±0.6	0.4 ±0.6
	0.05	40	3.3 ±0.5	1.7 ±0.8	1.8 ±1.0
	0.1	20	2.6 ±1.1	1.4 ±0.8	0.1 ±0.3
	0.5	4	3.1 ±1.5	2.0 ±0.9	0.8 ±1.6
	1	2	3.1 ±0.4	1.9 ±2.6	0.0 n/a
3.5	0	0[b]	2.8 ±2.3	1.4 ±2.0	0.0 n/a
	0.05	70	3.3 ±1.1	1.4 ±0.7	0.8 ±1.2
	0.1	35	3.4 ±0.6	1.5 ±0.4	1.3 ±1.2
	0.5	7	3.0 ±1.2	1.8 ±0.7	1.7 ±2.9
	1	3.5	-	-	-
5	0	0[b]	3.0 ±0.6	1.5 ±0.6	0.7 ±0.8
	0.05	100	3.8 ±0.6	2.1 ±0.9	0.6 ±1.1
	0.1	50	3.2 ±0.8	2.0 ±0.5	1.4 ±1.0
	0.5	10	3.6 ±1.6	1.8 ±0.5	2.5 ±2.8
	1	5	3.3 ±0.8	3.1 ±0.8	0.3 ±0.9

[a] As mean of all surviving explants ±95% confidence limits

[b] For ease of calculation, treatments with no NAA are assumed to have a BAP/NAA ratio of 0 although traces of endogenous plant growth regulators may be present giving a true ratio of far greater than 100.

Although results indicated that there was high variation and no significant differences existed between treatments, it is notable that shoot regeneration was maximal with a cytokinin : auxin ratio of between 5 : 1 and 50 : 1 (BAP: 8.9 to 22.2 μM). When the mean number of shoots regenerated was plotted against the cytokinin to auxin ratio (irrespective of concentration), a good correlation was observed. A best-fit curve ($y = 0.24xe^{-0.040x}$) using the observed results, shows a peak of axillary shoot formation with the BAP:NAA ratio at 25 : 1 (see Figure 6.1.). The presence of one outlier with low shoot induction response (BAP:NAA = 20) does suggest the occurrence of two peaks in activity, however the lack of explants, prevented verification of this possibility.

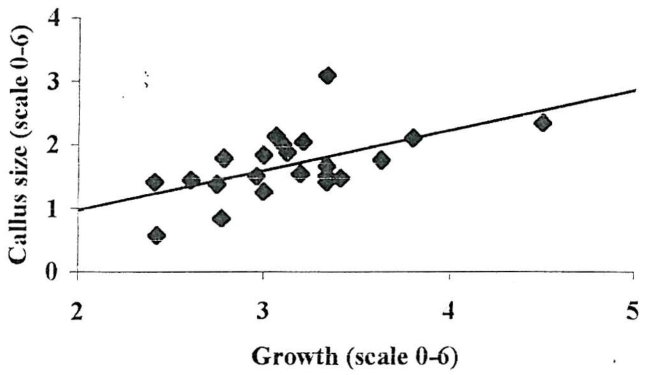
Figure 6.1. Graph showing model of *in vitro* shoot induction in *V. paradoxa* seedlings



[a] Errors bars indicate one standard error, i.e. 67% confidence limits
[b] Outlier not included in best-fit calculation
[c] BAP/NAA ratio calculated using mgl^{-1} concentrations as given above (Table 6.3.), points represents the mean for each ratio

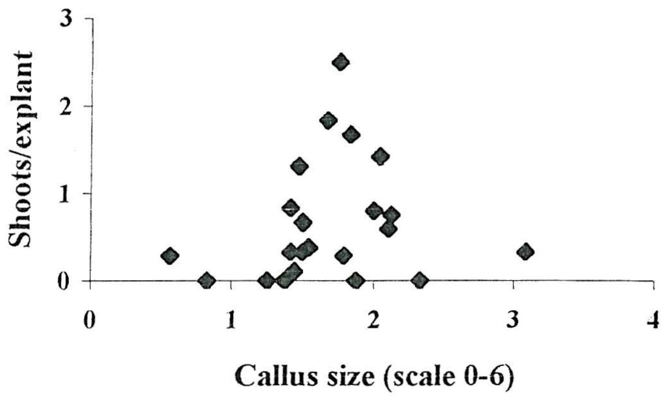
Other relationships noted included the significant and positive linear relationship of mean callus size with mean growth (Figure 6.2.). Although the correlation ($r = 0.565$, $p<0.01$) was relatively weak, it should be emphasised that an arbitrary scale was used for these measurements that did not take into account actual volume of callus or direct measurements on growth rate. The number of shoots also appeared to be related with callus size, as most shoots were induced only when the callus size was between 1.4 and 2.2. The relationship, however, is non-linear and it has not been possible to fit a best-fit model (Figure 6.3.)

Figure 6.2. Mean callus size vs. mean growth of *in vitro* propagated *V. paradoxa*



Each point represents the mean of each treatment after 42 days

Figure 6.3. Mean number of shoots vs. mean callus size of *in vitro* propagated *V. paradoxa*



Each point represents the mean of each treatment after 42 days

6.4. Root induction

Although time and material prevented optimisation of axillary shoot formation, any *in vitro* cultured shoots formed were used to test whether adventitious roots could also be stimulated *in vitro*. Adventitious roots were induced when regenerated axillary shoots of *V. paradoxa* were cultured for 70 days on root regeneration media containing MS macro- and micronutrients at quarter-strength. 8% of cultures formed one root per explant on media with 4.9 μM IBA and 8% formed three roots per explant on media containing 14.8 μM IBA (see Plate 6.2.). No signs of rooting were observed when shoots were cultured on media containing MS macro- and micronutrients at half-strength. The statistical significance of these findings is unknown due to the limited results available at the time the experiment was completed (after 70 days). Despite the fact that these results demonstrate a low success rate, it should be noted that 76% of cultures were still healthy at this point. Unfortunately when the experiment was then left with a technician (during the final field trip of this research) the majority of cultures died when they were accidentally subbed into media containing full-strength MS media.

Plate 6.2. Roots induced after 70 days culture on quarter-strength MS media (IBA 14.8 μM)

It is of interest to note that in terms of 'time to root', these observations are very comparable to those made while attempting to root *V. paradoxa* cuttings during field experiments in northern Ghana using poly-propagators (Lovett et al, 1997; Opoku-Ameyaw et al, 1997) and under mist in heated greenhouses in England (P. Lovett, unpubl.). Under these conditions, the formation of the first hormone induced adventitious roots usually occurred between 90 and 120 days after setting, with a minimum recorded time of 67 days using mist with under-heating. In poly-propagators in Ghana, it was also observed that many more cuttings had rooted after re-checking the experiment 180 days since first set, though the mortality rate of cuttings had also increased significantly by this time (Table 6.4.).

Table 6.4. Rooting leafy cuttings of *V. paradoxa* at CRIG sub-station Bole, Ghana

Woodtype[a]	SW	SW	SW	SH	SH	SH	HW	HW	HW	**Mean**
Rooting powder[b]	None	No. 2	No. 3	None	No. 2	No. 3	None	No. 2	No. 3	
Observations after 4 months of being set[d]										
%A[c]	44	33	0	78	44	44	67	56	33	**44**
%C	44	33	89	22	56	22	11	22	33	**37**
%D	11	0	0	0	0	11	11	0	22	**6**
%R	**0**	**33**	**11**	**0**	**0**	**22**	**11**	**22**	**11**	**12**
Observations after 6 months of being set[d]										
%A	11	33	0	33	11	22	44	33	11	**22**
%C	0	0	11	0	0	0	0	0	0	**1**
%D	33	22	44	44	44	11	33	11	56	**33**
%R	**56**	**44**	**44**	**22**	**44**	**67**	**22**	**56**	**33**	**43**

All cuttings (10cm long, 3 leaves, diagonal cut) set in mid-August in a poly-propagator (Leakey et al, 1990)

[a] Woodtype: SW – Softwood (this seasons growth); SH – Semi-hardwood (more than one seasons growth); HW – Hardwood (more than one years growth)

[b] Leafy cuttings were washed in clean water and dipped in Seradex ® rooting powder (No. 2 and No. 3).

[c] Results are presented as percentage of all cuttings set: alive with no signs of callus or roots (%A), alive with signs of callus but no roots (%C), roots visible (%R) or dead (%D)

[d] All cuttings rooted at four months were immediately transplanted and the total results given at 6 months

Chapter 7. Conclusions and recommendations

7.1. Anthropic domestication of *V. paradoxa*

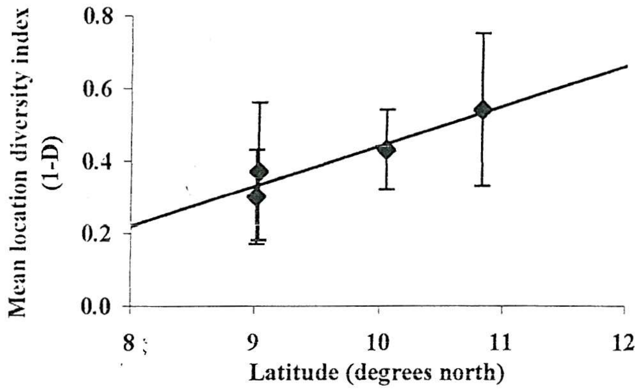
As results in Chapter four demonstrate, traditional anthropic agroforestry practices have clearly had a significant impact on both the woody species composition and on the population structure of trees on farmland in northern Ghana as compared to area of unmanaged 'natural' woodland. It is also apparent that the majority of trees recorded on cultivated land are maintained because their non-timber products are locally valued and cultural management tools (traditional laws or taboos) have evolved to prevent cutting without careful consultation with senior community members concerned with land management. On cultivated land in the West Gonja District, the most frequent species recorded was the sheanut tree (*V. paradoxa*), forming over 80 % of the woody biomass. Further north, in the Upper West Region, *V. paradoxa* biomass reduced to only 40 to 60 % of the total on farmland, as the contribution by other NTFP species increased, e.g., *Parkia biglobosa* (from 7 % in Bole to 36 % in Wa) and *Diospyros mespiliformis* (from 4 % in Bole to 8 % in Nandom).

The diversity of woody species and total woody biomass recorded on cultivated land was also higher in the north. Despite the lack of significant differences between cultivated sites, the significant positive trend ($r = 0.956$, $p = 0.044$) of mean woody species diversity, with increasing latitude (Figure 7.1.) and aridity, is particularly surprising considering the opposite is usually seen in tropical forests (Givnish, 1999):

> The number of woody species in tropical forests tends to increase with precipitation, forest stature, soil fertility, rate of canopy turnover and time since catastrophic disturbance, and decrease with seasonality, latitude, altitude, and dbh.

As the data on farm distances illustrates, there is a higher intensity of land pressure at northwest sites and although environmental differences confound comparisons to unmanaged woodland or between locations, this evidence shows that significant selection at the species level is occurring as a new ecological – 'agroforestry' – system is established following long-term and intensive anthropic management of cleared woodland. This also demonstrates that after removing 'unwanted' woody species, farmers are still capable of diversifying their risks (particularly for food security) by increasing the numbers of utilised species by protection or planting. This is most notable in areas when land pressure is high or where marginal conditions exist and tree planting was less common in areas where woody resources were still plentiful (West Gonja). Unless other reasons exist for the lack of responses from women, it is also of some concern that results show women are not being empowered in either tree planting or indigenous species selection during clearance.

