Attributes that make *Acacia karroo* dominant: stable N and C isotope analysis of nine *Acacia* species from KwaZulu-Natal (South Africa)

BY:
Theresa Nobuhle Mgidi

SUPERVISED BY:
Associate Professors J. J. Midgley & W. D. Stock

Submitted in fulfilment of the requirements for the Master of Science degree
Botany Department
University of Cape Town

2006

ABSTRACT

This study focuses on nine *Acacia* species from Hluhluwe-Umfolozi Park in KwaZulu-Natal, South Africa. The study was motivated by the need to understand the dominance of legumes in the process of bush encroachment in Southern Africa. *A. karroo* has been identified as a particularly invasive species in HUP, and determining what makes this *Acacia* species and any of the other acacias in HUP invasive formed the foundation of this study. The leaves of nine *Acacia* species found in HUP were analysed for stable nitrogen ($\delta\ ^{15}N$) and carbon ($\delta\ ^{13}C$) isotope ratios. The concentration of total nitrogen (N) in the leaves of these species was also measured, and both their mechanical and chemical defence mechanisms evaluated. The measured leaf $\delta\ ^{15}N$ values were used to determine the N_2-fixing capabilities of the nine *Acacia* species, including the variation between the nine species and the potential influence of external factors such as environmental differences between the two main sampling sites, Hluhluwe and Umfolozi. The $\delta\ ^{13}C$ values were used as indicators of water-use efficiency and were further correlated with the leaf size, and compared between the wet (Hluhluwe) and dry (Umfolozi) sampling sites. Total leaf N concentrations were correlated with the mechanical and chemical defence mechanisms of the nine *Acacia* species in order to establish whether or not there was a link between the two variables.

The $\delta\ ^{15}N$ values varied amongst the selected *Acacia* species. Those from the dry Umfolozi sites differed significantly from those of known non-fixing plants. The Hluhluwe *Acacia* species had $\delta\ ^{15}N$ values within the same range as those of the known non-fixing plants. Species differences (in relation to the presence of N_2-fixing bacteria) and environmental differences (in relation to rainfall and fire) were considered responsible for the lack of difference between the $\delta\ ^{15}N$ values of the acacias (+0.90 ‰) and known non-fixing plants (+1.49 ‰) sampled in Hluhluwe. The $\delta\ ^{13}C$ values of the selected *Acacia* species indicated that to some extent the species sampled from the drier Umfolozi sites (-28.24 ‰) were utilising available water more efficiently than those sampled from the wetter Hluhluwe sites (-29.19 ‰). Furthermore, the correlation between the $\delta\ ^{13}C$ values and water-use efficiency was such that the acacias with small leaves (e.g. *A. luderitzii*) were more water-use efficient than those with bigger leaves (e.g. *A. caffra*). The nine *Acacia* species differed in both their mechanical and chemical defence mechanisms. However, the *Acacia* species with the highest leaf N concentrations (e.g. *A. tortilis*) had the best defence whether mechanical, chemical or a combination of both and other adaptations.

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LIST OF ABBREVIATIONS AND UNITS

Hluhluwe-Umfolozi Park (HUP)
^{15}N natural abundance (δ ^{15}N)
^{13}C natural abundance (δ ^{13}C)
Nitrogen (N)
Nitrogen gas (N_2)
Total leaf nitrogen concentration ([N])
Carbon (C)
Carbon dioxide (CO_2)
Nitrate (NO_3^-)
Nitrite (NO_2^-)
Nitrogen-oxides (NO_2, NO, N^2O)
Ammonia (NH_4)
Ammonium (NH_4^+)
Species – singular (sp.)
Species – plural (spp.)
Degrees Celsius (° C)
Primary (1°)
Secondary (2°)
Analysis of Variance (ANOVA)
Standard Deviation (Std.Dev.)
Peedee Formation Standard (PDB)
Versus (vs.)

Milligrams (mg)
Centimetres (cm)
Milligrams per centimetre squared (mg/cm^2)
Litre (L)
Millilitres (mL)
Microlitres (µL)

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Associate Professors William Stock and Jeremy Midgley. Not only did they guide me and encourage me throughout the very long journey of my thesis, but they were my strongest and unwavering support structure even with matters way outside their supervisory scope. For their tremendous ability to motivate while remaining patient with me throughout the duration of my Masters, I am very grateful.

I would also like to thank the Botany Department of the University of Cape Town, for providing an environment that made it possible for me to pursue this path and all the support leading to where I am today. The Mellon Foundation is thanked for its financial support without which all this would not have been possible. I would also like to thank the University of Cape Town's Post-Graduate Funding system/structure for their contribution towards helping me meet my financial needs.

I would like to thank the staff of the Hluhluwe-Umfolozi Park's Research Centre for accommodating me and making it possible for me to collect all the data I needed for my study. In particular, I would like to thank the following: Dave Balfour for putting in place structures of assistance for me as well as allowing me the use of the Research Centre's facilities; Sue and Terry for their company and continuous support during my time at the park; Abednig, Thobile, S'ne, Zanele and Xolani for making me feel at home at the park and for the occasional help they provided when I needed it; and lastly S'celo Nkosi for the great effort he put into helping me collect all my data, a fate that was not easy but that he did with such enthusiasm and dedication, that my project could not have been anything but successful with his help and input.

Last but definitely not least, I'd like to thank my family and friends for putting up with me talking about my project non-stop throughout its duration.

CHAPTER 1:

INTRODUCING THE STUDY

1.1 Why investigate *Acacia* species?

The genus *Acacia* is one of the largest and widely distributed genera of trees and shrubs in Africa, occupying a diverse range of habitats (Pooley, 1993; Barnes *et al.*, 1996; Van Wyk and van Wyk, 1997). The motivation for focussing on acacias for this study relates to the process of bush encroachment, a serious problem in many southern African ecosystems. Most of the bush encroaching (i.e. invasive) species in southern Africa are legumes such as *Dichrostachys* (Roques *et al.*, 2001), and in Hluhluwe-Umfolozi Park (HUP) *Acacia karroo* has long been known to be an aggressive invasive (Whately and Porter, 1983; Skowno *et al.*, 1999; Bond *et al.*, 2001). With this in mind, I was interested in determining what attributes legumes have that make them invasive, particularly *A. karroo* in HUP. Is it because it is able to fix atmospheric N_2 better than the other *Acacia* species growing in its vicinity? Does it have the best resistance to herbivory which makes it possible for it to persist and spread more than the other acacias? Or is it because it has higher water-use efficiency in an environment where rainfall varies and water availability can be limited? The objectives aimed at getting answers to these three questions are described in section 1.3 below and are discussed in more detail in the following chapters of this study.

1.2 Why the $\delta^{15}N$ technique?

Since *A. karroo* and other legumes have been identified as some of the major invaders of savanna ecosystems, the role N_2-fixation plays in the process leading to invasiveness of some legumes was identified as the most important factor to be investigated in this study. N_2-fixing plants have access to an external source of N from atmospheric N_2 supplied by N_2-fixing bacteria, unlike non-fixing plants that utilise soil N. Symbiotic N_2-fixation by legumes plays a key and fundamental role in maintaining soil fertility and the continued productivity of natural and cultivated ecosystems. However, its contribution towards making plants such as *A. karroo* successful invaders in HUP and the extent to which it results in the alteration of soil fertility such that non-fixing plants are out-competed remains unclear. In order to be able to assess the role of N_2-fixation in promoting the invasiveness of legumes, its quantification is important (Gathumbi *et al.*, 2002) and to this effect many techniques have been developed for that purpose (Unkovich and Pate, 2000; Tsialtas *et al.*, 2004).

Realistic estimates of N_2-fixation are best determined under field conditions. The dynamics of the N cycle in the field are different from those found under laboratory conditions, rendering the field experimental approach more accurate in its estimations. A number of methods (e.g. total N difference, ureide assay, acetylene reduction assay, and ^{15}N isotope techniques) have been developed to measure N_2-fixation. The choice of a particular method depends on the type and site of the experiment, the available resources, and the species and/or system under investigation (Giller, 2001). ^{15}N isotope techniques have been widely used to obtain estimates of the percentage of N derived from N_2-fixation of a legume during its growing period (Gathumbi *et al.*, 2002). Parrotta *et al.* (1994) suggested that the $\delta\ ^{15}N$ method for estimating N_2-fixation is a useful technique for investigating N_2-fixation in large tree species under field conditions. However, it is important to note that such a method of estimating N_2-fixation especially when applied to deep rooted shrubs and trees and in undisturbed natural ecosystems, can be limited and can produce results with errors (Danso *et al.*, 1993; Pate *et al.*, 1993; Stock *et al.*, 1995; Handley and Scrimgeour, 1997; Boddey *et al.*, 2000; Spriggs *et al.*, 2003; Okito *et al.*, 2004). Since there is no single method that is perfect for such an analysis at present, the $\delta\ ^{15}N$ method is well-suited for the qualitative assessment required for this study.

1.3 Study objectives

The measurements resulting from the $\delta\ ^{15}N$ analysis of the nine *Acacia* species from HUP and their reference plants were used to investigate the three main objectives mentioned in section 1.1 above, each of which had hypotheses that were tested. The first objective was aimed at determining which of the nine *Acacia* species of HUP was the best N_2-fixer, was it *A. karroo* as expected? The second objective involved assessing the defence mechanisms of the nine *Acacia* species, does *A. karroo* have the best resistance against herbivores. The last objective was aimed at investigating the water-use efficiency of nine *Acacia* species in order to determine if *A. karroo* had that advantage over the other HUP acacias. The hypotheses tested as part of each of the above-mentioned objectives are listed below (sections 1.3.1, 1.3.2 and 1.3.3).

1.3.1 ^{15}N isotope ecology

This part of the study involved comparing the stable ^{15}N isotopic compositions of the nine *Acacia* species from HUP (e.g. differences within and between the acacias) in order to determine their N_2-fixing ability and/or strength. The following two hypotheses were tested in relation to this objective:

- N_2-fixation abilities of the acacias differ among species;

- N_2-fixation abilities of the acacias are affected by environmental conditions.

Chapter 2 elaborates on this part of the study and the results of the investigated hypotheses were used to make conclusions about the role of N_2-fixation in the invasive nature of legumes and more specifically *A. karroo* in HUP.

1.3.2 Comparative defence ecology

The defence mechanisms of the nine *Acacia* species selected for this study were investigated by testing the following hypotheses:

- The acacias differ in their mechanical and chemical defence mechanisms;
- Acacias with high leaf N have the greatest allocation to defence.

This is discussed in more detail in Chapter 3, where the selected *Acacia* species ability to resist herbivory is measured by comparing their mechanical and chemical defence strategies, and correlating this to total leaf N concentration [N]. The results of this part of the study were used to arrive at a conclusion with regards to *A. karroo* being the best defended of the HUP acacias, and how that may be linked to its success as and invader in HUP.

1.3.3 ^{13}C isotope ecology

The third objective involved using stable ^{13}C isotope measurements to investigate the water-use efficiency of the nine *Acacia* species, and determining how water-use efficiency is affected by rainfall variation and leaf size. The following hypotheses were tested:

- The acacias differ in water-use efficiency;
- Acacias with small leaves have higher water-use efficiency;
- Acacias from drier areas have higher water-use efficiency.

This part of the study is presented in Chapter 4 and its results were used to draw conclusions on whether *A. karroo* has higher water-use efficiency than the other HUP acacias.

The general conclusions of this study is presented in Chapter 5. This refers back to the three questions asked at the beginning of this chapter with regards to the attributes that make *A. karroo* invasive in HUP over the other acacias growing in the same vicinity. This last chapter also elaborates on other research contributions arising from this study. These include providing insight into the extent of the impact N_2-fixation has on soil dynamics, presenting a framework for interpreting herbivory results, and highlighting some potential implications of global change impact predictions on the water-use efficiencies of plants.

CHAPTER 2:

USING THE δ ^{15}N METHOD TO DETERMINE N_2-FIXATION ABILITY OF NINE SOUTH AFRICAN ACACIAS

2.1 Nitrogen fixation and plant δ ^{15}N

Nitrogen inputs to ecosystems can occur through two processes, biological fixation and atmospheric deposition. Both of these processes involve plants interacting with soil and soil microbes to obtain N (Hawkes, 2003). It is well-known that the highest reserve of N is in the atmosphere (about 78%), and that most plants cannot directly utilise this source of N. N_2-fixing legumes, however, are able to convert atmospheric N_2 into forms of N that they can use for growth through symbiotic bacteria (Ibijbijen *et al.*, 1996; Knops *et al.*, 2002). In nutrient-poor systems, N fixed by these bacterial associations gives an advantage to the leguminous plants (Evans and Ehleringer, 1993) over non-fixing plants that are reliant on soil N for their growth. Furthermore, N_2-fixing plants contribute substantially to the overall N economy of the systems in which they occur (Allaway *et al.*, 2000; Gathumbi *et al.*, 2002), particularly environments with nutrient-poor soils.