Figure 7.1. Increase in woody species diversity on farmed land with increasing latitude



Error bars indicate 95% confidence limits (Woodland, 1-D = 0.81±0.03)

In this cyclical farming system, field observations and inventory results revealed that the main selection stage of *V. paradoxa* occurs when fallow (rarely virgin woodland) is cleared. Most immature individuals are removed and only certain large trees are then maintained on cultivated land. 'Unwanted' mature sheanut trees were observed as being actively killed by ring barking and then left to dry while still standing. Over time, after many cycles of cultivation and fallow, results show that this has led to significant changes in the *V. paradoxa* population structure as compared to that seen in the unmanaged, 'natural' environment. Although preferential conditions that would favour all sizes and most species of trees, are present in the open farmland (more light and less competition) a larger than expected proportion of mature *V. paradoxa* individuals (dbh > 20cm) was recorded in cultivated areas (48-94%) in comparison to woodland areas (40%). The proportion of all other woody species larger than this size was, however, similar to woodland near Bole but not at northern sites.

Lack of significant differences between the medium intensity landuse site (around Mandari) and unmanaged areas, in terms of total species numbers, *V. paradoxa* biomass and population structure, is probably due to inventory of insufficient plots for the given variation. Visual clarification from the ground or satellite images, however, clearly demonstrates that there is a notable difference in landuse. Results therefore indicate that the land near Mandari is either better managed; has longer fallow periods; or that farmland has been cleared recently cleared from unmanaged woodland. The similarity of the Mandari *V. paradoxa* population profile to that observed on more intensively managed sites near Wa (< 5 km from the regional capital) illustrates that sheanut trees selection on farmland is independent of human population density and is more likely to be influenced by management decisions or farming methods employed.

Important considerations for *V. paradoxa* conservation and management are implied by differences in farming methods between sites as deep ploughing reduces regeneration and damages mature tree root systems. The local elders near Mandari strictly enforce traditional methods and inventory results show that this site has a healthy population structure with many immature trees. Farmland around Bole, in contrast, has a much higher incidence of tractor ploughing; many damaged mature trees and large proportion of plots lacking any regeneration. Fallow length is also decreasing at this site and many fields are now permanently farmed with the use of fertilisers. Field observations and interviews in other regions support this view since a similar *V. paradoxa* population structure was observed on farmland near Nandom in the Upper West Region of Ghana, an area where cattle ploughing is widespread and fallows are also shorter.
Interviews with local people revealed that male farmers are actively selecting specific sheanut trees when land is cleared for cultivation from fallow or woodland. The selection criteria used for mature trees are based primarily on fruit productivity (as a function of age, health and size) and competitive effects with annual crops (determined by tree size, leaf density and spacing) – the latter is also the main reason for pruning. These views are further confirmed by similarities in the mean total woody biomass and *V. paradoxa* biomass between farmland sites in each ecological area. In support of other studies (Kater et al., 1992; Kessler, 1992; Bagnoud et al., 1995; Boffa, 1995; Jonsson et al., 1999) these results demonstrate that farmers are actively and consciously maintaining a careful balance between crop and tree productivity. The unwanted mature trees are not wasted but are utilised for household uses, e.g. building poles, mortars and fuelwood. Since farmers do not appear to be 'systematically' maintaining this species with the specific aim of 'improving the breed' this form of management is best termed as evidence for '*unconscious*' selection of *V. paradoxa*.

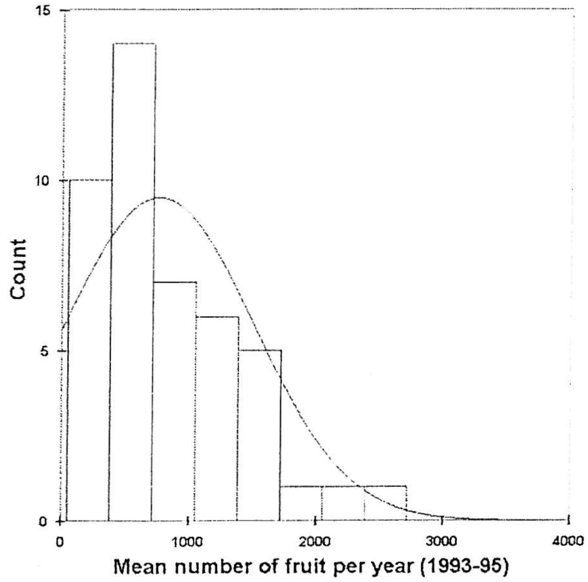
Aside from the field observations and interviews, other evidence for this proposed selection for tree productivity comes from data on fruit yield. Although no yield data was collected during this study, only one tree was found with fruit in unmanaged woodland whereas all trees (>20cm dbh) on farmland had at least some fruit during the harvest season. Other research, on a random sample of 53 trees (dbh > 10cm) in parklands at Thiougou, Burkina Faso (north of Navrongo), confirms this observation (J-M. Boffa, unpubl.). After a three-year study on individual fruit yield, only two trees (dbh = 10 & 14 cm) were recorded as not producing a single fruit during the period. Further analysis of this data reveals that although the tree population is apparently normally distributed by size class, the mean annual yield is heavily skewed to the right, i.e. more productive trees are present than expected (Table 7.1. & Figure 7.2).

Table 7.1. 'Normality' of *V. paradoxa* productivity at Thiougou, Burkina Faso

Character	Mean	Std. Dev.	Number of trees	Kurtosis (K)	Std Error of Kurtosis (SEK)	Ratio of K/SEK	Skewness (S)	Std Error of Skewness (SES)	Ratio of S/SES
Tree dbh	**25.4**	**8.2**	**52**	**-0.59**	**0.65**	**-0.91**	**-0.12**	**0.33**	**-0.36**
Fruit yr^{-1} (1993-95)	**763**	**742**	**53**	**4.57**	**0.64**	**7.14***	**1.76**	**0.33**	**5.33***

* Values > 2 indicate potentially significant departure from a normal distribution

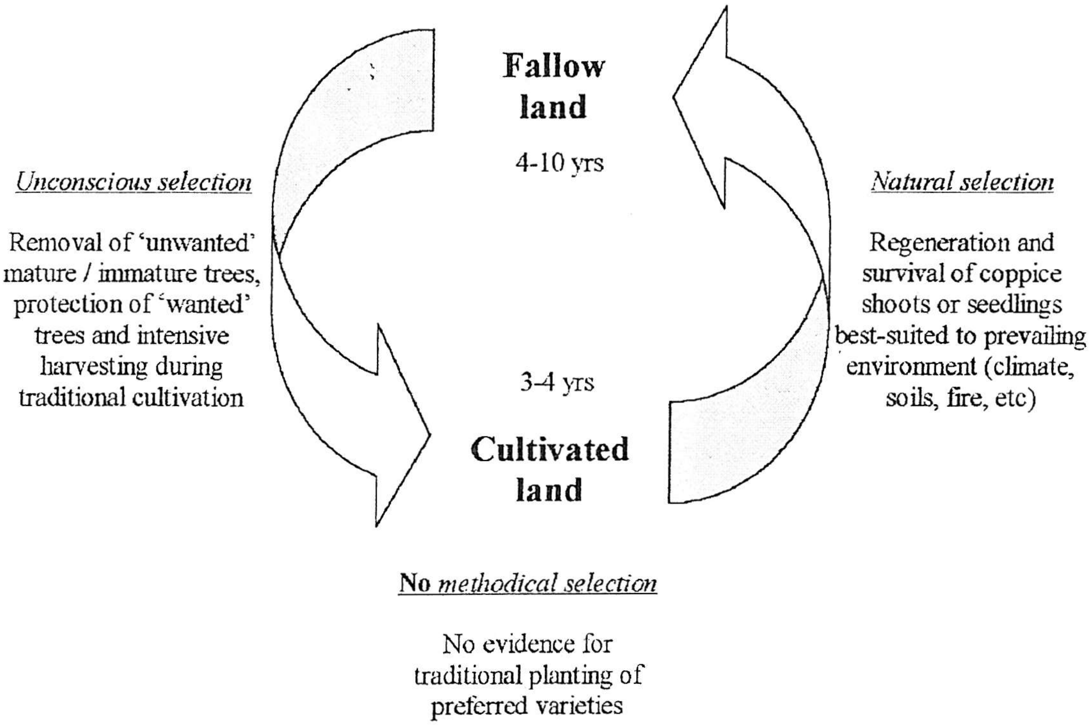
Figure 7.2. Histogram of skewed *V. paradoxa* yield at Thiougou, Burkina Faso



Data analysed and presented with kind permission of Dr J-M Boffa

New recruits for farmland sheanut populations are selected from regeneration by not cutting and then protecting from fire, during the cyclical clearing of fallow land (Figure 7.3.). The criteria used to select immature sheanut trees were based on spacing, health and speed of growth (following a fallow cycle, only saplings > 1m kept). This form of management can best be termed as '*natural*' selection since no other traits except vigour was identified, i.e. only those immature trees best-suited to the environment will be maintained. Since *V. paradoxa* is rarely traditionally planted – only one of the 130 farmers interviewed stated that he ever planted without external advice – no evidence exists for '*methodical*' selection of any traits.

Figure 7.3. The farm-fallow cycle of northern Ghana and selection of *V. paradoxa*



Apart from the characters described above, other preferred traits included fruit palatability, seed size and resistance to mistletoe, but since these were not given as reasons for tree selection during clearing, no firm evidence exists for any form of selection for these traits. Men stated that they like large sweet fruit, also an important product of *V. paradoxa*, but were not convincing as to which seed size was supposedly better for oil production. Women, who are the sole processors of shea butter in the area, clearly stated that small seeds give a higher yield of oil. Since laboratory analysis showed no correlation of seed size with fat/oil content (percentage by dry weight), the only woman who liked big seeds stating that 'less would be needed to fill basin', is probably wise in her laziness. This is because the same mass of small objects would be expected to occupy a lower volume than an identical mass of larger objects with the same density, i.e. a container of small seeds would be expected to yield more oil than the same vessel filled with large seeds having identical oil content and density. As most items are measured and traded by volume in northern Ghana, this preference is therefore understandable. It is, however, also conceivable that oil is more easily extracted from certain seed types using traditional methods, and local views still deserve further research.

Other '*unconscious*' anthropic selection pressures on *V. paradoxa* traits are also theoretically possible – but currently unproven – following the intensive harvesting and land cultivation during the germination period. These are particularly relevant in the study area since Osei-Amaning (1996) suggests that most immature trees in fallow land around Bole result from coppice. Although further research is required to determine the exact period when seedlings become established during the farming cycle, it is suggested that the traditional two week ban, coupled with thorough collection of viable seeds, would encourage survival of varieties that either fruit early, abscise on maturity or germinate immediately. It is also of note that traditional bans on early harvesting, are a common occurrence in many areas of West Africa (J-M. Boffa, pers. comm., 1999). Mature seeds that remain on the tree or had long viability would be more likely to be collected for oil production since germinated seeds are usually discarded. Cryptogeal germination of *V. paradoxa* also occurs during the cultivation period and new shoots do not appear above ground until planting and weeding has been completed, four to eight weeks later. Since the land is usually burnt four to six months later, the phenology of this unusual characteristic, which probably originally evolved in response to fire (Jackson, 1974) or aridity, may now be protecting seedlings from the intensive but shallow hand cultivation (using short-handled hoes) commonly practised throughout the region. This mechanism would also provide the seedlings with an immediate purchase in loose cultivated soil, during a period of heavy rainstorms. As with other cultivated crops, it is therefore possible that '*unconscious*' selection has affected the natural dispersal mechanisms of *V. paradoxa* by increasing survival rates, in a man-made environment, of those varieties that fruit, abscise or germinate earlier.

The length of time for alleles controlling for '*domesticated*' traits to become fixed in a population varies between species, though it has been postulated that populations of cultivated wild lentils could develop 100% indehiscence in as little as twenty generations (Blumler & Bryne, 1991). The Gonja conquered the indigenous Vagla and Safalba ethnic groups near Bole, after migrating from Mali in the sixteenth century. Agriculture was probably already well established at this point as the latter groups are still given a degree of control over land and tree management. It is also likely that the rural population densities, though not as nearly high as present, were relatively large as there has been considerable military activity and ethnic migration in the area (Goody, 1969). Two hundred years ago, well before the rapid population growth of the last century, many areas of semi-arid West Africa were already intensively managed, as Park (1799) describes from a site thought to be in present day Mali:

> …we passed a large town called Kabba, situated in the midst of a beautiful and highly cultivated country, bearing a greater resemblance to the centre of England than to what I should have supposed had been the middle of Africa. The people were everywhere employed in collecting the fruit of the shea trees, from which they prepare the vegetable butter mentioned in former parts of this work. These trees grow in great abundance all over this part of Bambarra. They are not planted but are found growing naturally in the woods; and in clearing land for cultivation, every tree is cut down but the shea.

Even with a low population density and long fallow periods, tree density is still reduced by over 90% (>300 ha^{-1} in woodland down to ca. 30 ha^{-1} in farmland) at every farm cycle. *V. paradoxa* selection/survival rates of immature trees at each clearance cycle are also usually ca. 1 in 10 or higher, as this and other studies that compare farm and fallow populations indicate (Hall et al., 1996; Schreckenberg, 1996). Assuming an average farm cycle of 15 years (the current maximum fallow length) and similar agricultural practices, this period would have allowed at least 30 *V. paradoxa* generations to establish since the arrival of the Gonja. Although yield data does not yet exist for West Gonja, it is conjectured that this amount of time would have allowed time for selection of the healthiest and most productive trees, as other data suggests has already been the case in an area just north of the study area. Assuming that *V. paradoxa* was also growing and utilised in other northern areas, particularly into the Saharan areas where agricultural is postulated to have started, the number of 'selected' generations could be much higher (over 300) which would have allowed ample time for the phenological 'domesticated alleles' to have increased in frequency. As evidence described earlier suggests, the climatic conditions probably deteriorated ca. 3000 yrs bp and was accompanied by a migration south of agricultural people. This, it is believed, would have effectively squeezed the 'selected' *V. paradoxa* genepool through an agricultural 'bottleneck' into areas previously occupied by tropical moist forest, and normally outside of its ecological range.

Without firm archaeological data on the active management and utilisation of *V. paradoxa* (currently only available for the last 1000 years) many of these suggestions are still speculative. Evidence from this and other studies, however, is revealing that anthropic pressures on the woodlands on semi-arid West Africa may be much larger than once supposed. It is therefore proposed that the agroforestry parklands, and *V. paradoxa* populations therein, are not just 'relics' of natural woodland but are the direct result of anthropic selection following many centuries of traditional cultivation and fallow. Furthermore, evidence and hypotheses are offered that may refute earlier assertions that *V. paradoxa* is a just 'wild' tree species despite evidence for centuries of management. Although *'methodical'* selection is non-existent, as preferred varieties are not planted, there is evidence (from field observations, interviews with farmers and other research near the study area) for other forms of selection. Results indicate that healthy, productive individuals are being specifically protected, whilst undesirable varieties are selectively removed, with the result that yield traits no longer have a normal distribution in some populations. It is proposed that the maintenance of healthy trees that are well suited to these agroforestry systems, coupled with the intensive harvesting and cultivation across the species range, has led to *V. paradoxa* becoming semi-domesticated following '*unconscious*' and '*natural*' selection mechanisms. Further research, however, is required to compare parkland populations with 'wild' trees in unmanaged woodland in order to clarify whether other potentially domesticated traits exist and to identify any potentially useful undomesticated traits, e.g. long viability and slow fruit abscission on maturity.

7.2. Phenotypic variation and genetic diversity

As with many tree species, it is apparent that the environment plays a large part in determining the architecture, growth and size of a mature sheanut tree, as distinct types were observed under different climatic and vegetation conditions. Tall straight boled, high canopy individuals were common in southwest woodland where water availability was probably less of a limiting factor to survival than competition for light. Large but short boled trees, with spreading canopies, were frequent in open farmed parklands where light was clearly not a limiting factor. The high variation for height, basal area and number of stems is probably indicative of the wide range of age classes from which samples were obtained. It is also currently unknown as to what degree genetics or light levels effect tree architecture at the sapling stage, therefore, unless tree age and site history can be accurately determined, these variables are not recommended for *V. paradoxa* diversity studies. The relationship determined for canopy and dbh during this study, is also remarkably similar to that given for a sample from Benin:

$cd = 0.197dbh + 2.03, r = 0.793, p < 0.001$ (Lovett & Haq, 2000a)
$cd = 0.197dbh + 1.24, r = 0.848, p = 0.001$ (Schreckenberg, 1996)

No relationship was seen for leaf with tree size, and leaf variation was much lower than that obtained for tree size characters. Leaf size variability therefore indicates that the care taken in choosing samples from the most accessible southern branch of mature trees, has prevented these results from being confounded by age or location of the leaf on the tree. Reduced size at more northern latitudes and lower altitudes is presumably an adaptation to control evapotranspiration rates where arid conditions or high temperatures would be expected to increase water loss (cf. Hegazy & El Amry, 1998). This hypothesis would also explain higher leaf variation in areas where high rainfall or low temperatures would prevent evapotranspiration rates being one of the major limiting factors to tree survival, e.g., at higher altitudes. A significant clinal trend of decreasing petiole length with increasing aridity was observed and the role of this organ in controlling water balance deserves further attention. Pigment changes during conditions of water stress are probably indicative, as with other plant species, of a protection mechanism preventing ultraviolet damage to tissues with low water levels (Ryan et al., 1998).

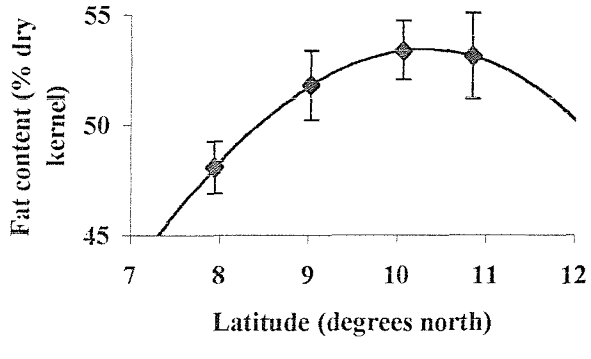
Edaphic influences on seed size are conspicuous, as quantitative variation was significantly associated with altitude, which in turn, closely correlated with geological variation and soil type. Large seeds were frequent on the granite uplands where crops indicative of high fertility were grown whereas shrubby trees with small seeds were common on soils where petroferric or stony conditions were prevalent. Although these small trees were obviously mature, the lack of growth studies means the influence of age cannot yet be excluded with certainty. It is suggested that large seeds produce competitively superior seedlings and have been naturally selected at fertile sites where vigorous vegetation is supported. Climatic influences on seed size are less significant, although a weak trend of decreasing size, and lighter coloured seeds, was apparent towards hotter, drier localities. These observations potentially indicate a mechanism adapted to control temperature prior to germination (cf. Fenner, 1992).

Results also demonstrate that a wide degree of inter-tree morphological variation exists at sites along the latitudinal transect, however, comparisons between sites at a location and around the study area, clearly illustrates that a significant proportion of this variation is influenced by currently unquantified local [environmental] factors. Along the latitudinal transect, inter-site variation was sometimes apparent for leaf size, but was significant for seed size at certain locations again highlighting the susceptibility of this characteristic to edaphic variation. In comparison, LfL/LfW and NtL/NtW1 ratios showed no significant correlation with any location parameter though variability was evident at all sites. NtW1/NtW2 showed only a weak but significant positive correlation with latitude and on a study area wide basis, no significant differences were identified for this character between any sites, although on a locational basis (along the latitudinal transect) significant inter-tree variation was recorded. Since the wide scale environmental effects on these variables is apparently low, inter-tree variation of seed or lamina shape at each site is therefore offered as a possible indicator of genetic diversity in the study area until data on local scale environmental variation is available.

In addition, no significant correlations with location were observed for lamina/seed ratios (except a weak relationship of Lf/NtW2 with latitude) though a small positive and significant relationship was identified for seed with leaf size. Genetic diversity may therefore be further illustrated by the existence of trees that produce seeds with a size different from that predicted by the leaf size. Daboya, a low-lying site on alluvial soils on the west side of the White Volta River, was notable for trees with small leaves (similar to populations towards the east which also had small seeds) but with larger than expected seeds. Edaphic factors at this site cannot be ruled out as being the major influence on seed size variation, as this character appears to be more susceptible to the environment and may therefore be demonstrating higher levels of phenotypic plasticity than leaf size. A second possibility relating to different speeds or ease of geneflow for seed and leaf characters, might also explain this finding. It is suggested that proximity to the riverine corridor could serve as a more effective dispersal route for animals carrying fruit with maternal genes controlling for large seeds (found to the north and west) whereas leaf size may be determined by paternal genes which are only disbursed over relatively short distances by insects. This second theory, however, does not explain the low LfL/NtL ratios found at Bole and Polmakom suggesting that higher levels of seed phenotypic plasticity, in response to edaphic variation, seem more likely. As shade affects on crop yield have been noted for *V. paradoxa* (Kessler, 1992), the possibility of genetically influenced large seed, small leaf varieties deserves further attention as a desirable morphoagronomic character for tree improvement.

Trees with high fat content were more common in northern parts of the study area, a result comparable to that given by Adu-Ampomah et al. (1995). A latitudinal transect from 8 to 11 °N (Figure 7.4.) in the West of Ghana revealed that fat content appeared to peak at ca. 10 °N before a slight decrease at the most northerly site ($r^2 = 0.99$ for 2nd order polynomial best-fit curve).

Figure 7.4. Variation of fat content along a latitudinal transect in northern Ghana



Error bars indicate 95% confidence limits, points represent location mean (tree n per location = 20 to 30)

This transect was conducted at locations which were thought to have similar edaphic conditions, i.e. on granite, although a number of significant differences were found to exist between sites for seed size. In contrast no significant differences existed between sites for fat content although when sites were combined by either geographic or climate station area the southwest mean was significantly lower than all other areas. This suggests an environmental influence from climatic gradients found in the study area and with further study, it may be possible to identify optimum conditions for fat production. It is, also conceivable that fat content is genetically determined and high yielding (by fat content) varieties can be identified and selected for tree improvement programs. Genetic control of this trait would also allow for the possibility of unconscious or natural selection, following long-term integration in agroforestry systems, of landraces that have superior resources at germination and more likely to produce fast growing healthy saplings that would be selected for protection by farmers. Methodical selection of any visually recognisable trait has already been shown to be non-existent and in addition, no correlation has been found between seed size/shape and fat content, although many local shea butter processors were convinced that they could identify small seed types that gave higher fat yields during traditional processing. If earlier suggestions are correct and *V. paradoxa* is in the process of becoming domesticated, it is feasible that 'weedy' varieties have co-evolved to survive in the traditional farming systems (Harlan, 1992). From current results, it is suggested that these are the low fat varieties (ca. 30 %) that may have been maintained on farmland because of their 'false' productivity. This possibility is further supported by the normal distribution for fat content in the southwest, an area where domestication is likely to have been occurring for the shortest period (see below), as compared to the higher kurtosis and negative skewness values for fat content at other more northerly areas. This would suggest that where selection of highly productive trees has been happening for the longest periods (away from the forest margin) the numbers of lower fat (weedy) varieties has increased enough to skew the fat content distribution.