2.1.1 *Measuring plant* $\delta^{15}N$

The δ ^{15}N method has been used to estimate the proportion of N derived from the atmosphere by N_2-fixers. It is based on the differences in isotopic composition between plant available soil N and atmospheric N_2 (Reiter *et al.*, 2002). The method relies on there being some non-fixing 'reference' plants known to access soil N only, whose δ ^{15}N measurements can be compared to those of the legumes under investigation. Plant available soil N is naturally enriched in ^{15}N compared to atmospheric N, which has δ ^{15}N values defined as 0.00 ‰ (Redecker *et al.*, 1997;

Okito *et al.*, 2004). Thus $\delta\ ^{15}N$ values higher than 0.00 ‰ are expected from analysed soil samples and from plants known to be utilising soil N only; and $\delta\ ^{15}N$ values lower than or around 0.00 ‰ are expected from plants utilising atmospheric N_2 (such as N_2-fixing legumes). Even though the use of the $\delta\ ^{15}N$ method to estimate N_2-fixation is known to have some disadvantages (Shearer and Kohl, 1993; Stock *et al.*, 1995; Spriggs *et al.*, 2003; Okito *et al.*, 2004), other studies have shown that this method is adequate for quantitatively estimating N_2-fixation in plants under field conditions (Parrotta *et al.*, 1994; Gathumbi *et al.*, 2002).

2.1.2 Variation in plant $\delta^{15}N$

A number of factors are known to have an influence on plant $\delta\ ^{15}N$. The influence of two of these factors will be discussed in this study: firstly, the differences in the effectiveness of different rhizobium species responsible for the nodulation of N_2-fixing plants (Galiana *et al.*, 1990; Galiana *et al.*, 2002); and secondly, the differences in the environmental conditions under which the sampled plants were grown (Lauenroth and Sala, 1992; Snyman and Fouche, 1993; O'Connor *et al.*, 2001; Oesterheld *et al.*, 2001; Snyman, 2004). The environmental conditions that can have an impact on plant $\delta\ ^{15}N$ that are of that are pursued in this study include the following: soil N, and fire as a consequence of variation in annual rainfall.

The influence of soil N on the N isotopic composition of the plants has been linked to spatial heterogeneity effects within and between sampling sites, whereby these spatial effects may affect soil $\delta\ ^{15}N$ measurements (Aranibar *et al.*, 2003). The link between annual rainfall and fire was considered because of the potential impact of fire on both soil and plant N. A characteristic of savanna habitats is that wet sites have tall uniform stands of grass ideal for supporting more frequent and intense fires after the rainy season, whereas the drier sites have shorter and patchy stands of grass associated with fewer and less intense fires (Lauenroth and Sala, 1992; Snyman and Fouche, 1993; O'Connor *et al.*, 2001; Oesterheld *et al.*, 2001; Bond *et al.*, 2001; Snyman, 2004). Since past studies have shown that fire has an effect on nutrient (including N) cycling and distribution, and that fire intensity can have biological, chemical and physical effects on soils thereby impacting on nutrient reserves (Aranibar *et al*., 2003; Okito *et al*., 2004), the difference in annual rainfall (and consequently fire regimes) between Hluhluwe and Umfolozi is likely to influence the $\delta\ ^{15}N$ measurements of the plants and soils within HUP.

2.1.3 Study objectives

This part of the study is aimed at estimating the ability of the nine *Acacia* species from HUP to fix atmospheric N_2. This is indicated by $\delta\ ^{15}N$ measurements lower than or close to 0.00 ‰ as expected of N_2-fixing plants with bacterial associations that discriminate against the heavier ^{15}N isotope. Furthermore, the known difference in annual rainfall between Hluhluwe and Umfolozi is likely to influence the selected acacias' ability to fix N_2, whereby significant differences between the $\delta\ ^{15}N$ measurements of the acacias (and their reference plants) sampled from Hluhluwe and those sampled from Umfolozi would be expected.

2.2 Materials and Methods

2.2.1 *Materials and study sites*

Material

The leaves of nine species of the genus *Acacia* and six different known non-fixing tree species occurring in the same vicinity as the acacias were sampled for the purposes of this study (Table 2.1). Many similar studies used leaf samples to measure whole-plant $\delta\ ^{15}N$ (Hobbie *et al.*, 2000; Miller and Bowman, 2002), as it is often difficult to access root material and impossible to sample whole-plant $\delta\ ^{15}N$ in field experiments (Yoneyama *et al.*, 1991; Evans *et al.*, 1996; Högberg *et al.*, 1999; Robinson *et al.*, 2000; Kolb and Evans, 2002). Two of the selected reference plants (e.g. *Euclea* species) were not the ideal plants to compare with the acacias as they are evergreen while the acacias are deciduous, a difference that could have an impact on the $\delta\ ^{15}N$ measurements. However, these plants were selected because they were repeatedly found growing along side the *Acacia* species under investigation and were amongst the a few tree species within the vicinity closest in age to the sampled acacias. Soil samples were also collected in order to identify the differences between plants utilising soil N (e.g. known non-fixing plants) and those with access to atmospheric N_2 such as N_2-fixing plants (Högberg, 1997; Handley and Raven, 1992; Martinelli *et al.*, 1999; Evans, 2001; Robinson, 2001).

Study sites

The *Acacia* species and the known non-fixing plants used in this study were collected at different sites in Hluhluwe-Umfolozi Park (HUP), a game park of 96 000km^2 in KwaZulu-Natal, South Africa (Figure 2.1) contains an immense diversity of grasses and trees. Hluhluwe is the wetter northern section of the park and is characterised by a hilly topography, while Umfolozi makes up the drier southern component of the park west of the town Mtubatuba. The park is generally hot and humid, typical of sub-tropical regions. Most rain falls during the months of September to April, with an annual rainfall between 750mm and 1000mm. As a result of the difference in annual rainfall between Hluhluwe and Umfolozi, a fire frequency and intensity gradient exists (Balfour and Howieson, 2001).

2.2.2 Methods

Sample collection

Leaf material was randomly sampled from five individual mature trees of each of the nine selected *Acacia* species. In Hluhluwe, leaves of *A. nilotica*, *A. karroo*, *A. burkei*, *A. robusta* and *A. caffra* were collected at four different sites per species. In Umfolozi, leaves of *A. nigrescens*, *A. tortilis*, *A. luderitzii* and *A. grandicornuta* were sampled and this was repeated at five different sites.

At each sites leaf material from five individual trees known not to be fixers of N_2 (reference plants) were sampled. These were generally the same sizes as the sampled acacias. Two of the selected *Acacia* species, *A. nilotica* and *A. karroo*, were sampled from both Hluhluwe and Umfolozi. This was done in order to determine the impact of a variation in environmental conditions between Hluhluwe and Umfolozi.

Under each sampled acacia and reference plant in both Hluhluwe and Umfolozi sites, soil was also sampled to a depth of 5 cm. This was done with the notion that the soil $\delta\ ^{15}N$ measurements could be used to distinguish between plants utilising soil N and those fixing atmospheric N_2. The soil $\delta\ ^{15}N$ was also used to identify any soil property effects (e.g. soil N, soil moisture, and fire) that could have an impact on the $\delta\ ^{15}N$ of the selected acacias and their reference plants. However, the soils of *Rhus sp.* and *Sclerocarya birrea* sampled from Hluhluwe as well as *Rhus sp.* and *Ziziphus mucronata* sampled from Umfolozi were contaminated after sampling and therefore could not be analysed for $\delta\ ^{15}N$.

$\delta\ ^{15}N$ procedure

All plant leaf samples were dried at 70 °C for 48 hours and then ground up using a Retch Mill ball-mill (Brinkmann Instruments Inc., NY, USA) until the leaf material was powder fine. Approximately 2 mg of the ground material of the acacias and the reference plants were measured for their stable isotopic composition in the Finnigan-MAT 252 mass spectrometer after combustion in a Carlo Erba CHN analyser. The system allowed for the simultaneous measurement of plant $\delta\ ^{15}N$ and the total N concentration ([N]). The measured $\delta\ ^{15}N$ and [N] values were adjusted using the University of Cape Town's laboratory standard Nasturtium before statistical analysis.

Statistical analyses

The mean $\delta\ ^{15}N$ measurements of the *Acacia* species, the reference plants and the soil sampled from Hluhluwe and Umfolozi sites were tabulated (see Table 2.2). T-test analyses were performed on these measurements and used to compare the following:

i. the mean $\delta\ ^{15}N$ values of the acacias and those of their reference plants, at both sites;
ii. the mean $\delta\ ^{15}N$ values of the acacias and reference plants sampled from Hluhluwe and with those of the same plants sampled from Umfolozi;
iii. the mean $\delta\ ^{15}N$ values of the soils sampled under the acacias and those sampled under the reference plants, at both sites;
iv. the mean $\delta\ ^{15}N$ values of the soils sampled from under the acacias and reference plants from Hluhluwe and those of the soils sampled from under the acacias and reference plants from Umfolozi.

Lastly, simple or one-way ANOVAs were calculated between the mean $\delta\ ^{15}N$ values of the *Acacia* species (*A. karroo* and *A. nilotica*) sampled in both Hluhluwe and Umfolozi (i.e. $\delta\ ^{15}N$ values of *A. karroo* sampled from Hluhluwe compared to those of *A. karroo* sampled from Umfolozi, and similarly for *A. nilotica*).

2.3 Results

2.3.1 *Comparison $\delta^{15}N$ amongst species and soils within Hluhluwe*

The mean $\delta^{15}N$ values of the *Acacia* species sampled within Hluhluwe ranged from +0.22 ‰ for *A. caffra* to + 1.67 ‰ for *A. burkei*, with an overall mean $\delta^{15}N$ value of +0.90 ‰ (Table 2.2). The mean $\delta^{15}N$ values of the reference plants within the same part of HUP ranged from +0.56 ‰ for *E. racemosa* and +2.21 ‰ for *Rhus sp.*, and their overall mean $\delta^{15}N$ value was +1.49 ‰ (Table 2.2). Even though the overall mean $\delta^{15}N$ values of the acacias and the non-fixing reference plants in Hluhluwe were different (+0.90 and +1.49 ‰), these differences were statistically insignificant ($p = 0.21$). In general, of all the *Acacia* species sampled in this part of the park, *A. nilotica* and *A. burkei* had mean $\delta^{15}N$ values that were uncharacteristically high for potentially N_2-fixing leguminous plants. *E. divanorum* and *E. racemosa* also had uncharacteristically low mean $\delta^{15}N$ values for non-fixing reference plants.

Soil sampled under the acacias and the reference plants in Hluhluwe was found to have similar mean $\delta^{15}N$ values (Table 2.3). The mean $\delta^{15}N$ values of the soil sampled under both the acacias and the reference plants were much higher than those of the plants. The difference between the mean $\delta^{15}N$ values of the soil sampled under the *Acacia* species and those sampled under the reference plants was found not to be significant ($p = 0.44$).

2.3.2 *Comparison of $\delta^{15}N$ amongst species and soils within Umfolozi*

The mean $\delta^{15}N$ values of the *Acacia* species sampled from Umfolozi were generally low, with an overall mean $\delta^{15}N$ value of +0.03 ‰ (Table 2.2). When the overall mean $\delta^{15}N$ value of the *Acacia* species was compared to that of the reference plants, they differed significantly ($p = 0.01$).

The overall mean $\delta^{15}N$ values of the soil sampled from under the Umfolozi acacias was +4.03 ‰, and that of soil sampled under the reference plants was +3.96 ‰ (Table 2.3). When the overall mean $\delta^{15}N$ values of the soil sampled under the acacias were compared to those of soil sampled under the reference plants, they did not differ significantly (p = 0.93).

2.3.3 Comparison of $\delta^{15}N$ between Hluhluwe and Umfolozi

The mean $\delta\ ^{15}N$ values of the acacias sampled in Hluhluwe were higher (overall mean of +0.90 ‰) than those sampled in Umfolozi (overall mean of +0.03 ‰), and this difference was significant (p = 0.04). The mean $\delta\ ^{15}N$ value of *A. nilotica* sampled from Hluhluwe was +1.40 ‰ and -0.44 ‰ for the Umfolozi samples, this difference was significant (p=0.05). Similarly, the mean $\delta\ ^{15}N$ value of *A. karroo* sampled from Hluhluwe was +0.43 ‰ and -0.85 ‰ for the Umfolozi sampled replicate, these differed significantly (p= 0.01).

The mean $\delta\ ^{15}N$ values of the soil sampled under the acacias from Hluhluwe did not differ (p = 0.21) from the soil sampled under acacias in Umfolozi. Similarly, the mean $\delta\ ^{15}N$ values of the soil sampled under the reference plants from Hluhluwe and those of soil sampled under the reference plants from Umfolozi were not significantly different (p = 0.41) (i.e. plant values differed between the two sites, but not the soil values).

2.4 Discussion

2.4.1 *N_2-fixation abilities of acacias differ between species*

Differences between the δ ^{15}N measurements of the selected *Acacia* species within both Hluhluwe and Umfolozi were an indication of the plants' varying N_2-fixing abilities. The mean δ ^{15}N values of most (except *A. burkei* and *A. nilotica* from Hluhluwe) of the *Acacia* species under investigation were lower than those of the reference plants, and were close to 0.00 ‰ as is expected of N_2-fixing plants utilising atmospheric N_2 (Redecker *et al.*, 1997; Okito *et al.*, 2004). However, the difference observed from the Hluhluwe acacias and their reference plants was not statistically significant. On the other hand, the mean δ ^{15}N values of the Umfolozi acacias (*A. luderitzii*, *A. grandicornuta*, *A. nigrescens*, *A. tortilis*, *A. karroo*, and *A. nilotica*) differed significantly from those of their reference plants (p= 0.01). This suggests that the Umfolozi acacias and their reference plants access different sources of N. Since it is expected that the reference plants (known to be non-fixers) only utilise soil N, the results of this study suggest that the Umfolozi acacias were most likely accessing atmospheric N_2.

The mean δ ^{15}N values of *Acacia* species of some of the Hluhluwe acacias were uncharacteristically high (e.g. *A. burkei* and *A. nilotica*), and thus these did not differ significantly from their reference plants (p = 0.44). It was this study's conclusion however, that the Hluhluwe *Acacia* species were most likely also utilising atmospheric N_2 but that they were possibly weaker N_2-fixers than the other species from HUP which led to their mean δ ^{15}N values being very similar to those of their reference plants. Differences in rhizobium species and their effectiveness from one location to the next, are known to affect the δ ^{15}N of legumes (Galiana *et al.*, 1990; Spriggs *et al.*, 2003). This is a result of mycorrhizal fungi discriminating against the heavier ^{15}N isotope during transfer of N from the fungus to the host plant. Therefore, the variation between the δ ^{15}N values of the nine *Acacia* species was concluded to possibly be a reflection of the effectiveness of the rhizobium strains associated with each of the selected *Acacia* species and the sites from which they were sampled. It was the conclusion of this part of the study therefore, that the strongest N_2-fixers were the *Acacia* species with the lowest mean δ ^{15}N values (closest to that of atmospheric N_2), as a result of being associated with the most effective rhizobium strains. Moreover, the relatively high mean δ ^{15}N values of the Hluhluwe acacias and the significant difference between the *Acacia* species ($p = 0.05$) suggested that differences in the environmental conditions between the two parts of HUP also had an impact. This is discussed in more detail in the following section.

2.4.2 N_2-fixation abilities of acacias are affected by environmental conditions

The δ ^{15}N values of the soil sampled under the acacias and the reference plants in Hluhluwe and Umfolozi were found to be similar ($p = 0.21$ and $p = 0.41$). Therefore, soil N was excluded as a potential factor of influence on the mean δ ^{15}N values of the acacias and their reference plants in HUP. Rainfall, fire frequency and intensity were the only other environmental factors known to differ between Hluhluwe and Umfolozi. Hluhluwe being the wetter part of HUP has more frequent and possibly intense fires than in Umfolozi where fire is limited by the availability of dry season grass fuel (Bond *et al.*, 2001). Frequent and intense fires are known to result in the even re-distribution of soil nutrients, including ^{15}N, to all plants growing within the burnt vicinity. The close similarity between the δ ^{15}N values of the Hluhluwe acacias and their reference plants in Hluhluwe could have been a reflection of this phenomenon. Fewer fire incidences are expected in the drier Umfolozi part of the park, and thus the distinction between the N_2-fixing acacias and their reference plants was evident from their δ ^{15}N values.

Tables

Table 2.1: The *Acacia* species and the known non-fixing (reference) plants used to test for N_2-fixing ability.

HLUHLUWE		UMFOLOZI	
***Acacia* plants**	**Reference plants**	***Acacia* plants**	**Reference plants**
Acacia nilotica (L.) Willd. ex Delile [Fabaceae]	*Euclea divinorum* Hiern. [Ebenaceae]	*Acacia nilotica* (L.) Willd. ex Delile [Fabaceae]	*Euclea divinorum* Hiern [Ebenaceae]
Acacia karroo Hayne [Fabaceae]	*Euclea racemosa* Murr. [Ebenaceae]	*Acacia karroo* Hayne [Fabaceae]	*Euclea racemosa* Murr [Ebenaceae]
Acacia burkei Benth. [Fabaceae]	*Spirostachys Africana* Sond. [Euphorbiaceae]	*Acacia nigrescens* Oliv. [Fabaceae]	*Spirostachys Africana* Sond. [Euphorbiaceae]
Acacia robusta Burch. [Fabaceae]	*Rhus sp.* [Anacardiaceae]	*Acacia tortilis* (Forssk.) Hayne [Fabaceae]	*Rhus sp.* [Anacardiaceae]
Acacia caffra (Thunb.) Willd. [Fabaceae]	*Sclerocarya birrea* (A. Rich.) Hochst. [Anacardiaceae]	*Acacia grandicornuta* Gerstner [Fabaceae]	*Ziziphus mucronata* Willd [Rhamnaceae]
		Acacia luderitzii Engl. [Fabaceae]	

Table 2.2: Mean $\delta\ ^{15}N$ results of the *Acacia* species and the known non-fixing (reference) plants sampled from Hluhluwe and Umfolozi.

HLUHLUWE PLANTS (20 leaf samples per species)				UMFOLOZI PLANTS (25 leaf samples per species)			
***Acacia* species**	**$\delta\ ^{15}N$ (‰)**	**Reference plants**	**$\delta\ ^{15}N$ (‰)**	***Acacia* species**	**$\delta\ ^{15}N$ (‰)**	**Reference plants**	**$\delta\ ^{15}N$ (‰)**
Acacia nilotica	1.40	*Sclerocarya birrea*	2.13	*Acacia nilotica*	-0.44	*Ziziphus mucronata*	1.40
Acacia karroo	0.43	*Euclea divanorum*	0.56	*Acacia karroo*	-0.85	*Euclea divanorum*	0.48
Acacia burkei	1.67	*Euclea racemosa*	0.9	*Acacia nigrescens*	0.82	*Euclea racemosa*	0.95
Acacia robusta	0.79	*Spirostachys africana*	1.61	*Acacia tortilis*	0.10	*Spirostachys africana*	2.19
Acacia caffra	0.22	*Rhus sp.*	2.21	*Acacia grandicornuta*	0.26	*Rhus sp.*	2.04
				Acacia luderitzii	0.30		
Overall Mean	0.90	Overall Mean	1.49	Overall Mean	0.03	Overall Mean	0.41

Table 2.3: Mean δ ^{15}N results of the soil sampled from under both *Acacia* species and the known non-fixing (reference) plants in Hluhluwe and Umfolozi.

HLUHLUWE SOIL (20 soil samples per species)				**UMFOLOZI SOIL (25 soil samples per species)**			
***Acacia* species**	**δ ^{15}N (‰)**	**Reference plants**	**δ ^{15}N (‰)**	***Acacia* species**	**δ ^{15}N (‰)**	**Reference plants**	**δ ^{15}N (‰)**
Acacia nilotica	3.60	*Euclea divanorum*	3.62	*Acacia nilotica*	2.18	*Euclea divanorum*	3.39
Acacia karroo	3.75	*Euclea racemosa*	3.21	*Acacia karroo*	3.35	*Euclea racemosa*	3.70
Acacia burkei	3.58	*Spirostachys africana*	3.79	Acacia nigrescens	4.26	*Spirostachys africana*	4.79
Acacia robusta	2.54	-	-	*Acacia tortilis*	3.97	-	-
Acacia caffra	2.81	-	-	*Acacia grandicornuta*	5.56	-	-
				Acacia luderitzii	4.84		
Overall Mean	3.26	Overall Mean	3.54	Overall Mean	4.03	Overall Mean	3.96

- samples contaminated before they could be analysed

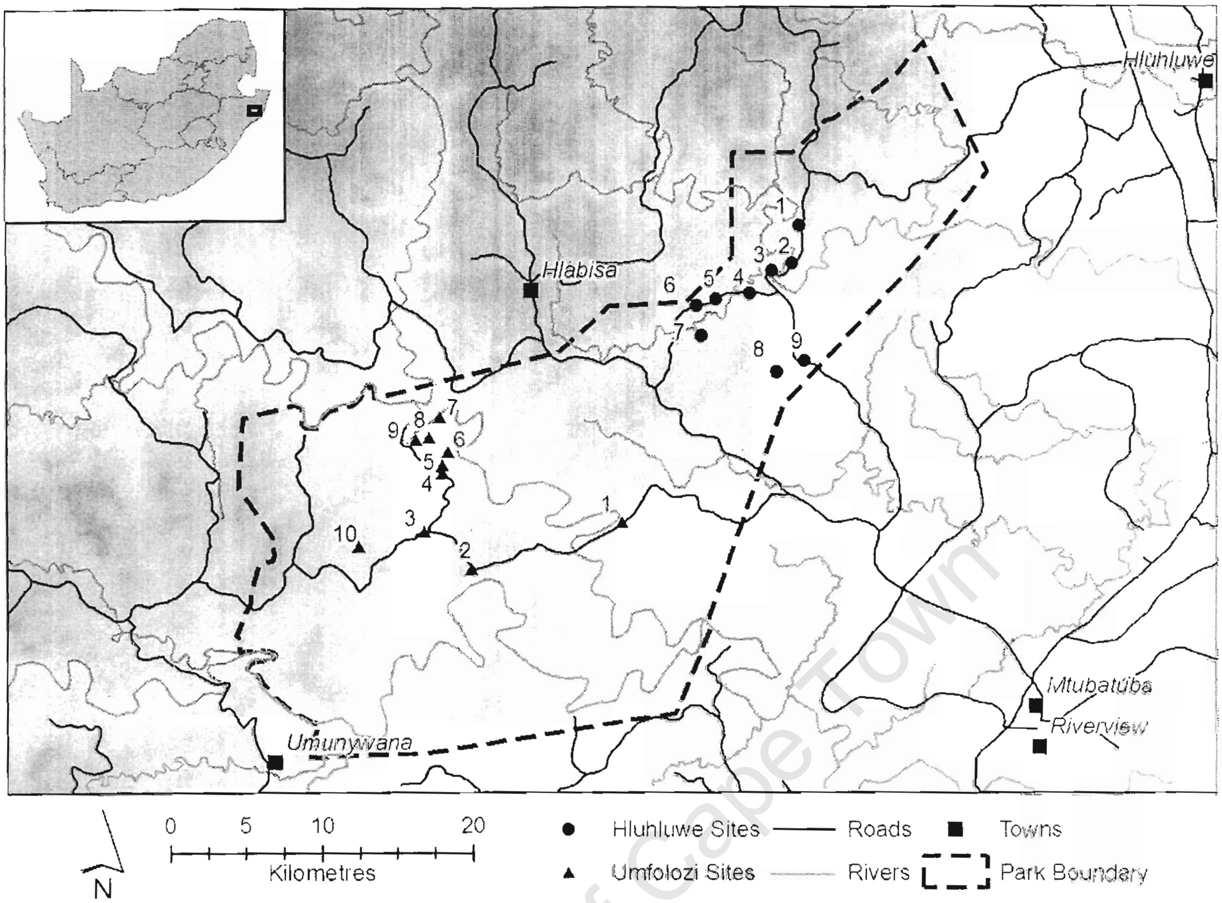


Figure 2.1: Hluhluwe and Umfolozi sampling sites, the following plants were collected at each site:

In Hluhluwe

Site 1 = *A. nilotica*, *A. burkei*, *E. divanorum*, *E. racemosa*, *Rhus sp.*
Site 2 = *A. nilotica*, *A. burkei*, *A. karroo*, *E. divanorum*, *E. racemosa*, *Rhus sp.*, *S. birrea*
Site 3 = *A. robusta*, *A. caffra*, *A. nilotica*, *A. karroo*, *A. burkei*, *E. divanorum*, *S. birrea*
Site 4 = *A. robusta*, *A. nilotica*, *E. divanorum*, *S. birrea*
Site 5 = *A. robusta*, *A. caffra*, *S. africana*
Site 6 = *A. robusta*, *A. burkei*, *Rhus sp.*, *E. divanorum*
Site 7 = *A. caffra*, *A. karroo*, *Rhus sp.*, *E. divanorum*
Site 8 = *A. robusta*, *A. burkei*, *E. racemosa*, *S. africana*
Site 9 = *A. karroo*, *A. caffra*, *Rhus sp.*, *S. africana*

In Umfolozi

Site 1 = *A. nilotica*, *A. karroo*, *A. tortilis*, *A. nigrescens*, *E. divanorum*, *E. racemosa*, *S. africana*
Site 2 = *A. nigrescens*, *A. grandicornuta*, *A. leuderitzii*, *E. racemosa*, *S. africana*
Site 3 = *A. tortilis*, *A. leuderitzii*, *A. nigrescens*, *S. africana*, *E. racemosa*
Site 4 = *A. grandicornuta*, *A. nigrescens*, *A. tortilis*, *S. africana*, *E. racemosa*, *E. divanorum*
Site 5 = *A. grandicornuta*, *A. leuderitzii*, *S. africana*, *E. racemosa*
Site 6 = *A. tortilis*, *A. nigrescens*, *A. grandicornuta*, *A. leuderitzii*, *S. africana*, *Z. mucronata*
Site 7 = *A. grandicornuta*, *A. leuderitzii*, *A. tortilis*, *E. racemosa*, *E. divanorum*
Site 8 = *A. karroo*, *A. nilotica*, *S. africana*, *E. divanorum*, *E. racemosa*, *Z. mucronata*
Site 9 = *A. nilotica*, *A. karroo*, *S . africana*, *E. divanorum*, *E. racemosa*
Site 10 = *A. karroo*, *A. nilotica*, *E. divanorum*, *E. racemosa*, *Rhus sp.*

CHAPTER 3:

COMPARATIVE ECOLOGY OF THE DEFENCE CHARACTERISTICS OF NINE SOUTH AFRICAN *ACACIA* SPECIES

3.1 Plant defence mechanisms

Plants have developed a multitude of defence mechanisms against herbivores (Karban and Baldwin, 1997; Arimura *et al.*, 2005). Such defensive action by plants may negatively affect herbivores' physiology or feeding biology (Cooper and Owen-Smith, 1986; Arimura *et al.*, 2001). Plant defence mechanisms can be classified as either direct or indirect (Price *et al.*, 1980). Direct defences may prevent herbivores from feeding via physical and chemical barriers (i.e. constraints on nutritional requirements, feeding-time or on the capacity to process food in the alimentary canal). Indirect defences work by attracting the herbivores' enemies, such as parasites or predators, thereby actively reducing the number of feeding herbivores (Turlings *et al.*, 1990; Dicke *et al.*, 1990b, 1999; Takabayashi and Dicke, 1996; De Moraes *et al.*, 1998).