As it has been noted that the oil content of other seeds can vary according to changes in temperature (Fenner, 1992). Environmental effects (apparent as either phenotypic plasticity or genetic adaptation) may also explain the differences in fatty acid profiles between the western and eastern subspecies. Since germination processes would require seed oils to be in the correct physical condition at a given temperature, i.e., liquid.

Higher stearate to oleate ratios would be favoured at the low altitude, high temperature sites in the west whereas the reverse would be expected on the higher and cooler East Africa plateau. Confirmation of any genetic difference between these proposed sub-species would therefore require genetic profiling using DNA markers and/or growth to maturity in the reverse environment.

In Ghana, well-spaced and healthy natural regeneration of *V. paradoxa* is customarily maintained on farmland. Although evidence presented above suggests that selection of high yielding (total harvest) varieties is occurring, no evidence exists for any selection of particular fruit or seed types. No correlation of fat content was found with seed size or shape and the only possible evidence for unconscious selection of fat content suggests that 'weedy' low fat varieties may have been unknowingly maintained because of their 'false' productivity. The major cause of variation in seed size between sites is linked with edaphic conditions through changes in altitude, bedrock, soil type or water availability. Coupled with analysis of inter-site and inter-tree variation it appears that seed size is also more susceptible to changes in edaphic conditions then leaf size and larger seeds are more commonly found with fertile, well watered conditions. Leaf size, appeared to be more strongly linked to climatic conditions, probably because evapotranspiration rates are related to temperature and water availability. These conditions are less dependent on soil fertility and therefore natural selection would be less affected by edaphic variation. Results also indicate that the site coefficient of variation of leaf size is linked to climate, offering evidence that morphological variation of this character can increase when limiting conditions are minimised.

Although significant differences in seed breadth and depth size exist between sites, their coefficients of variation appear to be less effected by edaphic variation. For example, the two sites at Wa have different edaphic conditions although there is virtually no difference in the coefficient of variation of seed breadth and depth, although seed length variation is higher at the drier site. Site variation in seed breadth and depth is therefore assumed to be independent of edaphic variation and it is almost certain that this trait is not being used as any form of anthropic criteria either for maintaining trees or for selective harvesting. Since all samples were taken from cultivated land in agroforestry parklands, it is proposed that the protection afforded following incorporation into this farming system has reduced any natural selection influences on seed breadth and depth. In the absence of any ecological explanation, except possibly high predation, no mechanism for why there has been an increase in the variation of these traits is offered except time. Consequently, it is proposed that after long periods of incorporation in a local farming system and without any natural or anthropic selection pressures, these traits are able to increase in variation, without any detriment to the survival of the *V. paradoxa* population.

If this hypothesis is correct, the question therefore remains as to why the site coefficient of variation of seed breadth and depth significantly increases towards the northeast at areas that are thought to be both drier and less fertile. Increasing evidence indicates that warmer, wetter periods occurred during the Holocene and expansion of evergreen vegetation, from rainforest in the southwest or southeast of Ghana, was possible (Talbot & Johannessen, 1992; Lézine & Vergnaud-Grazzini, 1993; Dupont & Welnelt, 1996; Salzmann & Waller, 1998). These studies also suggest, that ecological zones, as a result of either climatic or anthropic influences, were moving south again by about 3,000 years ago, a period at which agriculture had probably already been practised in West Africa for at least 2,000 years (Ehret, 1984; Blumler, 1996). As the current distributions of *V. paradoxa* indicate, this slow growing species cannot grow on waterlogged sites or compete with fast growing vegetation unless seasonal fires are frequent. Given the above assumptions, *V. paradoxa* populations integrated into traditional agroforestry systems would therefore be expected to exhibit the highest seed diversity at sites where suitable semi-arid conditions (for both the tree and the farming system) have existed for the longest period. Results from this study demonstrate that the variation of all recorded seed characteristics increase towards the northeast, perpendicular to moist forest boundary and the movement of the inter-tropical convergence zone. Since climatic shifts will probably have followed the same direction, it is suggested that conditions suitable for *V. paradoxa* have existed in northeast Ghana longer than in the southwest. As the variation of *V. paradoxa* seed breadth is both significantly and positively related to distance in this direction, this character is offered as a potential non-molecular indicator of long-term agriculture. If this proposal can be confirmed, it is also possible that variation of this morphological character could be calibrated and used to describe climatic changes or even anthropic influences across the species range.

Although current literature and field observations suggest that certain tree types or varieties do exist, significant environmental influences have confounded the identification of causes of inter-tree variation and the clarity of multivariate analysis when all characteristics are used. Clearer clusters were revealed, however, when a set of phenotypic descriptors not correlated with location was used for the analysis. The resultant groups also corresponded closely to those produced using the original quantitative variables, but with far less overlap. It is therefore assumed, that by choosing leaf and seed ratios not correlated with location, confounding environmental influences on inter-tree variation have been lessened simply by excluding those descriptors that are most strongly affected. The remaining variability is offered as indicative of baseline genetic diversity levels (not inclusive of that adapted to environment) occurring in the study area.

Evidence from the weak geographic separation between eastern and western populations, determined using isozyme genotypes, confirms the purported genetic diversity offered from phenotypic ratios. The suggestion that genetic isolation is low, is further demonstrated by the lack of geographical segregation of groups obtained from multivariate analysis and the high estimates obtained for geneflow ($Nm = 19.9$) in the study area. This confirms existing evidence which suggests that *V. paradoxa* has an outcrossing reproductive strategy, e.g. low levels of self pollination; no known barriers to insect cross pollination; and potentially long distance dispersal of seed by humans, birds, bats or primates (Hall et al., 1996). Without constraints to dispersal, genotypes important to survival are not isolated and subsequent adaptation to a given environment could easily occur, e.g. metabolic genes or leaf form. In areas where healthy trees are selectively protected, high geneflow could then allow non-limiting phenotypes to increase in diversity, e.g. seed form.

Since clinal trends suggest that environmental influences are large, genotypic and phenotypic interactions cannot readily be accurately resolved. Results do, however, indicate that *V. paradoxa* populations in Ghana exhibit a wide range of genetic diversity throughout the 9,320 km^2 study area, offering enormous scope for tree improvement. Potentially superior varieties have also been seen (with high fat content or useful morphoagronomic characters) though without effective clonal methods to capture this diversity and proof that these are genetically controlled characters, it is still not possible to offer farmers improved planting stock. Higher densities of mature trees were seen on agricultural land throughout the study area, as compared to unmanaged woodland, with no evidence for environmental barriers to geneflow. If assumptions regarding seed variation are correct, and in the absence of other ecological evidence from 'wild' unmanaged populations, initial indications from this research, are that diversity centres, and initial incorporation into the traditional farming system, lie to the northeast of the study area. It is important to add, that these may not necessarily coincide with the true 'centre of origin' of this dryland species as evidence may well have been erased by millions of years of climatic fluctuations and the closest living relatives are most likely found in the remaining areas of moist forest, e.g. *Baillonella toxisperma*, a member of Sapotaceae that is also utilised for oil by indigenous people and has been recorded from the high forest in Nigeria, Cameroon, Gabon and DR Congo.

7.3. Capture of genetic diversity

If 'superior' varieties of *V. paradoxa* can be identified, it is fundamental for any tree improvement program that this genetic diversity is captured and multiplied. Unfortunately, the out-crossing nature, lengthy maturation period and recalcitrant seeds of this species have so far made this difficult. Many different methods of vegetative propagation have been attempted although none have yet been standardised or proven effective for large-scale multiplication. Grafting is thought to be limited by latex production, although recent grafting success in Mali and in Burkina offers evidence that this problem can be overcome, and attempts at air-layering are confounded by environmental conditions (strong winds and desiccating conditions). Significant advances have also been made rooting cuttings at CRIG, although the methods being tested still require the correct propagation conditions to be maintained for a minimum of three months, even before transplanting can take place.

In developing a methodology for the clonal propagation of a potentially economically valuable tree species, it is important that techniques can be considered 'appropriate' to a given situation. In deciding this, attention should be given to stakeholder demand and availability of resources (skill, funds and equipment). When asking for comments from local farmers on improvement of the sheanut industry, improved planting stock was one of the major reasons offered to encourage tree planting although improved management, extension and land tenure, were cited as more important. Due to the recalcitrant nature of this species, this highlights the need for clonal propagation, however, no questions were asked during this study as to what methods should be used or whether production of planting stock should be centralised or community based. It is therefore hoped that future research will make full use of the results offered from this study and ensure that the guidelines of the Convention on Biodiversity are adhered to, i.e. aim to empower local people by offering opportunities to voice opinions and provide the capacity required to achieve these goals. In Ghana, CRIG has the mandate for research on *V. paradoxa*, the facilities and the trained staff necessary for high technology propagation though due to lack of funds, the northern research site in the sheanut tree zone was inadequately provided for. Although research is continuing throughout the region on low and hopefully more appropriate technology, to date no effective or standardised methodology is yet available to local communities. In addition, this and other studies have highlighted that some populations of *V. paradoxa* may be in dire need of conservation. It is therefore recommended that every type of propagation method be attempted, even if it may initially prove to be complex, labour intensive and expensive. Laboratory based *in vitro* technology may not at first glance seem to be the most appropriate technology to attempt, until one considers that once capacity has been established and the methods standardised, it can be a very cost effective means to produce large quantities of high quality (sterile) planting stock and conserve germplasm of this recalcitrant species. The route to achieving this goal can also support development as infrastructure can be used for other species and offer well paid employment for skilled staff. For these reasons it is hoped that results reported here will encourage the development of standardised *in vitro* propagation techniques and the means to provide high quality planting stock.

Reduced survival, growth and callus formation of explants in shoot regeneration media containing MS macro- and micronutrients at full strength confirms other studies on woody plant species where macro- and micronutrients at full strength have had inhibitory effects. As demonstrated, this inhibition on *in vitro* growth can sometimes be overcome by diluting the medium strength or by decreasing the total ionic strength through a reduction in the concentration of macro-elements (Badji et al., 1993).

Numerous morphogenic responses are reported for other Sapotaceae in the presence of different plant growth regulators (Jordan & Oyanedel, 1992; Singh & Bansal, 1993; Rout & Das, 1993). Likewise, this study has shown that explants from *V. paradoxa* seedlings also demonstrate a variable response to different concentrations and combinations of the plant growth regulators BAP and NAA. A large variation in response was also noted between accessions cultured on the same media. Since the seedlings were obtained from randomly sampled populations, the main source of this variation is probably due to the existence of genetic diversity as suggested from the phenotypic studies. Comparable variation, in terms of responsiveness to *in vitro* culture, has also been ascribed to the genetic diversity of another dryland member of Sapotaceae, *Argania spinosa*, using material collected from wild populations in Morocco (Nouaim & Chaussod, 1994).