Both mechanisms can be either constitutive (always expressed) or inducible (appear only when needed) (Arimura *et al.*, 2005). Some variations in the effects of plant defence mechanisms exist. These might be due to the efficacy of the molecular form of some of the compounds involved, or can be dependent on the matrix of other plants from which the herbivore must choose its food (Robbins *et al.*, 1987). Moreover, it is important to note that while defence mechanisms derive some benefit to the plant, whether constantly or transiently expressed, their benefits come at a cost (Baldwin and Preston, 1999). A plant's responses to herbivory may channel C and N resources away from vegetative and reproductive growth into protective mechanisms (Walling, 2000).

Traditionally, plant defence mechanisms have been divided into two main categories: mechanical and chemical defences. The first category comprises of a series of physical barriers that assist a plant to avoid being eaten. In the second category are a variety of substances that are toxic, repellent, or that render plant tissues indigestible to animal. The two defence mechanisms are discussed briefly in the following sections, and their role in the defence of the nine *Acacia* species selected for this study is investigated.

3.1.1 Mechanical defence

Mechanical defence mechanisms used by plants as protection against herbivores include: spines, thorns, leaf hairs, waxes, bark, stems, and highly lignified or silica loaded tissues that physically deter herbivores (Duffey and Stout, 1996). It has been shown that herbivores select food that gives a maximum intake (mass) per unit of time (Belovsky *et al.*, 1991; Knops *et al.*, 2000), and for food items of adequate nutritional quality (e.g. high in N which is essential for protein synthesis). Spines and thorns prevent herbivores from achieving their maximum nutritional intake by increasing the amount of time they have to spend eating as they attempt to avoid these. The presence of spines or hooks can be extremely variable both within and between individual plants. These variations are due to factors such as local impacts of herbivory and plant size, age, and height above ground (Milewski *et al.* 1991; Midgley and Ward, 1996). The influence of some of these factors will be discussed in this chapter.

3.1.2 Chemical defence

Defoliation is costly to a plant, because it represents an immediate loss of C and other nutrients, in turn forcing the plant to allocate additional energy and nutrients to construct new leaves (Scholes and Walker, 1993). As a survival strategy, a plant may invest in the synthesis of anti-herbivory metabolites if the total benefit to the plant exceeds the costs of the defence mechanism (Coley *et al.*, 1985). The defensive chemicals present in a plant usually reflect the relative availability of C and N in the environment in which the plant is growing. There are generally two types of 2° compounds that make up a plant's chemical defence, quantitative and qualitative compounds (Fox, 1981). Quantitative 2° compounds tend to reduce the herbivore's ability to digest or assimilate nutrients (e.g. tannins may reduce protein assimilation); whereas qualitative 2° compounds are toxic after a certain amount has been consumed. However, such defences are thought to be costly for plants and are usually reflected in trade-offs with other plant functions such as growth and reproduction (Briggs and Schultz, 1990).

3.1.3 Study objectives

This part of the study was aimed at exploring the mechanical (spinescence) and chemical (2° compounds) defence mechanisms of the selected nine South African *Acacia* species, and to determine whether or not a correlation exists between both defence mechanisms and [N]. The latter part was examined because of the known nutritional value of N, a major plant nutrient essential for protein synthesis in herbivores (Mattson, 1980; Crawley, 1983), which leads to plants with higher [N] being preferred by herbivores. Spine and hook lengths, their densities, how this relates to leaf length, and tannin concentrations were measured for each of the selected acacias and comparisons were made. The measured spine and hook lengths, their densities, leaf lengths, and tannin concentrations of the selected acacias were then correlated to [N]. The *Acacia* species with the highest [N] were expected to be better defended (e.g. spines and tannins) than the acacias with less [N] (Mattson, 1980; Crawley, 1983).

3.2 Materials and Methods

3.2.1 Materials

The nine *Acacia* species sampled from HUP for Chapter 2 and listed in Table 2.1 were used for this part of the study.

3.2.2 Methods

Spine and hook length

Twenty spine and/or hook pairs were measured from a single randomly selected branch, from five trees per *Acacia* species sampled. The spines and hooks were measured from the tip of the spine or hook to its base (area where the spine or hook joins the branch). This resulted in four spine or hook length mean values per *Acacia* species in Hluhluwe (Figure 3.2a) and five spine or hook length mean values per *Acacia* species in Umfolozi (Figure 3.2b).

Spine and hook density

Spine or hook density was measured as the distance between each of 20 spine or hook pairs per randomly selected *Acacia* species branch from which the spine and hook length measurements were taken. Overall, nineteen measurements of the distance between spines or hooks were recorded for each of the five trees per *Acacia* species sample in Hluhluwe and Umfolozi. This resulted in four distance-between-spine or hook mean values for each *Acacia* species sampled from Hluhluwe (Figure 3.3a), and five mean distance-between-spine or hook mean values for each of the *Acacia* species sampled from Umfolozi (Figure 3.3b).

Leaf length

The length of the leaves (made up of pinnules) of all nine *Acacia* species were measured from the tip of the leaf to the petiole. Two leaf length measurements were taken from ten individual trees of each of the nine *Acacia* species. This resulted in 20 leaf length measurements for each *Acacia* species from which the mean leaf length values per *Acacia* species were calculated for the Hluhluwe samples (Figure 3.4a) and those sampled in Umfolozi (Figure 3.4b). Leaf length was the only leaf measurement considered for this part of the study to compare with thorn length (instead of leaf area or specific leaf mass) due to field observations during sampling. The *Acacia* species with leaves that were longer than the thorns were easier to sample than those whose leaves were much shorter than the thorns. This observation raised the question of whether or not browsers would have the same experience while feeding.

2° compounds

Tannin concentrations were measured from the leaves of the nine *Acacia* species selected for this study. By analysing tannins, a broad measure of 2° compounds present in the selected acacias was achieved. Tannins, like other 2° compounds, are difficult to analyse and limitations exist with the methods of tannin analysis (Hagerman *et al.*, 1997). These limitations include the different responses given by the different phenolics, and the difficulty of securing appropriate standards (Hagerman, 1998). A qualitative analysis of the tannins in the selected *Acacia* species' leaves was chosen because even though quantitative analyses are more specific, they are time-consuming and costly, and such detailed results were not necessary to achieve the objectives of this study.

Numerous methods for determining tannins using their interaction with proteins have been devised. The radial diffusion method (Hagerman, 1987) seemed the most appropriate for this study, as it is a particularly simple protein precipitation method appropriate for situations where laboratory facilities are limited or where numerous samples are to be analysed. The different responses given by the different phenolics are pronounced with this type of assay, which makes the choice of standard very important. Tannic acid (from 0.0 mg to 1.0 mg, at intervals of 0.2 mg) was chosen as the standard for this study. Tannic acid solutions were then assayed for tannins using the same method as that used to analyse the leaf samples and a calibration curve was produced (Figure 3.1). The reagents, plate preparation and assay procedures are listed in the Appendix. Tannin concentrations of the selected *Acacia* species were estimated using the calibration curve. The estimated tannin concentrations were correlated with [N] (Figures 3.5a & b), spine or hook lengths (Figures 3.6a & b), and spine or hook density (Figures 3.7a & b) for each of the two parts of HUP.

3.3 Results

3.3.1 Spinescence and total leaf N concentration [N]

Spine and hook length

The Hluhluwe sampled *Acacia* species with hooks (*A. burkei* and *A. caffra*) had higher [N] than the acacias with spines (*A. nilotica*, *A. karroo* and *A. robusta*) (Figure 3.2a). The relationship between spine or hook length and [N] was indistinct for the Umfolozi sampled *Acacia* species, despite the similarities between both the spine lengths and [N] of the replicated *Acacia* species (*A. nilotica* and *A. karroo*) (Figure 3.2b). Correlation analyses between spine or hook length measurements and [N] of the acacias sampled from both parts of HUP showed that the two variables were inversely correlated for the Hluhluwe sampled acacias (r = -0.78), and directly correlated for the Umfolozi sampled acacias (r = +0.76). This meant that the *Acacia* species sampled from Hluhluwe with the highest [N] had hooks as a means of mechanical defence whereas, the Umfolozi sampled *Acacia* species with the highest [N] had long spines.

Spine and hook density

The relationship between the spine and hook density measurements of the nine *Acacia* species from Hluhluwe and Umfolozi and their [N] was uncertain (Figure 3.3a & b). Within each of the two parts of HUP, the density of the spines and of the hooks varied from densely packed (*A. luderitzii* at 1.11 cm and *A. tortilis* at 1.29 cm) to fairly sparse (*A. nilotica* at 2.14cm and *A. caffra* at 2.09cm). Moreover, there was similar variation in the [N] of the *Acacia* species with densely packed spines or hooks (*A. luderitzii* at 2.61%; and *A. tortilis* at 3.38%) and those with fewer spines or hooks per branch (*A. nilotica* at 2.26%; and *A. caffra* at 3.23%). When compared, spine and hook densities were found not to be related to [N] for both the Hluhluwe (r = -0.08) and the Umfolozi (r = -0.10) sampled *Acacia* species.

Spines, hooks and leaves

The relationship between the spine and hook lengths of the nine *Acacia* species and their leaf lengths varied considerably between the two parts of HUP (Figure 3.4a &

b). The Hluhluwe sampled *Acacia* species with hooks had leaves that varied in length from a mean value of 6.98cm (*A. burkei*) to that of 15.70cm (*A. caffra*), while the acacias with hooks sampled from Umfolozi had mean leaf length values that ranged from 4.95cm (*A. luderitzii*) to 7.48cm *(A. nigrescens*). The mean leaf length values of the acacias with spines sampled from Hluhluwe ranged from 7.98cm (*A. nilotica*) to 9.33cm (*A. karroo*); and for the Umfolozi samples *A. grandicornuta* had a mean leaf length value of 5.88cm. When hook and spine length measurements of the both the Hluhluwe and Umfolozi sampled *Acacia* species were compared to their leaf lengths, they were found to be strongly correlated. The *Acacia* species sampled from Hluhluwe with long spines had short leaves (r = - 0.92), and those with hooks had long leaves (r = - 0.97). The Umfolozi *Acacia* species with long spines also had long leaves (r = +0.98), whilst those with hooks had short leaves (r= +0.97).

3.3.2 2° compounds and total leaf N

Tannins

The relationship between the tannin and [N] of the selected *Acacia* species was similar between the two parts of HUP (Figures 3.5a & b). Of the five *Acacia* species sampled from Hluhluwe, *A. nilotica*, *A. karroo* and *A. robusta* were found to have tannins in their leaves, while *A. burkei* and *A. caffra* did not despite having higher [N] (Figure 3.5a). The leaves of *A. luderitzii* of the Umfolozi sampled *Acacia* species were found to contain tannins, as did the two replicated acacias, *A. nilotica* and *A. karroo*. The rest of the Umfolozi sampled acacias were found not to have tannins in their leaves despite having relatively higher [N] than *A. leuderitzii* (Figure 3.5b). The tannin and [N] measurements of the replicated *Acacia* species were similar between the two parts of HUP. The tannin concentration values of the selected *Acacia* species were found to be inversely correlated to their [N] for both the Hluhluwe (r = -0.61) and Umfolozi (r = -0.64) samples. This meant that acacias with low total [N] had higher concentrations of tannins in their leaves, for both parts of HUP.

3.3.3 Spinescence and 2^o compounds

Spine/hook length and tannins

A. nilotica had both the longest spines and the highest tannin concentration in its leaves of all the Hluhluwe sampled *Acacia* species (Figure 3.6a). *A. caffra* on the other hand, had the smallest hooks and no record of tannins in its leaves. Despite the variable measurements of spine or hook lengths and the tannin concentration of the rest of the Hluhluwe sampled acacias, the sampled *Acacia* species with the longest spines had the highest concentration of tannins in their leaves (Figure 3.6a). The Hluhluwe sampled acacias with either short spines or hooks had low or no tannins (r = +0.97) in their leaves. In Umfolozi, *A. luderitzii* leaves had the highest concentration of tannins despite having very short hooks (Figure 3.6b). The remaining Umfolozi sampled *Acacia* species had varying spine length measurements but no trace of tannins in their leaves. Overall, the correlation between the Umfolozi sampled *Acacia* species with long spines and their tannin concentration was much lower (r = +0.52).

Spine/hook density and tannins

The comparison of spine and hook density with leaf tannin concentrations varied with species for both the Hluhluwe and Umfolozi (Figures 3.7a & b). In Hluhluwe, the leaves of *A. nilotica* which had the least dense spines of the acacias sampled in this part of HUP, had the highest tannin concentration. *A. caffra* on the other hand, had a low hook density and no tannins in its leaves. The leaves of the Umfolozi sampled *Acacia* species with the lowest density of spines or hooks (*A. nigrescens*) had no tannins, and the leaves of one (*A. luderitzii*) of the two *Acacia* species with the highest density of spines or hooks had the highest concentration of tannins. Correlation analyses between spine and hook density and the tannin concentrations of the selected *Acacia* species differed between the two parts of HUP. The spine and hook densities of the acacias sampled from Hluhluwe were directly related to the tannin concentrations in their leaves (r = +0.50). A stronger but inverse relationship was found for the Umfolozi acacias (r= -0.96). This meant that the leaves of the Hluhluwe sampled acacias with the most densely packed spines or hooks had the highest concentrations of tannins, whereas the Umfolozi sampled acacias with densely packed spines or hooks had the lower concentration of tannins.

3.4 Discussion

3.4.1 The acacias differ in their mechanical and chemical defence mechanisms

Spine and hook length

Herbivores have different feeding responses to plants with spines and those with hooks. Plants with hooks (e.g. *A. burkei*, *A. caffra*, *A. nigrescens* and *A. luderitzii*) are considered to be weakly spinescent (Midgley *et al.*, 2001) compared to those with spines, as hooks are limited in their ability to protect leaves longer than they are from browsing herbivores. Plants with spines on the other hand (e.g. *A. nilotica*, *A. karroo*, *A. grandicornuta*, and *A. robusta*), depend on the length of their spines to increase their effectiveness in deterring herbivores from feeding on their leaves. The long spines of *A. nilotica*, *A. tortilis* and *A. grandicornuta* make it difficult for the herbivores to pick, prune, or strip their leaves, twigs and branches. *Acacia* species with short spines (e.g. *A. karroo* and *A. robusta*), even though compromised defensively compared to the acacias with longer spines, have an advantage over the acacias with hooks as their leaves are not as exposed to browsing herbivores.

Spine and hook density and strength

The length of spines and hooks is not the only means of mechanical defence the selected *Acacia* species have against herbivory. The density and strength of spines and hooks contributes towards the overall success of their defence. For example, *A. karroo* has spines and is expected to be better defended than the acacias with hooks (Midgley *et al.*, 2001), but its spines are weak and can be easily broken and consumed by browsing herbivores. This minimises its advantage over acacias with hooks, particularly the hooks with special adaptations that enhance their ability to limit herbivore damage. The *Acacia* species with hooks (*A. burkei*, *A. caffra*, *A. nigrescens* and *A. luderitzii*) were expected to be defensively compromised compared to those with long spines, but were found to be more effective when their hooks were densely packed along the branches (e.g. *A. luderitzii*). Dense hooks make picking of leaves more time consuming, and pruning and stripping of the leaves and twigs painful to the browsing herbivores. This discourages herbivores from feeding on the plants. Similarly, spines and hooks that are sparsely distributed along the branches of the trees (e.g. *A. nilotica*, *A. caffra*, *A. nigrescens*, and *A. robusta*) minimise the constraints on the browsing herbivores by allowing them to achieve their nutritional requirements within a short period of time.

Spines, hooks and leaves

Previous studies (Gowda, 1996; 1997) have argued that spine or hook length and density should be considered in relation to the length of the associated leaves. Plants with leaves that are larger than the spines or hooks protecting them (e.g. *A. caffra*, *A. karroo*, *A. robusta*, *A. nigrescens*, *A. burkei*, and *A.nilotica*) are not as effective defensively as their leaves are easily accessible to browsing herbivores. The acacias with leaves smaller than the spines protecting them (*A. tortilis*, *A. luderitzii* and *A. grandicornuta*) reduce browsing more successfully as they present less food mass to the herbivores (Myers and Brazely, 1991).

Other adaptations

The defence of the selected *Acacia* species with leaves longer than their spines or hooks (*A. caffra*, *A. karroo*, *A. robusta*, *A. nigrescens*, *A. burkei*, and *A.nilotica*) should have been disadvantaged mechanically. However, these acacias had other adaptations that enhanced their defences against herbivores. These adaptations included: having densely packed spines or hooks (e.g. *A. burkei*); the presence of small hooks on the underside of the leaves (e.g. *A. caffra*); having very strong and curved spines (e.g. *A. nilotica*); and either having a large single-stemmed growth form with leaves beyond the reach of herbivores (e.g. *A. nigrescens*) or the ability to resprout (e.g. *A. karroo*). The *Acacia* species selected for this study that were protected by long spines (e.g. *A. tortilis*, *A. luderitzii* and *A. grandicornuta*) also had additional adaptations improving their effectiveness in protecting their leaves from herbivory. *A.tortilis* not only has densely packed long spines protecting its small leaves, but has the additional advantage of densely packed hooks. This particular *Acacia* species has relatively weak spines, but the presence of hooks minimises that disadvantage. *A. grandicornuta* not only has long and strong spines but it also has randomly occurring enlarged spines in which ants, that further protect its leaves, reside. Another example of a mechanical defence adaptation that minimises the disadvantage of having hooks instead of long spines as a means of protection is shown by *A. luderitzii,* which has three hooks per leaf node instead of two, with each hook facing a different direction.

2° compounds

Another constraint that can be imposed on herbivores and other foragers is the presence of 2° compounds such as tannins. These reduce the herbivore's capacity to process food in the alimentary canal (Dade *et al.*, 1990). Tannin concentrations in the leaves of the acacia plants investigated in this study varied between the selected species. The *Acacia* species with leaves strongly defended mechanically by either long spines or densely packed spines and hooks (e.g. *A. nilotica* and *A. luderitzii*) also had high concentrations of tannins in their leaves, despite the expectation that such species would not be in need of additional defence mechanisms. However, it became apparent that the leaves of both *A. nilotica* and *A. luderitzii* trees were relatively larger than the spines and hooks protecting them, making them easily accessible to browsing herbivores. Therefore, the presence of tannins in the leaves of these *Acacia* species is a necessary additional defence mechanism in order to minimise foliage loss. *A. karroo* and *A. robusta* were found to have relatively lower concentrations of tannins than *A. nilotica* and *A. luderitzii,* despite their mechanical defence mechanisms being weaker than those of the latter two species. *A. karroo* and *A. robusta* leaves were protected by reasonably long spines, but these were either not particularly strong (*A. karroo*) or had large gaps between them (*A. robusta*), making the leaves vulnerable to stripping and pruning from browsing herbivores. The presence of tannins in their leaves of the latter species possibly serves to minimise such disadvantages.

It was not surprising to find that *A. tortilis* and *A. grandicornuta* were two of the five *Acacia* species found not to have tannins in their leaves. The leaves of these two *Acacia* species, as already discussed in the sections above, are very well protected mechanically. The lack of tannins in the leaves of the remaining three *Acacia* species (*A. nigrescens, A. burkei* and *A. caffra*) was more surprising, as these acacias appeared not to have good mechanical defence mechanisms and were thus expected to have 2° compounds such as tannins as a means of overcoming that disadvantage. However, it became apparent that these species did not need to invest in the production of tannins to deter herbivores as they had other adaptations that were useful against herbivory. *A. nigrescens* and *A. burkei* are large single-stemmed trees and this ensures that most of their leaves are out of reach of browsing herbivores. *A. caffra* is adapted to herbivory by having the ability to rapidly recruit (by resprouting) after being browsed upon.

3.4.2 Acacias with high leaf N have the greatest allocation to defence

Mechanical defence

The relationship between mechanical defence (spine and hook length and density) and [N] of the nine *Acacia* species differed between Hluhluwe and Umfolozi. Spine and hook lengths of the acacias sampled from Hluhluwe were inversely correlated to [N]. This meant that the Hluhluwe sampled *Acacia* species with the highest [N] either had short spines or had hooks, and those with the low [N] had long spines. Such a correlation supports the conclusion by Briggs (1990) which states that plants with leaves that are low in N have more C available to play a role in defence mechanisms. However, the spine and hook length measurements of the Umfolozi sampled *Acacia* species were directly correlated to [N]. This implies that the Umfolozi sampled *Acacia* species with the highest [N] had the longest spines and those with short spines or hooks had lower [N].

The direct correlation between these variables in this part of HUP is possibly due to the influence of N_2-fixation. This could have been masked in the case of the Hluhluwe sampled acacias as the excess N they obtained through N_2-fixation was probably lost through either the even re-distribution of nutrients from frequent fires occurring there due to the higher annual rainfall, or because of having less efficient N_2-fixing symbionts (see section 2.4 in chapter two). The process of N_2-fixation has an effect on the C to nutrient balance of legumes (Briggs, 1990) leading to a trade-off between nodulation activities and the development of defence mechanisms. Hence the direct correlation between the development of spines and hooks and [N] of the N_2-fixing Umfolozi sampled acacias. The inverse correlation between spine and hook density and [N] of the acacias sampled in both parts of HUP, was less in agreement with reports by Briggs (1990). This was probably due to the general complexity of mechanical defence mechanisms within each of the sampled acacia plants. The relationship between [N] and spine and hook density may be overshadowed by other adaptations to herbivory. Overall, however, it was apparent that the highest [N] was found in the leaves of the *Acacia* species with the most complex or elaborate mechanical defence mechanisms (*A. tortilis*, *A. caffra* and *A. grandicornuta*), and this supports the notion that leaves with more N are more vulnerable to herbivory (Mattson, 1980; Crawley, 1983) and therefore require more elaborate defence strategies.

Chemical defence

As already mentioned above, plant species limited in N have more C available for use in defence (Briggs, 1990). This is particularly relevant where 2° compounds such as tannins, whose composition is based in C, are concerned. Of the nine *Acacia* species selected for this study, the highest concentration of N was found in the leaves of the strongest N_2-fixers, which were not limited in N. These N rich species had the lowest concentrations (or none) of tannins (e.g. *A. tortilis*, *A. caffra* and *A. grandicornuta*). Supporting actively fixing nodules affects the allocation of C-based to defence chemicals such as tannins because the large C sink associated with N_2-fixation reduce the amount of C available for other metabolic, defence and structural purposes.

Consequently, it was the leaves of the *Acacia* species with the lowest concentrations of N that had the highest concentrations of tannins (e.g. *A. karroo*, *A. nilotica*, *A. robusta* and *A. luderitzii*). These species were the most limited in N of the selected *Acacia* species and thus had more C available, which was then used to produce the C-based defence chemicals. Furthermore, these N-limited *Acacia* species had the most simple mechanical defence mechanisms (e.g. basic long spines of *A. karroo*) in comparison to the rest of the acacias, which were less limited in N and had more elaborate mechanical defence mechanisms (e.g. the specialised spine and hook combination of *A. tortilis*).

Figures

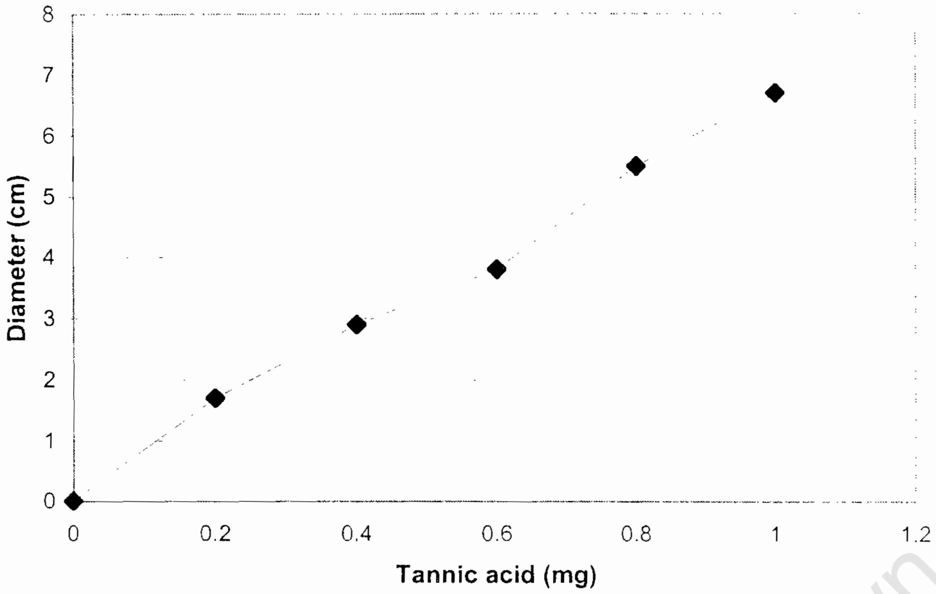
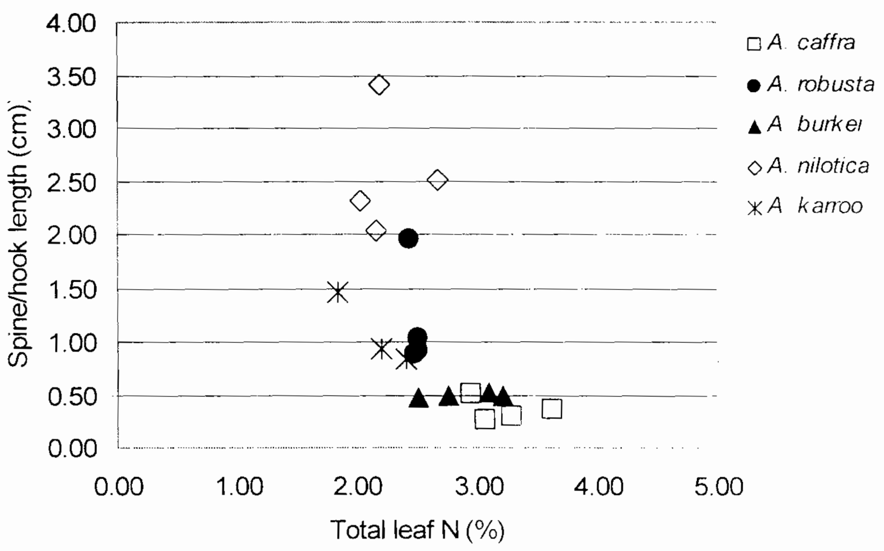
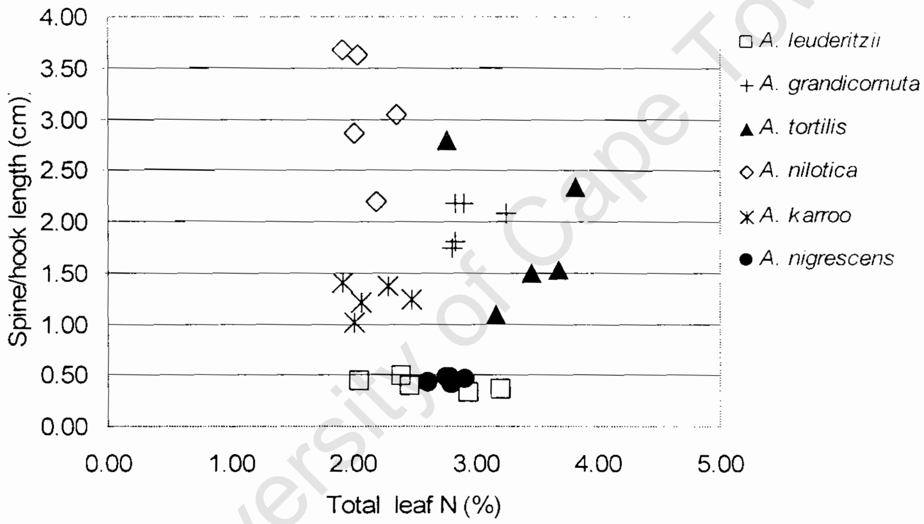