The observed 'ratio' effect could also account for some of the high variation in shoot proliferation since only a small change in response to the BAP:NAA ratio would induce a large change in numbers of axillary shoots induced. Such a change could be a result of many factors including variation in genotype or responsiveness at different BAP concentrations. It has frequently been observed that plants exhibit growth and shoot proliferation in response to a high cytokinin:auxin ratio whilst root induction usually occurs in the presence of high auxin:cytokinin levels (Taiz & Zeiger, 1991). However, few reports have been seen on the result of cytokinin:auxin ratios during *in vitro* propagation of tropical tree species. The optimal cytokinin:auxin ratio for inducing shoot proliferation in Mahogany was given as 100-200:1 at the first sub-culture and 25:1 at the second sub-culture (Mathias, 1988; Leakey & Newton, 1994). Villarreal & Rojas (1996) showed maximal root induction of *Mimosa tenuiflora* when the auxin:cytokinin ratio was greater than 10:1. This apparent effect of cytokinin:auxin ratios on shoot or root induction during *in vitro* culture may therefore be important for other woody plant species including *V. paradoxa.*

Although other methods may be more 'appropriate', this is the first report to describe both shoot regeneration and root induction during *in vitro* culture of the scientifically important tropical oleaginous species *V. paradoxa*. Thereby demonstrating that in the absence of other low technology methods, micropropagation techniques may offer a potential method for clonal propagation. Although few cultures exhibited adventitious shoot formation, the induction of axillary shoots could still prove to be a useful means of multiplication since young trees of this species normally exhibit only apical growth, with axillary branches generally forming after at least 5-6 years (Delolme, 1947).

7.4. Recommendations for future research

As mentioned earlier, much attention is now being paid to those woody plants termed as 'Cinderella' species that provide economic or environmental benefits through traditional utilisation but have been neglected by mainstream domestication (Leakey & Newton 1994). *V. paradoxa* is just such a species and although traditional management may have resulted in semi-domesticated varieties, with regard to current agroforestry requirements this species still has many undesirable characteristics. Trees take up to 20 years to mature, the seeds are recalcitrant and the heterozygous populations produce a highly variable crop in terms of quantity and quality. Despite evidence for the existence of a diverse genetic resource base, superior 'true-to-type' individuals are difficult to maintain as this species has an out-crossing breeding system and successful research into vegetative propagation methods is still in its infancy (Bonkoungou, 1987; Grolleau, 1989; Opoku-Ameyaw et al., 1997; Lovett et al., 1997). In order to improve our understanding of this species the following recommendations are offered based on this study's findings.

With the aim of identifying 'wild' varieties that may be useful in tree improvement programs or require conservation. It is recommended that further study be made on the genetic variability of many of the phenotypic traits of this species. Potential traits that could demonstrate high levels of variation, and may have been affected during unconscious or natural selection, are shown in Table 7.1. together with characteristics thought most likely to occur in wild populations of *V. paradoxa*. These are suggested on the basis of observed variability, the maintenance criteria described above, coupled with intensive and rapid harvesting of viable seeds, and the environmental conditions prevalent in the agroforestry parklands.

With regard to current requirements, many of these potentially 'domesticated' traits, that may have resulted from improve survival on farmland, are also some of the most undesirable ones. This is simply because *V. paradoxa* seeds are not reserved for planting and therefore contrast with those more commonly recorded in other cultivated plants (Heiser, 1988). For example, unconscious selection has led to many domesticated annual crops having non-dehiscent seeds because these are more likely to be harvested and therefore planted the following season. In contrast, it is proposed that an early harvest ban and intensive collection has resulted in *V. paradoxa* varieties with fruits that abscise on maturity, and germinate immediately. These are characters not conducive to an improved industry particularly if there is a need to harvest ripe, high quality fruit from the tree or maintain viable germplasm for conservation.

Table 7.2. Potential agronomic traits of *V. paradoxa* recommended for further research

Trait	**Proposed wild-type phenotype** (as compared to domesticated traits)
Seed viability	Longer
Cryptogeal germination	Non-existent or during high fire risk period
Fruiting period	Late in the rainy season
Masting	Extreme irregularity
Mean yield	Low
Fruit pulp/seed ratio	Low
Fruit pulp palatability	Poor
Fruit abscission on maturity	Slow
Simultaneous ripening	High
Seed coat	Thick
Seed shape variation	Low
Fat content	Low
Tree form	Straight bole, high canopy
Leaf/seed size ratio	High
Coppice ability	Low
Fire resistance	Low

It is possible, however, that wild varieties have been virtually eliminated from the West African parklands because of the widespread selection pressures in these man-made environments. Evidence for cycles of shifting ecological zonation also suggests that the more humid regions, uninhabitable by *V. paradoxa*, may have migrated first north and then south. If these theories are correct, man and his agroforestry system, might have effectively wiped out any 'wild' individuals when the domesticated relatives of this species recolonised southern areas. It is therefore proposed that the best chance of finding undomesticated trees, and desirable traits for tree improvement, will be in the southernmost areas of the species range in areas of woodland that have had little or no history of cultivation. In contrast the most highly selected productive trees are most likely to be found in the northern areas of this species range.

Results of this study indicate that although site means are generally reliable, it can be seen that the requirements for viable germplasm, transport logistics and large numbers of widely distributed sites resulted in rather small sample sizes (trees per site ≤ 12). If future country wide studies are planned, that hope to build on this research by identifying causes of inter-tree variation, it is recommended that transport logistics are minimised by careful selection of fewer locations using as many secondary sources of information as possible prior to commencing field work (meteorological data, soil maps, population surveys, aerial or satellite imagery). In a country such as Ghana, four main locations (NE, W, E and SW) with at least three sites at each location are suggested (total 12 sites). Sites should then be situated in different intensities of landuse (high, medium, low) in an area with similar environmental conditions, and farms identified with owners having a range of socio-economic characteristics. With the aim of adhering to the Convention on Biodiversity guidelines, participatory questionnaires must be conducted so that local stakeholders are aware of the research objectives and a needs assessment can be conducted. It is advised that a minimum of 30 trees are selected at each site (total 360 trees), all located in one area of known characteristics (landuse history, soil variation, etc), and any locally preferred individuals in the population distinguished. In the absence of high resolution environmental variation and individual age data, that require expensive soil surveys or tree rings for dendrochronology techniques, this sampling method would aim to identify these influences by comparing the characteristics of each tree to its nearest neighbours. Since ca. 10% of tree leaf or seed means were not proven to be normally distributed, when using a sample size ≤ 20, it is recommended that a minimum of 30 samples are collected for each morphological feature and if dimorphism is to be assessed, > 100 samples per tree should be collected at throughout a season. If the aim is to identify superior varieties for propagation, research should focus on variability and genetic control of locally preferred agronomic characters, e.g. annual harvest, fat content, palatability, tree/crop interactions, short maturation and disease resistance.

Due to the environmental influences on phenotype, recalcitrant seeds of this species and difficulties resolving isozymes, it is recommended that other biochemical markers are also used to determine genetic diversity across this species range. Any such method should allow easy collection of viable samples and the ability to resolve variation at the genetic level, e.g. field drying of leaf samples with silica gel for use with DNA based techniques. Since population degradation has been suggested, it would be beneficial for conservation programmes that these methods are also used to identify centres of diversity. In addition, any such studies should aim to determine the impact of anthropic management through assessment of diversity in different landuse intensity to allow recommendation of the best practices for maintenance of genetic diversity.

Conflicts of interests, between traditional and national natural resource management policies, have been noted in many countries, i.e. a total ban on cutting any tree instigated by colonial foresters, as opposed to selective protection through traditional management systems. This type of policy could, in theory, prevent ongoing improvement of a genepool following the removal of trees with undesirable traits, and can also be very detrimental to local communities by branding a large proportion of traditional farmers as criminals of the state. Participatory inventory is required in semi-arid Africa to enable scientific assessment of the true status of the agroforestry parklands, identify the existence of any traditional management tools and to highlight current threats to the sustainability of utilisation. As ethnobotanical research reveals that many species are utilised for food security or other livelihood needs, and on-farm maintenance of perennial species is an excellent means to diversify risks in marginal environments, these inventories should not just focus on *V. paradoxa*. Results from this research should then be developed with the aim of encouraging and empowering local communities to manage their own resources sustainably.

Recent development projects in Uganda, that aim to ensure that value is added locally to products of *V. paradoxa* subsp. *nilotica* (Masters & Puga, 1994), demonstrate that regeneration of this species can be encouraged by linking conservation to market forces (Lovett et al., unpublished). Since this and other studies show that *V. paradoxa* is an integral component of an agricultural system capable of providing a wide range of economic benefits. It is therefore recommended that future conservation efforts can be made more effective when local awareness on the value of indigenous products is increased and the capacity to link industry to local production is encouraged.

Evidence from the population structure of this species at sites with different land management methods, illustrate that fallow length and ploughing methods can have significant effects on the population dynamics of *V. paradoxa*. It is also clear that modernisation of agriculture is having detrimental effects on this species regenerative capacity. This is most notable following a decrease in fallow length and the widespread use of deep mechanised ploughing. It is therefore recommended that further study be made on the differences between traditional and modern farming systems to precisely identify what effects these practises have on the physiology, tree/crop interactions and regenerative capacity of *V. paradoxa*. It is also important to determine at what point seedlings become established and the role that coppicing rootstock plays.

Since results from participatory surveys revealed that there is a demand for advice on improving management of these agroforestry parklands, technical information needs to be disseminated and incorporated into effective national extension services. Current land and tree tenure mechanisms need further investigation with the aim of developing new strategies to encourage tree planting, particularly by women in areas not affected by deep mechanised planting,;e.g. on farm boundaries, in orchards and near compounds. Means should also be sought to encourage women to participate in research that aims to develop improved processing techniques and business capacity at a local level.

With assistance from industry and indigenous technical knowledge, locally desirable germplasm (from both domesticated and wild populations) should be selected, multiplied and their incorporation into traditional agroforestry systems encouraged. As evidence suggest that clonal propagation methods will be required for the capture of 'superior' genetic diversity from *V. paradoxa*. It is suggested that further research be undertaken to standardize propagation techniques for this species. *In vitro* techniques have only been tested on juvenile material, it is therefore recommended that culture media and plant growth regulators are optimised for shoot and root regeneration of explants taken from mature trees. Once techniques have been developed superior varieties should be selected and multiplied for the production of high quality planting material for farmers. To ensure conservation of *V. paradoxa* and development of this valuable resource in the immediate future, it is recommended that priority be given to the provision of training on local selection and appropriate propagation techniques (planting seed, grafting or rooting cuttings). This, it is hoped, will allow 'methodical' selection and continuation of a tree improvement process first started by African farmers thousands of years ago.

References

Abbiw DK (1990) *Useful plants of Ghana, West African uses of wild and cultivated plants*. Intermediate Technology Publications and The Royal Botanic Gardens, Kew, London. pp. 66-67.

Adegoke EA, Akisanya A, Naqvi SHZ (1968) Studies of Nigerian medicinal plants: 1. a preliminary survey of plant alkaloids. *Journal of West African Science Association*, **13**: 13-33.

Adomako D (1985) Prospects for the development of the shea nut industry in Ghana. *Cocoa Research Institute of Ghana, Technical Bulletin No.* **11**: 1-12.

Adu-Ampomah Y, Amponsah JD, Yidana JA (1990) Sheanut germplasm collection. *Cocoa Research Institute of Ghana, Annual Report 1988/89*: 97-98.