Figure 3.1: Calibration curve used to calculate tannin concentrations of nine *Acacia* species using tannic acid as a standard.



(a)



b)

Figure 3.2: Spine and hook length measurements of *Acacia* species sampled from (a) Hluhluwe and (b) Umfolozi against their total leaf N concentrations.

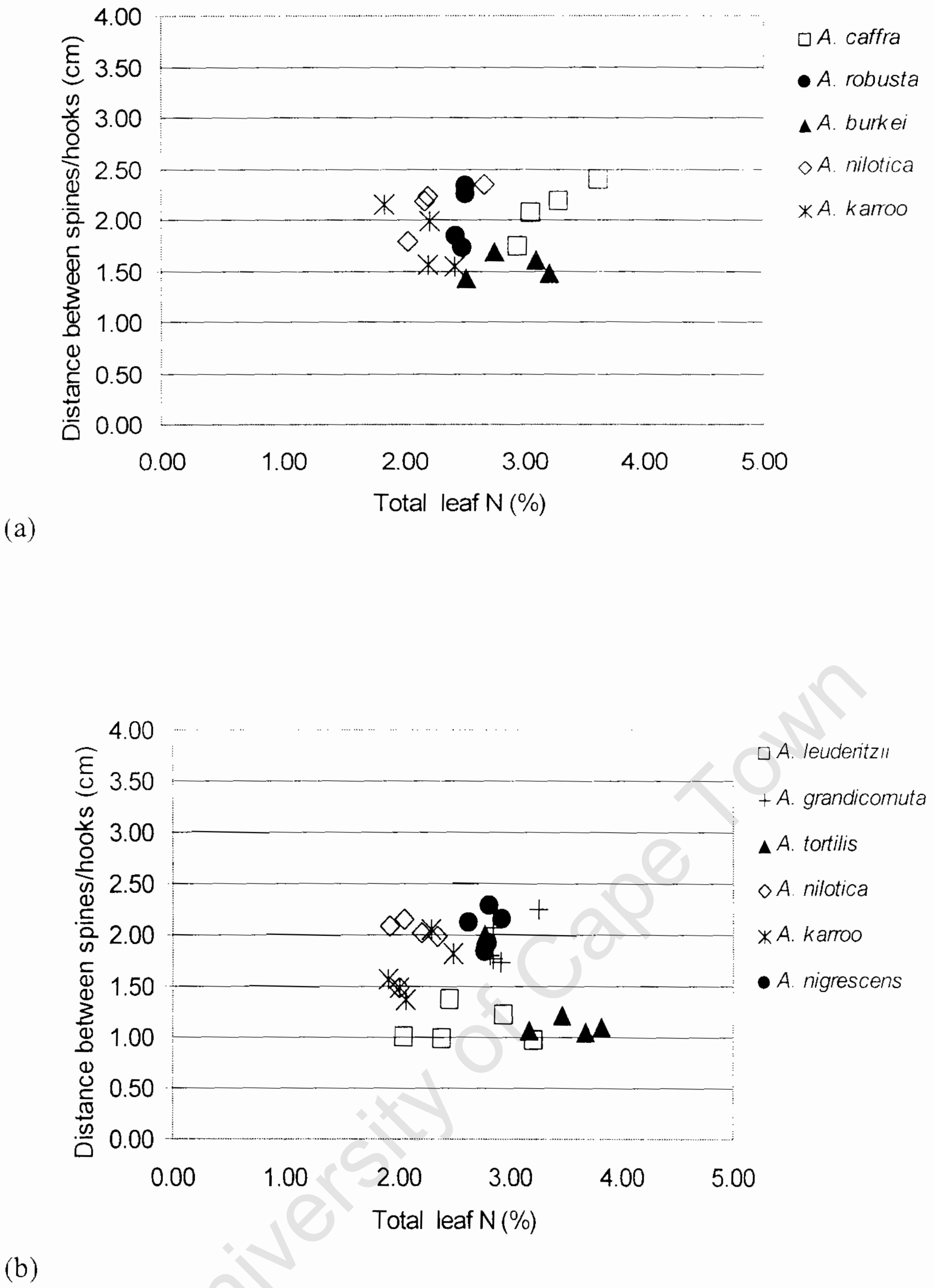


Figure 3.3: Distance between spine and hook measurements of *Acacia* species sampled from (a) Hluhluwe and (b) Umfolozi against their total leaf N concentrations.

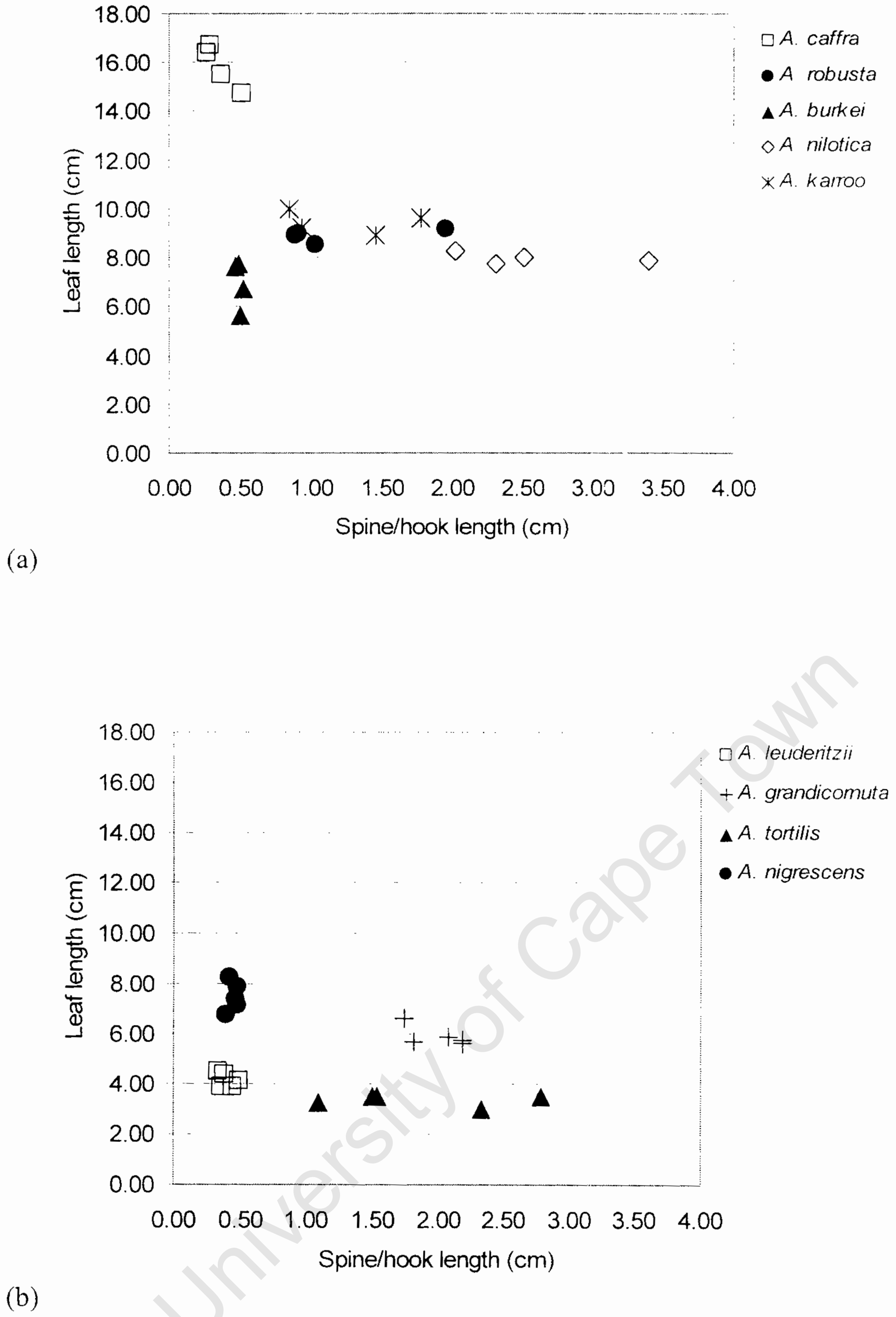
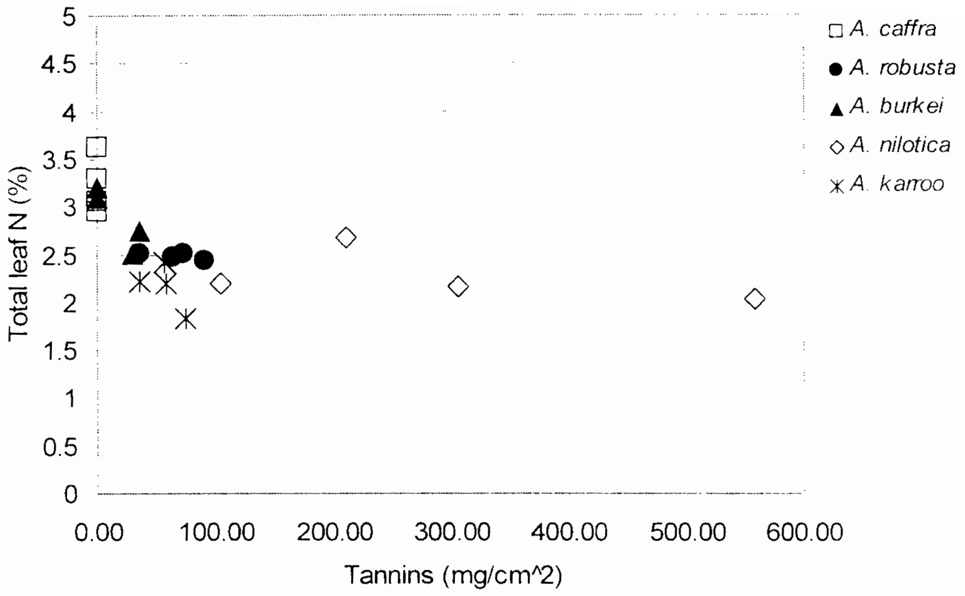
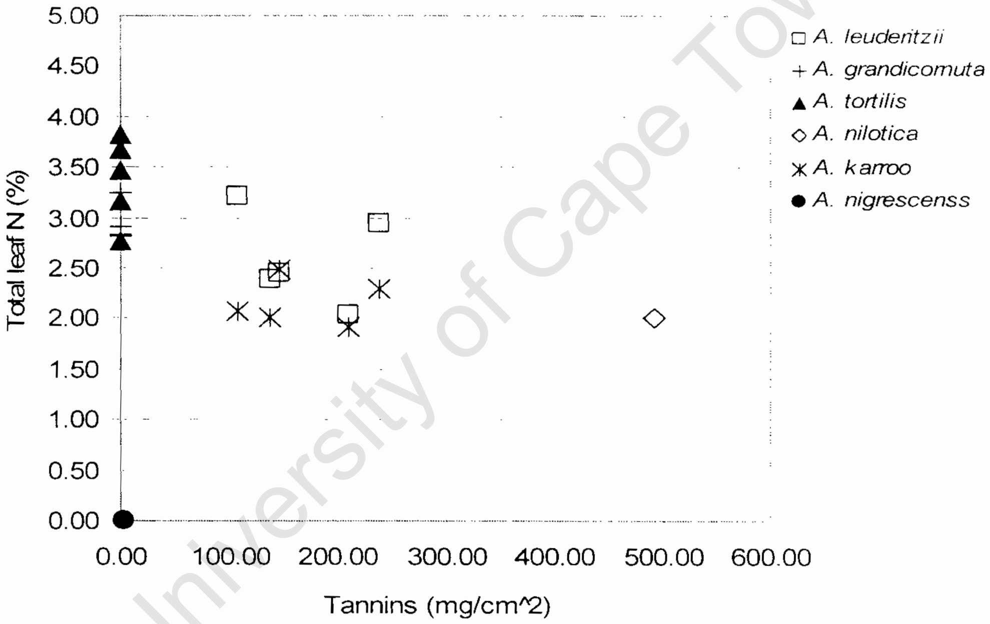


Figure 3.4: Leaf length measurements of *Acacia* species sampled from (a) Hluhluwe and (b) Umfolozi against their spine and hook length measurements.