Adu-Ampomah Y, Amponsah JD, Yidana JA (1995) Germplasm collection of sheanut (*Vitellaria paradoxa*) in Ghana. *Plant Genetic Plant Resources Newsletter*, **102**: 37-38.

Agbahungba G, Depommier D (1989) Aspects du parc a karités-nérés (*Vitellaria paradoxa* Gaertn. F., *Parkia biglobosa* Jacq. Benth.) dans le sud du Borgou (Benin). *Bois et Forêts des Tropiques*, **222**: 41-54.

Ahuja MR (1993) *Micropropagation of woody plants*. Kluwer Academic Publishers, Netherlands.

Amobi CC (1973) Periodicity of wood formation in some trees of southern Guinea savanna in Nigeria. *Nigerian Journal of Forestry*, **3 (2)**: 48-50.

Amponsah JD, Adu-Ampomah Y (1988) Sheanut breeding programme: germplasm collection. *Cocoa Research Institute of Ghana, Annual Report 1986/87*: 97-98.

Amponsah JD, Adu-Ampomah Y (1989) Sheanut germplasm collection programme. *Cocoa Research Institute of Ghana, Annual Report 1987/88*: 81-83.

Amponsah JD, Adu-Ampomah Y, Yeboah JM (1985) Sheanut germplasm collection, preliminary survey of tree populations in the northern and upper regions of Ghana. Unpublished report. 12 pp.

Anon (1991) The shea butter tree's untapped riches. *Spore CTA bulletin*, **32**: 5.

Anon (1997) Changing fortunes for oilseeds? *The Agronomist*, **2/97**: 9-11.

Anon (1998) Kassardjan to increase production. *Daily Graphic*, (unknown date) Ghana.

Arends JC (1976) Somatic chromosome numbers of some African Sapotaceae. *Acta Bot. Neerl.*, **25 (6)**: 449-457.

Ata JKBA, Fejer D (1976) Allantoin in shea kernel. *Ghana Journal of Agricultural Science*, **8 (2)**: 149-152.

Aubréville A (1950) *Flore forestière soudano-guineénne*. Société d'Editions Géographiques, Maritimes et Coloniales, Paris. 533 pp.

Aubréville A (1966) Toujours à propos du 'karité' Africain: *Vitellaria* Gaertn. F. ou *Butyrospermum* Kotschy? *Taxon*, **15**: 216.

Awoleye F (1995) Effects of seed sources on the growth of seedlings of *Vitellaria paradoxa* in the southern Guinea savanna of Nigeria. *Nigerian Journal of Botany*, **8**: 65-69.

Axtell BL, Fairman RM (1992) Minor oil crops. Part I - Edible oils. *FAO Agricultural Services Bulletin. No.* **94**: 121-125.

Badifu GIO (1989) Lipid composition of Nigerian *Butyrospermum paradoxum* kernel. *Journal of Food Composition and Analysis*, **2 (3)**: 238-244.

Badji S, Mairone Y, Ndiaye I, Merlin G, Danthu P, Neville P, Colonna JP (1993) *In vitro* propagation of the gum arabic tree (*Acacia senegal* (L.) Willd.) 1. Developing a rapid method for producing plants. *Plant Cell Reports*, **12**: 629-633.

Baehni C (1965) ...Car tel est notre bon plaisir... *Taxon*, **14**: 42.

Bagnoud N, Schmithusen F, Sorg J-P (1995) Shea tree and locust bean tree parklands in southern Mali: economic analysis of trees associated with crops. *Bois et Forêts des Tropiques*, **244**: 9-23.

Baker HG (1962) Comments on the thesis that there was a major centre of plant domestication near the headwaters of the River Niger. *Journal of African History*, **3 (2)**: 229-233.

Baker JG (1877) Sapotaceae. In: Oliver D (ed.) *Flora of Tropical Africa, Vol.* **III.** *Umbelliferae to Ebenaceae*. Reeve, Ashford. pp. 497-509.

Belknap K, Wagner G (2000) Lulu - assessment report from New Sudan. Paper presented at the "The Shea tree (*Vitellaria paradoxa* subsp. *nilotica*): First Regional Conference for Eastern and Central Africa". Lira, Uganda, June 26-30, 2000.

Benneh G (1987) Land tenure and agroforestry land use systems in Ghana. In: Raintree, JB (ed.) *Land, trees and tenure, proceedings of an international workshop on tenure in agroforestry.*, ICRAF and the Land Tenure Center. Nairobi and Maddison.

Blumler MA (1996) Ecology, evolutionary theory and agricultural origins. In: Harris, DR (ed.) *The origins and spread of agriculture and pastoralism in Eurasia*, UCL Press Ltd, London.. pp. 25-50.

Blumler MA, Byrne R (1991) The ecological genetics of domestication and the origins of agriculture. *Current Anthropology*, **12 (1)**: 23-54.

Boffa J-M (1995) Productivity and management of agroforestry parklands in the Sudan zone of Burkina Faso, West Africa. *Ph.D. thesis*, Purdue University, West Lafayette, Indiana. 101 pp.

Boffa J-M (1999) Agroforestry parklands in sub-Saharan Africa. *FAO Conservation Guide No.* **34**. (draft).

Boffa J-M, Yameogo G, Nikiema P, Taonda JB (1996) What future for the shea tree? *Agroforestry Today*, **8 (4)**: 5-9.

Bokdam J (1977) Seedling morphology of some African Sapotaceae and its taxonomical significance. *Mededelingen Landbouwhogeschool Wageningen*, **77-20**: 1-84.

Bolaji SB (1980) Paper from tropical (Nigerian) hardwoods. *Chemical Age of India*, **31 (3)**: 247-250.

Bonga JM, Dunzan DJ (1987) *Cell and tissue culture in forestry*. Martinus Nijhoff, The Hague.

Bonkoungou EG (1987) Monographie du karité, *Butyrospermum paradoxum* (Gaertn. F.) Hepper, espèce agroforestière à usages multiples. Institute de Recherche en Biologie et Ecologie Tropicale. Ougadougou, Burkina Faso. 69 pp.

Bonkoungou EG (1997) Report on seed collection of *Vitellaria paradoxa* in Burkina Faso, Mali, Senegal and Uganda. ICRAF and IPALAC. 19 pp.

Bonkoungou EG, Alexandre D-Y, Ayuk ET, Depommier D, Morant P, Ouadba J-M (1994) Agroforestry parklands of the West African semi-arid lands. Conclusions and recommendations of an international symposium. Ouagadougou. 25-27 October 1993. ICRAF/SALWA. 18 pp.

Booth FEM, Wickens GE (1988) Non-timber uses of selected arid zone trees and shrubs in Africa. *FAO Conservation Guide. No.* **19**. 176 pp.

Boussim J (1991) Contribution a l'étude des *Tapinanthus* parasites du karité au Burkina Faso. *Thèse de troisème cycle*. Université de Ougadougou, Ougadougou. 152 pp.

Boussim J, Sallé G, Guinko S (1993) *Tapinanthus* parasites in du karité au Burkina Faso. *Bois et Forêts des Tropiques*, **238**: 45-65.

Brookman-Amissah J, Hall JB, Swaine MD, Attakorah JY (1980) A re-assessment of a fire protection experiment in north-eastern Ghana savannah. *Journal of Applied Ecology*, **17**: 85-99.

Bureau of Ghana Languages (1986) *Language guide* [various versions]. Ghana Publishing Corporation.

Chevalier A (1943) Le karité ou arbre à beurre: essai monographique. *Revue Internationale de Botanique Appliquée et d'Agriculture Tropicale*, **23**: 100-120.

Chevalier A (1946) L'arbre à beurre d'Afrique et l'avenir de sa culture. *Oléagineux* **1**: 7-11.

Chevalier A (1948) Nouvelles recerches sur l'arbre à beurre du Soudan. *Revue Internationale de Botanique Appliquée et d'Agriculture Tropicale*, **28**: 241-256.

Clark JD (1980) Early human occupation of African savanna environments. In: Harris DR (ed.) *Human Ecology in Savanna Environments*. Academic Press, New York. pp. 41-71.

Corbett JJ (1989) The shea butter tree: a model for rural development. *M.Sc. Environmental Forestry dissertation*, University of Wales, Bangor. 165 pp.

Dako JT, Watson J, Amoakwa-Adu M (1974) Available carbohydrates in Ghanaian foodstuffs. *Plant Foods for Man*, **1**: 121-125.

Dalziel JM (1937) *The useful plants of West Tropical Africa*. London, Crown Agents. pp. 350-354.

Delabre G, Paris P, Clamagirand B (1987) *Etude de marche et commercialisation des equipments villageois de frabication du beurre de karité au Mali*. Centre d'Echanges et Promotion des Artisans a Equiper, Paris.

Delolme JC (1947) Etude du karité à la station agricole de Ferkéssédougou. *Oléagineux*, **2**: 186-200.

Delwaulle JC (1979) Plantations forestières en Afrique tropicale sèche. Techniques et espèces a utiliser. *Bois et Forêts des Tropiques*, **187**: 3-30.

Desmaret J (1958) Observations sur la population de karités de Niangoloko, 1953 á 1957. *Oléagineux*, **13**: 449-455.

Dupont LM, Welnelt M (1996) Vegetation history of the savanna corridor between the Guinean and Congolian rain forest during the last 150,000 years. *Vegetation History and Archaebotany*, **5**: 273-292.

Eggeling WS (1940) *The Indigenous trees of the Uganda Protectorate*. Entebbe, Uganda. pp. 220-222.

Ehret C (1984) Historical/linguistic evidence for early African food production. In: Clark, J.D. & S.A. Brandt (eds.) *From Hunters to Farmers: The Causes and Consequences of Food Production in Africa*, University of California Press, Berkeley. pp. 26-35.

Eromosele IC, Eromosele CO, Kuzhhuzuzha DM (1991) Evaluation of mineral elements and ascorbic acid contents in fruits of some wild plants. *Plant Foods for Human Nutrition*, **41**: 151-154.

Fairhead J, Leach M (1996) *Misreading the African landscape: society and and ecology in a forest-savanna mosaic*. Cambridge University Press. Cambridge. 354 pp.

FAO (1968) *Ghana, land and water survey in the Upper and Northern Regions (Volume 1)*. FAO, Rome.

FAO (1977) Appendix **8**, forest genetic resource priorities. **8**. Africa. *Report of the Fourth session of the FAO panel of Experts on Forest Gene Resources*, held in Canberra, Australia, 9-11 March 1977, FAO, Rome. pp. 62-64.

FAO (1984) Appendix **8**, forest genetic resource priorities. **12**. Africa. *Report of the Fifth session of the FAO panel of Experts on Forest Gene Resources*, held in Rome, Italy, 8-11 December 1981, FAO, Rome. pp. 86-89.

FAO (1988a) Appendix **5**, forest genetic resource priorities. **10**. Africa. *Report of the Sixth session of the FAO panel of Experts on Forest Gene Resources*, held in Canberra, Australia, held in Rome, Italy, 8-11 December 1985, FAO, Rome. pp. 65-69.

FAO (1988b) Traditional food plants. *FAO Food and Nutrition Paper*, **42**: 1-593.

FAO-UNESCO (1977) *Soil Map of the World: 1: 5 000 000. 6. Africa*, UNESCO, Paris. 299 pp.

Fatubarin A (1985) The vegetation of Oro Forest Reserve in the southern Guinea savanna zone of Nigeria. I. Floristic composition. *Journal of Tropical Forest Resources* **1 (1)**: 4-12.

Fenner M (1992) Environmental influences on seed size and composition. *Horticultural Reviews*, **13**: 183-213.

Fleury J-M (1981) The butter tree. *International Development Research Centre Reports*. **10 (2)**: 6-9.

Franklin J, Hiernaux PHY (1991) Estimating foliage and woody biomass in Sahelian and Sudanian woodlands using a remote sensing model. *International Journal of Remote Sensing* **12 (6)**: 1387-1404.

Frimpong EB, Adomako D, Yeboah JM (1987) Vegetative propagation of shea, kola and *Pentadesma*. *Cocoa Research Institute of Ghana, Annual Report 1985/86*: 100.

Frimpong EB, Amonoo RS, Akuoko S (1993) Vegetative propagation of *Butyrospermum parkii* (shea nut) by cuttings. *Cocoa Research Institute of Ghana, Annual Report 1989/90*: 149.

Gaertner CF von (1807) *De fructibus et seminbus plantarum, supplementum carpologicae*, Fasicle **2(2)**. C.F.E. Richter, Leipzig. 256 pp.

Gegenheimer P (1990) Preparation of extracts from plants. *Methods in Enzymology*, **182**: 174-193.

Germani G, Luc M (1982) Studies on the 'voltaic chlorosis' of legumes caused by the nematodes *Aphasmatylenchus straturatus* Germani. 2. *Revue de Nematologie*, **5 (2)**: 195-199.

Gijsbers HJM, Kessler JJ, Knevel MK (1994) Dynamics and natural regeneration of woody species in farmed parklands in the Sahel region (Province of Passore, Burkina Faso). *Forest Ecology and Management*, **64**: 1-12.

Givnish TJ (1999) On the causes of gradients in tropical tree diversity. *Journal of Ecology*, **87**: 193-210.

Godi VG, Spensley PC (1971) Oil and oilseeds. *Tropical Products Institute Crop and Products Digest*, **1**: 1-170.

Goody J (1969) *Comparitive Studies in Kinship: Marriage Policy and Incorporation in Northern Ghana*. Routledge and Kegan Paul Ltd, London. pp. 152-153.

Grigsby WJ, Force JE (1993) Where credit is due: forests, women, and rural development. *Journal of Forestry*, **91 (6)**: 29-34.

Grolleau A (1989) Contribution a l'etude de la multiplication vegetative par greffage du karité (*Vitellaria paradoxa* Gaertn. F. = *Butyrospermum paradoxum* Hepper). *Bois et Forêts des Tropiques*, **222**: 38-40.

Hall JB, Aebischer DP, Tomlinson HF, Osei-Amaning E, Hindle JR (1996) *Vitellaria paradoxa*: a monograph. School of Agricultural Sciences Publication Number: **8**. University of Wales, Bangor. 105 pp.

Hall JB, Hindle JR (1995) Epitypification of *Vitellaria paradoxa* C.F. Gaertn. (Sapotaceae). *Taxon*, **44 (3)**: 409-410.

Hallé F, Oldeman RAA, Tomlinson PB (1978) *Tropical trees and forests. An architectural analysis*. Springer, Berlin. 441 pp.

Hamilton AC, Perrott RA (1981) A study of altitudinal zonation in the montane forest belt of Mt. Elgon, Kenya/Uganda. *Vegetatio*, **45**: 107-125.

Harlan JR (1971) Agricultural origins: centers and noncenters. *Science*, **174**: 468-474.

Harlan JR (1992) *Crops and Man*. 2nd edn. Madison, Wisconsin: American Society of Agronomy/Crop Science Society of America. pp. 65-99, 177-191.

Harris R (1998) Recommendations for improved shea nut butter processing in northern Uganda. Report of a field visit, March 11-20th, 1998. Agribusiness Development Centre. IDEA Project.

Hegazy AK, El Amry MI (1998) Leaf temperatures of desert sand plants: perspectives on the adaptability of leaf morphology. *African Journal of Ecology*, **36 (1)**: 34-43.

Heiser CB (1988) Aspects of unconscious selection and the evolution of domesticated plants. *Euphytica*, **37**: 77-81.

Hemsley JH (1961) Notes on African Sapotaceae: III. The genera Aningeria, Malacantha, and Butyrospermum in East Africa. *Kew Bulletin*, **15 (2)**: 277-91.

Hemsley JH (1968) Sapotaceae. In: Milne E, Polhill RM (eds.) *Flora of Tropical East Africa*. Crown Agents. London. pp. 47-50.

Henry AN, Chithra V, Nair NC (1983) *Vitellaria* vs. *Butyrospermum* (Sapotaceae). *Taxon*, **32**: 286.

Hepper FN (1962) Proposal to conserve 6362 *Butyrospemum* Kotschy against *Vitellaria* Gaertn. F. (Sapotaceae). *Taxon*, **11**: 226-227.

Hess T, Stephens W, Thomas G (1996) Modelling NVDI from decadel rainfall data in the north east arid zone of Nigeria. *Journal of Environmental Management*, **48**: 249-261

Holland JH (1922) *The useful plants of Nigeria, Bulletin of Miscellaneous Information, Additional Series* **9**: 1-963.

Hopkins, B. (1968) Vegetation of the Olokemeji Forest Reserve, Nigeria.V. *Journal of Ecology*, **56**: 97-116.

Hutchinson J, Dalziel JM (1963) *Flora of West Tropical Africa* 2nd edn, Vol. 2. Crown Agents. London. 544 pp.

Hyman EL (1991) A comparison of labor-saving technologies for processing shea nut butter in Mali. *World Development*, **19 (9)**: 1247-1268.

Iltis H (1983) From teosinte to maize: The castastrophic sexual transmutation. *Science*, **222**: 886-894.

Irvine FR (1974) *West African Crops*. Oxford University Press. London. pp. 68-70.

Itoh T, Tamura T, Matsumoto T (1974) Sterols and methylsterols in some tropical and subtropical vegetable oils. *Oléagineux*, **19 (5)**: 253-258.

Jachmann H, Croes T (1991) Effects of browsing by elephants on the *Combretum – Terminalia* woodland at the Nazinga Game Ranch, Burkina Faso, West Africa. *Biological Conservation*, **57 (1)**: 13-24.

Jackson G (1968) Notes on West African vegetation. III. The seedling morphology of Butyrospermum (Gaertn. f.) Hepper. *Journal of West African Science Association*, **13**: 215-222.

Jackson G (1974) Cryptogeal germination and other seedling adaptions to the burning of vegetation in savanna regions: The origin of the pyrophytic habit. *New Phytologist*, **73**: 771-780.

Jahn SAA (1991) The traditional domestication of a multipurpose tree *Moringa stenopetala* Bak.F. Cuk. in the Ethiopian rift valley. *Ambio*, **20 (6)**: 244-247.

Jonsson K, Ong CK, Odongo JCW (1999) Influence of scattered néré and karité trees on microclimate, soil fertility and millet yield in Burkina Faso. *Experimental Agriculture*, **35 (1)**: 39-53.

Jordan M, Oyanedel E (1992) Regeneration of *Pouteria lucuma* (Sapotaceae) plants *in vitro*. *Plant Cell Tissue and Organ Culture*, **31**: 249-252.

Kahlheber S (1999) Indications for agroforestry: archaeobotanical remains of crops and woody plants from medieval Souga, Burkina Faso. In: Van der Veen M, (ed.) *The Exploitation of Plant Resources in Ancient Africa*. New York, Plenum Press. pp. 89-100.

Kar A, Mital HC (1981) The study of shea butter. 6. The extraction of shea butter. *Qualitas Plantarum Plant Foods for Human Nutrition*, **31 (1)**: 67-69.

Kater LJM, Kante S, Bundelman A (1992) Karité (*Vitellaria paradoxa*) and néré (*Parkia biglobosa*) associated with crops in south Mali. *Agroforestry Systems*, **18 (2)**: 89-105.

Kelley WA, Adams RP (1977) Preparation of extracts from Juniper leaves for electrophoresis. *Phytochemistry*, **16**: 513-516.

Kephart SR (1990) Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. *American Journal of Botany*, **77(5)**: 693-712.

Kershaw SJ (1982) Occurrence of aflatoxins in oilseeds providing cocoa-butter substitutes. *Applied and Environmental Microbiology*, **43 (5)**: 1210-1212.

Kershaw SJ, Hardwick E (1981) Heterogenity within commercial contract analysis samples of shea nut kernels. *Journal American Oil Chemists Society*, **58 (6)**: 706-710.

Kershaw SJ, Hardwick E (1986) Some contractual analysis statistics for commercial sheanut samples. *Oléagineux*, **41**: 567-570.

Kessler JJ (1992) The influence of karité (*Vitellaria paradoxa*) and néré (*Parkia biglobosa*) trees on sorghum production in Burkina Faso. *Agroforestry Systems*, **17 (2)**: 97-118.

Kisakye J, Vandereiden A, Moyini Y, Fine S, Murphy C, Tibasiimwa P (1997) A final evaluation - The Shea Project, a pilot phase. COVOL Uganda, Kampala.

Klee M, Zach B (1999) The exploitation of wild and domesticated food plants at settlement mounds in north-east Nigeria (1800 cal BC to today). In: Van der Veen M, (ed.) *The Exploitation of Plant Resources in Ancient Africa*. New York, Plenum Press. pp. 81-88.

Korthals-Altes FW (1982) *Traitment des noires de karité au Mali et en Haute Volta et proposition de recherche et de dévelopment d'un technologie appropriée, de transformation de karité au Mali*. Royal Tropical Institute, Amsterdam, 32 pp.

Kotschy T (1865) De plantis nilotico-aethiopicus knoblecherianis. *Sitzungsberichte der Koenig Akademie der Wissenschaften in Wien Mathematisch-Naturwissenschaftliche Klasse Abteilung 1*, **50**: 357-359.

Lamien N, Sidibé A, Bayala J (1996) Use and commercialization of non-timber forest products in western Burkina Faso. In: Leakey RRB, Temu AB, Melnyk M, Vantomme P (eds.) *Domestication and Commercialization of Non-Timber Forest Products in Agroforestry Systems, Non-wood Forest Products No.* **9**. FAO, Rome. pp. 51-64.

Leakey RRB., Mesén JF, Tchoundjeu Z, Longman KA, Dick JMcP, Newton AC, Matin A, Grace J, Munro RC, Muthoka PN (1990) Low-technology techniques for the vegetative propagation of tropical trees. *Commonwealth Forestry Review*, **69**: 247-357.

Leakey RRB, Newton AC (eds.) (1994) Domestication of tropical trees for timber and non-timber products. *MAB Digest* **17**, UNESCO, Paris. 97 pp.

Liengsiri C, Piewluang, Boyle TJB (1990) Characterization of isozymes of three tropical tree species – effect of extraction and running buffers on staining intensity and resolution. *Journal of Tropical Forest Science*, **3(2)**: 111-122

Lewicki T (1974) *West African food in the Middle Ages, according to Arabian sources.* Cambridge University Press, London.

Lézine A-M, Vergnaud-Grazzini G (1994) Evidence of forest extension in West Africa since 22,000 BP: a pollen record from the eastern tropical Atlantic. *Quaternary Science Reviews*, **12**: 203-210.

Lobreau-Callen D, Darchen R, Thomas A le, (1986) The contribution of palynology to knowledge of bee-plant relationships in the forest savannas of Togo and Benin. *Apidologie*, **17 (3)**: 279-306.