(a)



(b)

Figure 3.5: Total leaf N measurements of *Acacia* species sampled from (a) Hluhluwe and (b) Umfolozi against their tannin concentrations.

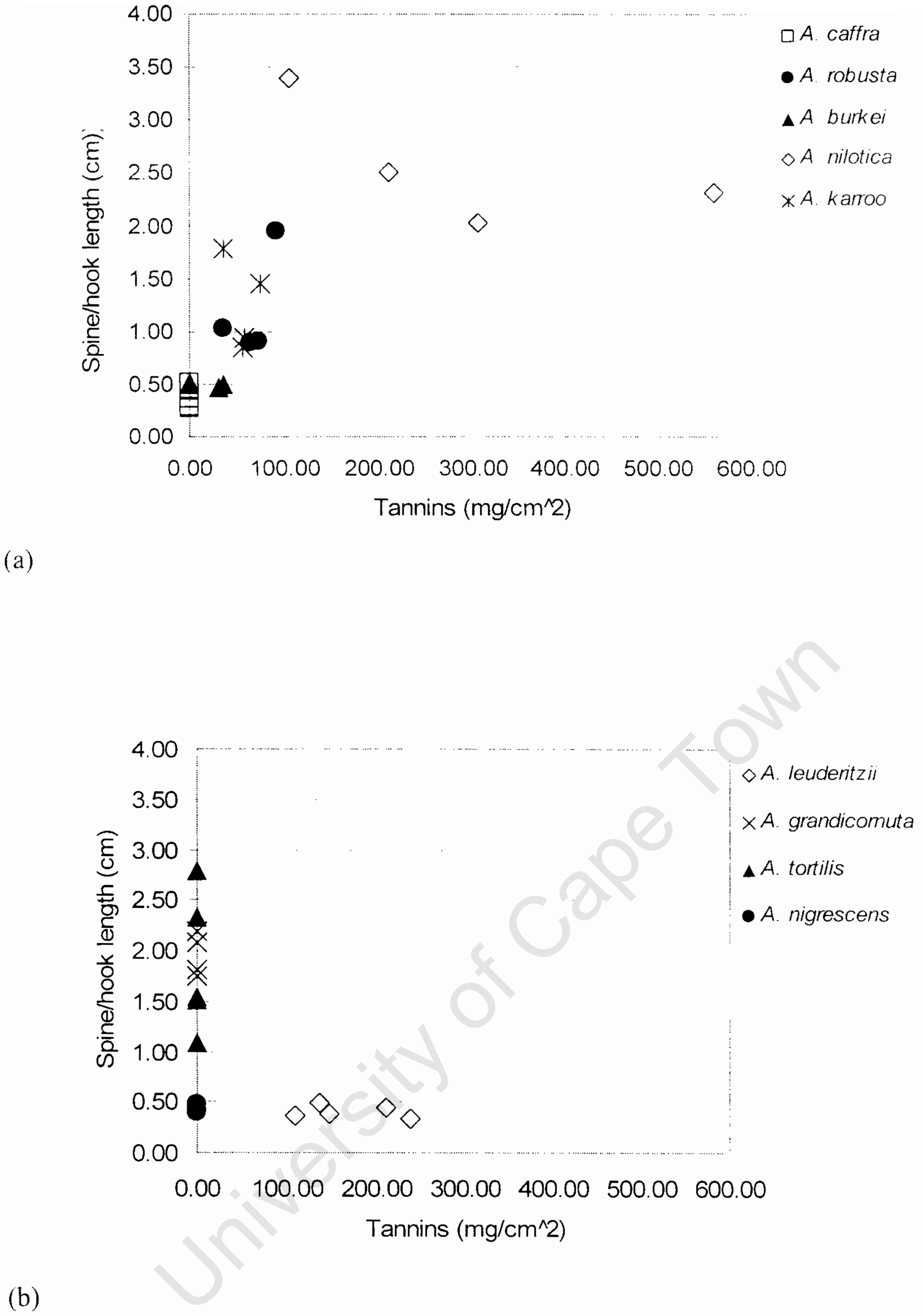
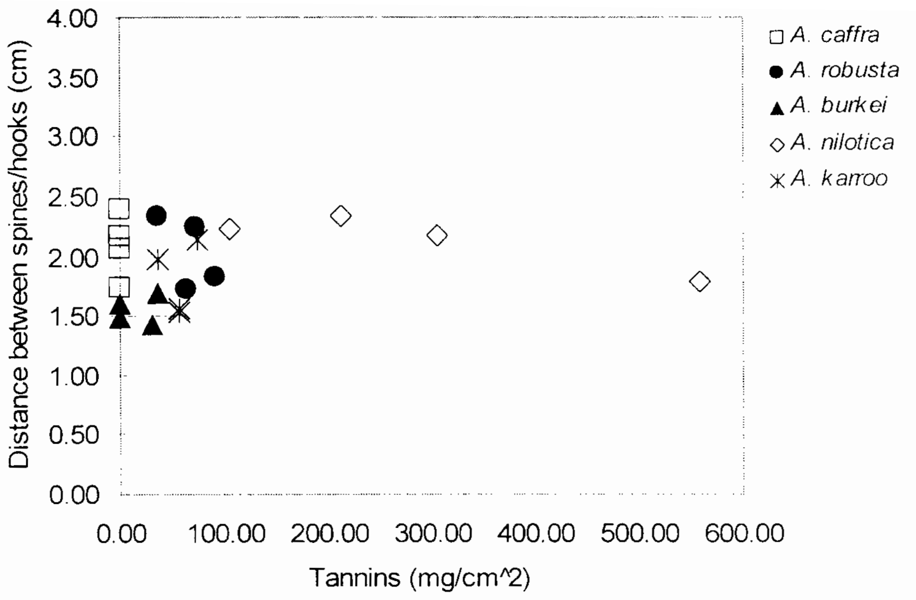
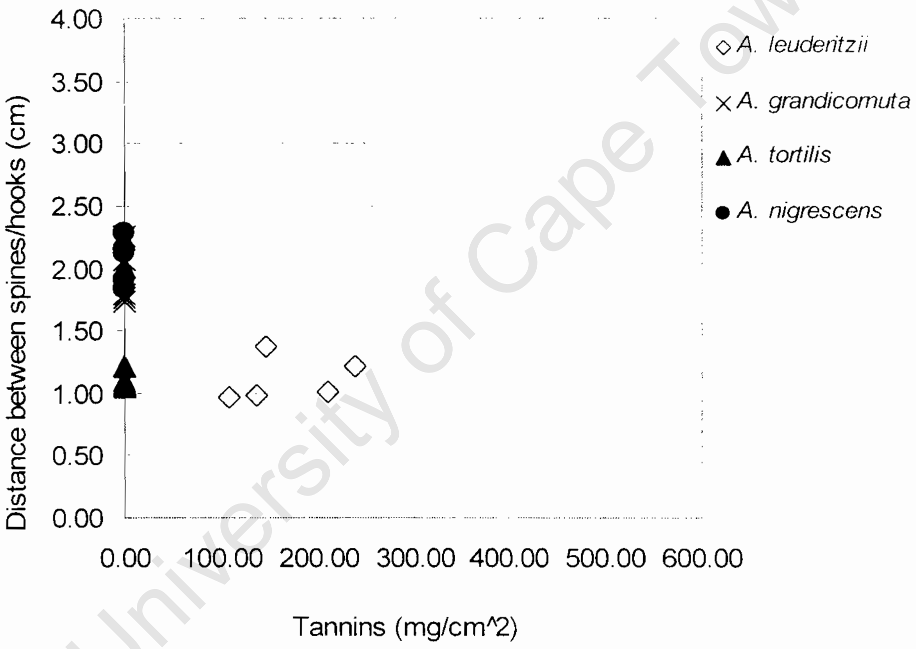


Figure 3.6: Spine and hook length measurements of *Acacia* species sampled from (a) Hluhluwe and (b) Umfolozi against their tannin concentrations.



(a)



(b)

Figure 3.7: Distance between spine and hook measurements of *Acacia* species sampled from (a) Hluhluwe and (b) Umfolozi against their tannin concentrations.

CHAPTER 4:

$\delta\ ^{13}$C OF NINE SOUTH AFRICAN *ACACIA* SPECIES: CORRELATION WITH LEAF ATTRIBUTES AND RAINFALL

4.1 Water-use efficiency and $\delta\ ^{13}$C

Water is not only essential for plant growth but it is also the required unit of exchange for the acquisition of CO_2 by plants (Condon *et al.*, 2002). However, plants differ in their capacity to regulate how much water is lost per unit C gained (i.e. water-use efficiency). This led to the use of $\delta\ ^{13}$C measurements as a measure of plant water-use efficiency (Richards and Condon, 1993; Newton *et al.*, 1996; Shaheen and Hood-Nowotny, 2005), where the variation in plant $\delta\ ^{13}$C measurements is largely a reflection of different physiological responses to environmental factors influencing stomatal conductance and carboxylation rates, and hence the concentration of intercellular CO_2 (Griffiths, 1993). The approach is based on the discrimination against ^{13}C by leaves during photosynthesis (Farquhar and Richards, 1984) and has been validated by many studies that identified a strong linear correlation between water-use efficiency and plant $\delta\ ^{13}$C (Yakir *et al.*, 1990; Condon *et al.*, 1993; Wright *et al.*, 1993; Sun *et al.*, 1996; Newton *et al.*, 1996; Saranga *et al.*, 1998; Leidi *et al.*, 1999; Brück *et al.*, 2000). The relationship is such that plants that are less water efficient have low (more negative) $\delta\ ^{13}$C measurements as a result of having less of the heavier C isotope, ^{13}C (Olbrich *et al.*, 1993; Ehdaie and Waines, 1993).

However, it is important to note that estimating water-use efficiency of plants using $\delta\ ^{13}$C measurements has largely been used in studies on crop plants (Yakir *et al.*, 1990; Wright *et al*. 1993; Leidi *et al.*, 1999; Earl, 2002). Relatively few studies on trees have used this technique and these have found the link between the two variables to be more complex (Olbrich *et al.*, 1993) than for crop plants. For a more accurate interpretation of water-use efficiency using plant $\delta\ ^{13}$C, details of the complete water balance of the plants are needed (Newton *et al.*, 1996). However, a significant number of experimental studies supported by experimental results on different tree species (Richards and Condon, 1993; Newton *et al.*, 1996; Sun *et al.*, 1996) have shown that plant $\delta\ ^{13}C$ values, are adequate indicators of plant water-use efficiency.

4.1.1 *Variation in plant $\delta\ ^{13}C$*

The $\delta\ ^{13}C$ values of C_3 plants typically range from -34 ‰ to -22 ‰ (O'Leary, 1995; Heaton, 1999). Within this range, plant $\delta\ ^{13}C$ measurements can vary due to a number of factors including the influence of genetic (Williams et al., 1991) and environmental differences (Farquhar *et al.*, 1989; Geber and Dawson, 1990; Smedley *et al.*, 1991). This study involved exploring the variation in plant $\delta\ ^{13}C$ measurements in response to leaf attribute differences such as water-use efficiency. Foliar $\delta\ ^{13}C$ measurements have in the past been used as an integrated measure of the response of photosynthetic gas exchange to environmental variables such as water availability (Meinzer *et al.*, 1992; Condon *et al.*, 2002). Furthermore, since plant water-use efficiency is known to vary depending on leaf size and availability of water, correlations between water-use efficiency and these two variables were also explored

4.1.2 *Water-use efficiency and leaf size*

Plant leaf area has been found to be closely correlated to $\delta\ ^{13}C$ and water-use efficiency (Wright *et al.*, 1993; Araus *et al.*, 1997b), and it is this correlation that was tested on the nine *Acacia* species selected for this study. It is theorised that plants maximise their fitness or water-use efficiency by decreasing both their leaf size and stomatal conductance in response to water availability (Dudley, 1996a; Nativ *et al.*, 1999; Arens *et al.*, 2000; Heschel and Riginos, 2005). Smaller leaves, therefore, increase plant fitness in dry conditions as the decreased surface area to volume ratio of small leaves inhibits desiccation (Geber and Dawson, 1990; Geber and Dawson, 1997; Pimentel *et al.*, 1999; Querejeta *et al.,* 2003).