Loomis WD (1974) Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles. *Methods in Enzymology*, **31**: 528-544.

Lovett PN (1993) Development of the "walking transect" method for indigenous forest survey: case study from Mbeya Mountain Forest Reserve, Tanzania. *M.Sc. Thesis*. University of Aberdeen, Aberdeen. 56 pp.

Lovett PN (1999) Recommendations for the applied research component of 'The Shea Project for Conservation and Development', expansion phase I, 1999 – 2003. Working report of a field visit to Uganda, January 12th-29th, 1999. (unpublished document).

Lovett PN, Haq N (2000a) Diversity of the sheanut tree (*Vitellaria paradoxa* Gaertn C. F.) in Ghana. *Genetic Resources and Crop Evolution*, **47**: 293-304.

Lovett PN, Haq N (2000b) Evidence for anthropic selection of the sheanut tree (*Vitellaria paradoxa*). *Agroforestry Systems* **48**: 273-288.

Lovett PN, Haq N (2000c) *In vitro* regeneration of *Vitellaria paradoxa*. (in prep.).

Lovett P, Azad AK, Paudyal K, Haq N (1997) Genetic diversity and development of propagation techniques for tropical fruit tree species. In: Smartt J, Haq N, (eds) *Domestication, Production and Utilization of New Crops*. COLORLINE Printers, Dhaka, pp. 292-293.

Mann RD (1990) Time running out: The urgent need for tree planting in Africa. *The Ecologist*, **20 (2)**: 48-53.

Masters ET (1992) *Shea in Uganda: a COVOL Preliminary Report*. Cooperative Office for Voluntary Organizations of Uganda, Kampala. 23 pp.

Masters ET, Puga A (1994) *The Shea Project for Local Conservation and Development*. A pilot project for community based conservation and development. Review copy. Edition 3. Cooperative Office for Voluntary Organizations of Uganda, Kampala. 56 pp.

Mathias PJ (1988) Micropropagation of the tropical hardwoods *Khaya ivorensis* A. Chev. and *Nauclea diderichii* (de Wild. and Th. Dur.) Merrill. *PhD Thesis*. University of Nottingham. 312 pp.

Maydell HJ (1986) *Trees and Shrubs of the Sahel; their characteristics and uses*. Deutsche Gesellschaft für Techniche Zusammenarbeit, Eschborn. pp. 202-207.

McVaugh R (1967) *Butyrospermum* Kotschy (1865) vs. *Vitellaria* C. F. Gaertner (1807). Conservation of generic names **VII**. Report of the committee for Spermatophyta. *Taxon*, **16**: 226-229.

McVaugh R (1970) Report of the committee for Spermatophyta. **XII**. *Taxon*, **19**: 289-294.

Menaut JC, Lepage M, Abbadie L (1995) Savannas, woodlands and dry forests in Africa. In: Bullock SH, Mooney HA, Medina E (eds) *Seasonally Dry Tropical Forests*. Cambridge University Press, Cambridge. pp. 64-92.

Mendez MVR, Lope JH (1991) La manteca de karité. *Grasas y Aceites*, **42**: 121-126.

Miège J (1954) Nombre chromosomiques et répartition géographique de quelques plantes tropicales et équatoriales. *Revue Cytologie et Biologie Végétale*, **15 (4)**: 312-348.

Miller, M.P. (1997) *Tools for population genetic analyses* (TFPGA) **1.3**: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.

Mital HC, Dove FR (1971) The study of shea butter. **1**. Physico-chemical properties. *Planta Medica*, **20 (3)**: 283-8.

Mital HC, Dove FR (1973) Shea butter as a base for ointments and creams. *Drug and Cometic Industry*, **113 (4)**: 46-47, 143-147.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, **15**: 473-497.

Murphy RW, Sites JW, Buth DG, Haufler CH (1996) Proteins: Isozyme electrophoresis. In: Hillis DM, Moritz C, (eds.) *Molecular Systemmatics*. Sinauer Associates, Sunderland, pp. 51-120.

Nei M (1972) Genetic distance between populations. *American Naturalist*, **106**: 283-292.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583-590.

Neiss T (1983) New shea butter technology for West African women. *GATE*, **2**: 15-17.

Neumann K, Kahlheber S, Uebel D (1998) Remains of woody plants from Saouga, a medieval West African village. *Vegetation History and Archaeobotany*, **7**: 57-77.

Neumann K (1999) Early plant production in the West African Sahel – new evidence. In: Van der Veen M, (ed.) *The Exploitation of Plant Resources in Ancient Africa*. New York, Plenum Press. pp. 73-80.

Nouaim R, Chaussod R (1994) Mycorrhizal dependency of propagated argan tree (*Argania spinosa*): I. Growth and biomass production. *Agroforestry Systems*, **27**: 53-65.

Ohler FMJ (1985) The fuelwood production of wooded savanna fallows in the Sudan zone of Mali. *Agroforestry Systems*, **3 (1)**: 15-23.

Opoku-Ameyaw K, Lovett P, Owusu GK (1997) Strategies for the conservation of the sheanut tree (*Vitellaria paradoxa* Gaertn. F.) in Ghana. Poster presented at "Gestion des Ressources Génétiques des Plantes en Afrique de Savanes" Bamako, Mali, February 24-28, 1997.

Osei-Amaning E (1996) Management of *Vitellaria paradoxa* in guinea savanna rangelands in Ghana. *Ph.D. thesis*. University of Wales. Bangor. 199 pp.

Ouédraogo AS (1995). *Parkia biglobosa* (Leguminosae) en Afrique de l'Ouest: biosystématique et amélioration. *Ph.D. Thesis*. Agricultural University Wageningen. 205 pp.

Park M (1799) Travels into the interior districts of Africa performed under the direction and patronage of the African Association in the years 1795, 1796 and 1797. In: Swift J (1983) *Travels into the Interior of Africa*. Eland, London. pp. 1-264.

Pennington TD (1991) *The genera of Sapotaceae*. Royal Botanic Gardens/New York Botanic Gardens, Kew/New York. 295 pp.

Pereira J (1983) The effectiveness of six vegetable oils as protectants of cowpeas and bambara groundnuts against infestation by *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) *Journal of stored products research*, **19** **(2)**: 57-62.

Peters CR, O'Brien EM, Drummond RB (1992) *Edible Wild Plants of Sub-Saharan Africa*. Royal Botanic Gardens Kew, London. 239 pp.

Petty JN, Guijt I, et al. (1995) *Participatory learning and action: a trainer's guide*. London, IIED.

Picasso G (1984) Synthèse des résultats acquis en matière de recherche sur le karité au Burkina Faso de 1950 à 1958. *Rapport de l'Institute de Recherches sur les Huiles et Oléagineux*, Paris. 45 pp.

Pilz GE (1980) Chromosome number reports **LXVII**. *Taxon*, **29**: 352-353.

Pritchard HW, Yameogo CS, Daws M (1996) *IPGRI Annual Report 1996*. http://www.iini.org/ipgri/ANNUREP/gdtxt.htm.

Pugansoa B, Amuah D, Wallace T (1991) Resources for Women: case study of the OXFAM sheanut loan scheme in Ghana. In: Wallace T, March C (eds.) *Changing perceptions on gender and development*. OXFAM publications, Oxford. pp. 236-244.

Pullan RA (1974) Farmed parkland in West Africa. *Savanna*, **3** **(2)**: 119-151.

Raison JP (1988) Les 'parcs' en Afrique: état des connaissances et perspectives de recherches. Centre d'Études Africaines, École d'Haute Études en Sciences Sociales, Paris. 117 pp.

Renes GJB (1991) Regeneration capacity and productivity of natural forest in Burkina Faso. *Forest Ecology and Management*, **41 (3-4)**: 291-308.

Roberts N (1998) *The Holocene. An Environmental History*. Blackwells Publishers Ltd. Oxford. 316 pp.

Rout GR and Das P (1993) Micropropagation of *Madhuca longifolia* (Koenig) MacBride var. latifolia Roxb. *Plant Cell Reports* **12**: 513-516.

Ruyssen B (1957) Le karité au Soudan. *Agronomie Tropicale*, **12 (2-3)**: 143-172, 279-306, 415-440.

Ryan KG, Markham KR, Bloor SJ, Bradley JM, Mitchell KA, Jordan BR (1998) UVB radiation induced increase in quercetin: kaempferol ratio in wild-type and transgenic lines of Petunia. *Photochemistry and Photobiology*, **68 (3)**: 323-330.

Sallé G, Boussim J, Raynal-Roques A, Brunck F (1991) Le karité: une richesse potentielle. Perspectives de recherche pour ameliorer sa production. *Bois et Forêts des Tropiques*, **228**: 11-23.

Sallé G, Raynal-Roques A, Tuquet C (1987) Field identification of some Loranthaceae parasitizing *Butyrospermum paradoxum* (Gaertn. F.) Hepper (Sapotaceae) in Mali and Burkina Faso. In: Weber HCh, Forstreuter W (eds.) *Parasitic flowering plants*. Proc. 4th ISPFP. pp. 715-717.

Salzmann U, Waller M (1998) The Holocene vegetational history of Nigerian Sahel based on multiple pollen profiles. *Review of Paleobotany and Palynology*, 100: 39-72.

Schmidt PR (1997) Archaeological views on a history of landscape change in East Africa. *Journal of African History*, **38**: 393-421.

Schreckenberg K (1996) Forests, fields and markets: a study of indigenous tree products in the woody savannas of the Bassila Region, Benin. *Ph.D. thesis*. University of London. 326 pp.

Schulman L, Junikka L, Mndolwa A, Rajabu I (1998) *Trees of Amani Nature Reserve N.E. Tanzania.* The Ministry of Natural Resources and Tourism, Tanzania. Helsinki University Printing House, Helsinki. 336 pp.

Simons T (1997) Tree domestication - better trees for rural prosperity. *Agroforestry Today*, **9 (2)**: 3-9.

Singh M, Bansal YK (1993) *In vitro* callus proliferation in a multipurpose tree species - Mahua (*Madhuca latifolia* Macb.). *Bionature*, **13 (2)**: 207-210.

Sokal RB, Rohlf JR (1997) *Biometry: the principles and practice of statistics in biological research.* 3rd ed. WH Freeman, & Co. New York. 850 pp.

Sokal RB, Braumann CA (1980) Significance tests for coefficients of variation and variability profiles. *Systematic Zoology*, **29**: 50-63.

Soltis DE, Haufler CH, Darrow DC, Gastony GJ (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal*, **73(1)**: 9-27.

Stahl AB (1986) Early food production in West Africa: rethinking the role of the Kintampo culture. *Current Anthropology*, **27 (5)**: 532-536.

Survey of Ghana (1966) *1:50,000 topographic maps of Ghana.* Accra.

Swetman T, Hammond L (1999) Rural women earn cash for "butter" from nuts in Ghana. Natural Resources Institute, University of Greenwich, UK. (unpublished). 3 pp.

Taiz L, Zeiger E (1991) *Plant Physiology*. Benjamin/Cummings Publishing Company Inc, California.

Talbot M, Johannessen T (1992) A high resolution palaeoclimatic record for the last 27,500 years in tropical West Africa from the carbon and nitrogen isotopic composition of lacustrine organic matter. *Earth and Planetary Science Letters*, **110:** 23-37.

Tawiah A (1994) Rural women's group exports shea-butter. *Daily Graphic*, Ghana. **27/6/94**: 1.