4.1.3 Water-use efficiency and rainfall

According to Ehleringer and Cooper (1988), leaf carbon isotope ratios increase in plant species along a soil moisture gradient from the relatively wetter wash to the relatively drier slope. This in turn indicates that the water-use efficiency of plants increases as soil water availability decreases (Smith and Nowak, 1990; Schulze *et al.*, 1991). Investigating this relationship between plant water-use efficiency (which can be estimated using $\delta\ ^{13}C$ measurements) and water availability through rainfall formed part of the objectives of this study.

4.1.4 Objectives

Three objectives, listed as hypotheses in Chapter 1, were investigated in this chapter. The main objective was to determine the water-use efficiency of the nine *Acacia* species selected for this study, using their $\delta\ ^{13}C$ measurements. Since water-use efficiency is known to be influenced by leaf size and water availability, correlations between plant $\delta\ ^{13}C$ and leaf length (as a measure of leaf size) within the two parts of HUP differing in water availability due to their annual rainfall, were explored. The leaves of the different acacias were visibly different in size, particularly in leaf length. Moreover, the two main sampling sites, Hluhluwe and Umfolozi, are known to differ in annual rainfall. Therefore, leaf length and annual rainfall were the ideal variables to correlate with water-use efficiency because of the observed differences between the selected acacias and the sampling sites, which in turn could be used to track and highlight the impact of their variations on water-use efficiency.

4.2 Materials and Methods

4.2.1 Materials

The nine *Acacia* species from HUP in KwaZulu-Natal used to investigate the N_2-fixing abilities of leguminous plants, as described in chapter 2 (see Table 2.1), were used for the ^{13}C isotope ecology study discussed in this chapter.

4.2.2 Methods

Measuring $\delta\ ^{13}C$

The $\delta\ ^{13}C$ measurements required to achieve the objectives of this study were obtained concurrently with the $\delta\ ^{15}N$ measurements discussed in chapter 2 (section 2.2.2), using a Finnigan-MAT 252 mass spectrometer. The means of the $\delta\ ^{13}C$ measurements of the nine *Acacia* species are presented in Table 4.1.

Measuring the leaves

The lengths of the leaves (made up of a number of pinnules) of the nine *Acacia* species under investigation were measured to represent leaf size. Two leaves were measured from ten individual trees of each of the selected *Acacia* species, resulting in 20 leaf length measurements for each species. The means of the 20 leaf length measurements for each of the nine *Acacia* species were calculated and tabulated (see Table 4.1).

Statistical analyses

The $\delta\ ^{13}C$ values of the nine *Acacia* species were compared using a one-way ANOVA in order to determine whether or not the selected acacias differed in their water-use efficiency. A correlation analysis was then performed between the $\delta\ ^{13}C$ and leaf size measurements of the nine acacias studied, aimed at assessing the role of leaf size in the water-use efficiency of the selected *Acacia* species. Lastly, a t-test was used to compare the $\delta\ ^{13}C$ values of the *Acacia* species sampled from Hluhluwe to those of *Acacia* species sampled from Umfolozi, in order to determine the influence of rainfall (dry vs. wet) on the water-use efficiency of the acacia plants under investigation.

4.3 Results

4.3.1 Variability in δ ^{13}C between the selected acacias

Of the nine *Acacia* species sampled from HUP, *A. karroo* had the lowest (most negative) mean δ ^{13}C value of -29.71 ‰ and *A. grandicornuta* the highest (least negative) at -27.49 ‰ (Table 4.1), a range typical from C_3 plants (O'Leary, 1981). Surprisingly, despite the similarity of the mean δ ^{13}C values of the nine acacias, they were found to differ significantly ($p = 0.03$).

4.3.2 Relationship between δ ^{13}C and leaf size

The leaves of the nine *Acacia* species varied in length from 3.35cm for *A. tortilis* to 15.70 cm for *A. caffra* (Table 4.1). The *Acacia* species which had high mean δ ^{13}C values had low mean leaf length values, and the acacias with low mean δ ^{13}C values had high mean leaf length values. The correlation between mean leaf length and δ ^{13}C indicated a weak inverse relationship between the two variables ($r = -0.51$).

4.3.3 Influence of rainfall variability on the δ ^{13}C measurements

The mean δ ^{13}C values of the nine *Acacia* species selected for this study were such that the Hluhluwe sampled acacias had slightly lower values (ranging from -29.71‰ to -28.52 ‰) than those of the acacias sampled from Umfolozi (which ranged from -28.27 ‰ to -27.49 ‰). The mean δ ^{13}C values of the acacias sampled from the two parts of HUP which differ in annual rainfall, were found to be significantly different ($p < 0.001$). This suggested that differences in environmental conditions between the two parts of HUP had an impact on the δ ^{13}C of the plants under investigation. However, when the mean δ ^{13}C values of the replicated acacias (*A. karroo* and *A. nilotica*) were compared, they were found to be similar in the two parts of the HUP ($p = 0.86$).

4.4 Discussion

4.4.1 The acacias differ in their water use efficiency

The nine *Acacia* species investigated in this study had δ ^{13}C measurements within the expected range of C_3 plants (O'Leary, 1995, Heaton, 1999). However, the δ ^{13}C measurements differed significantly between the selected species ($p < 0.001$). Previous studies have shown that the relationship between δ ^{13}C of plants and their water-use efficiency is such that low δ ^{13}C measurements are an indication of low water-use efficiency (Olbrich *et al.*, 1993; Ehdaie and Waines, 1993). In light of the above, the δ ^{13}C measurements of the nine *Acacia* species under investigation were used as indicators of each species' efficiency to utilise available water. It was concluded that *A. karroo,* which had the lowest mean δ ^{13}C value of the sampled *Acacia* species, was the least water-efficient of the selected *Acacia* species, followed by the rest of the acacias sampled from Hluhluwe. The Umfolozi sampled acacias had higher (less negative) δ ^{13}C measurements and were thus more efficient in utilising available water than the acacias sampled from Hluhluwe. *A. grandicornuta* was the most water-use efficient of all the *Acacia* species under investigation.

The differences between the δ ^{13}C measurements, and consequently water-use efficiency, of the selected acacias could be attributed to a number of genetic differences between the *Acacia* species (Flanagan and Ehleringer, 1998). Some relevant genetic differences that could be responsible for the δ ^{13}C results of this study include differences in leaf traits, vegetation type and form, and other physiological factors such as variations in stomatal conductance, which regulate the diffusion of CO_2 (Farquhar *et al.*, 1982; Farquhar and Sharkey, 1982; Field *et al.*, 1983; Farquhar *et al.*, 1989; Jones, 1992). Of these genetic factors that could be responsible for the variation in the δ ^{13}C measurements of the selected acacias, the influence of leaf traits such as leaf size was explored and is discussed below (3.4.2). This was in light of field observed size differences between the leaves of the acacias under investigation.

4.4.2 Acacias with small leaves have higher water-use efficiency

Leaf length (used as a measure of leaf size) and $\delta\ ^{13}C$ measurements of the nine *Acacia* species selected for this study were found to be indirectly correlated (r = -0.51). This meant that to some extent, *Acacia* species with the smallest leaves had the highest $\delta\ ^{13}C$ values. As already discussed in the section above, it was the acacias sampled from Umfolozi that had the highest (least negative) $\delta\ ^{13}C$ measurements and it was concluded that these had the highest water-use efficiency of the nine *Acacia* species under investigation (see section 3.4.1). As indicated by the correlation results, the Umfolozi sampled acacias with higher $\delta\ ^{13}C$ measurements had shorter leaves than the acacias sampled from Hluhluwe.

However, the correlation was not a strong one (r = -0.51) and a few of the sampled acacias did not display a clear indirect correlation between their leaf length and $\delta\ ^{13}C$ measurements. For example, *A. tortilis,* which had the smallest leaves of all nine acacias sampled, did not have the highest mean $\delta\ ^{13}C$ value (and consequently high water use efficiency); and *A. caffra* sampled from Hluhluwe, which had the largest leaves, had a relatively higher mean $\delta\ ^{13}C$ value than the other Hluhluwe sampled acacias. This indicates that the relationship between leaf size and water-use efficiency represented by the $\delta\ ^{13}C$ measurements of the nine *Acacia* species selected for this study is more complex than has been described.

In general, however, the correlation between the leaf length and $\delta\ ^{13}C$ measurements (as a measure of water-use efficiency) of the nine *Acacia* species under investigation was in accordance with the hypotheses and conclusions of previous studies (Donovan and Ehleringer, 1992; Dudley, 1996a; Geber and Dawson, 1997; Pimentel *et al.*, 1999; Nativ *et al.*, 1999; Arens *et al.*, 2000; Querejeta *et al.*, 2003; Heschel and Riginos, 2005) linking leaf size to water availability. These studies concluded that small leaves were a plant's attempt to increase its fitness in response to water shortages. This link between the water-use efficiency of the nine *Acacia* species and the availability of water (e.g. differences in annual rainfall) between the two main sampling locations, Hluhluwe and Umfolozi, was explored next and is discussed in the following section (3.4.3).

4.4.3 Acacias from the drier Umfolozi have better water use efficiency

Although past research showed very little difference between the $\delta\ ^{13}C$ values of plants sampled along an aridity gradient such as the one between Hluhluwe and Umfolozi, the highest $\delta\ ^{13}C$ values were recorded for plants sampled from the drier areas (Schulze *et al.*, 1991). Smith and Nowak (1990) also associated high water-use efficiency in plants with increased drought tolerance (i.e. with trees growing in dry areas). This is in accordance with what has been discussed in sections 3.4.1 and 3.4.2 above, where it was concluded that higher (less negative) $\delta\ ^{13}C$ measurements were an indication of *Acacia* species that utilised available water more efficiently.

Also evident from sections 3.4.1 and 3.4.2 is the distinct division in the $\delta\ ^{13}C$ measurements of the *Acacia* species sampled from Hluhluwe and those sampled from Umfolozi. When the mean $\delta\ ^{13}C$ measurements of the *Acacia* species sampled from Hluhluwe were compared to those sampled from Umfolozi, they were found to differ significantly ($p < 0.001$). The acacias sampled from Hluhluwe had mean $\delta\ ^{13}C$ measurements that were lower (more negative) than those of the acacias sampled from Umfolozi. These two parts of HUP differ in the amount of rainfall they receive annually, with Hluhluwe the more mesic part and Umfolozi the more arid. As expected, the acacias sampled from the drier Umfolozi were found to be the more efficient users of available water (indicated by the higher mean $\delta\ ^{13}C$ values) than the acacias sampled from the wet Hluhluwe. The results of this study support the findings of previous studies showing that environmental effects such as water availability or differences in annual rainfall influence plant $\delta\ ^{13}C$, such that plants from dry environments are linked to higher $\delta\ ^{13}C$ and water-use efficiency (Raven and Farquhar, 1990; Smith and Nowak, 1990; Schulze *et al.*, 1991; O'Leary, 1993; Newton *et al.*, 1996).

However, it is important not to completely eliminate species differences (i.e. genetic effects) as another potential factor responsible for differences between the mean $\delta\ ^{13}C$ measurements of the acacias sampled from Hluhluwe and those sampled from Umfolozi. Only two of the nine selected *Acacia* species were sampled from both Hluhluwe and Umfolozi. Therefore, the differences between the mean $\delta\ ^{13}C$

measurements of the acacias could be a reflection of having sampled and compared different *Acacia* species from the two parts of HUP. When the mean $\delta\,^{13}C$ measurements of the replicated *Acacia* species, *A. nilotica* and *A. karroo*, were compared between the two parts of the HUP, their mean $\delta\,^{13}C$ measurements were very similar ($p = 0.86$). This indicates that the differences between the $\delta\,^{13}C$ measurements of the *Acacia* species sampled from Hluhluwe and those sampled from Umfolozi were not only due to differences in environmental conditions (i.e. annual rainfall differences) within the two parts of HUP, but probably a combination of both environmental and genetic effects.

Tables

Table 4.1: Mean $\delta^{13}C$ and leaf length measurements of the *Acacia* species sampled from Hluhluwe and Umfolozi.

HLUHLUWE (20 leaf samples per species)			**UMFOLOZI (25 leaf samples per species)**		
***Acacia* species**	**$\delta^{13}C$ (‰)**	**Leaf length (cm)**	***Acacia* species**	**$\delta^{13}C$ (‰)**	**Leaf length (cm)**
Acacia nilotica	-28.52	7.98	*Acacia nilotica*	-28.27	-
Acacia karroo	-29.71	9.33	*Acacia karroo*	-29.60	-
Acacia burkei	-29.37	6.98	*Acacia nigrescens*	-28.07	7.48
Acacia robusta	-29.43	8.85	*Acacia tortilis*	-28.27	3.35
Acacia caffra	-28.93	15.70	*Acacia grandicornuta*	-27.49	5.88
			Acacia luderitzii	-27.71	4.15
Overall mean	-29.19	9.77	Overall mean	-28.24	5.22

- species not considered for this analysis therefore not measured

CHAPTER 5:

CONCLUSIONS

5.1 Outcomes of study

Handley and Raven (1992), Högberg (1997) and Robinson (2001) have shown how stable isotope studies can provide powerful clues and insights into ecological and physiological processes of ecosystems and plants. Similarly, the results of this study give insight into whether or not *A. karroo* is invasive in HUP because of its N_2-fixing ability, its defence mechanisms, and/or its ability to utilise available water efficiently. The findings of this study also provide useful information on the impacts of N_2-fixing plants such as the selected acacias on the soil in HUP. Furthermore, through investigating the defence mechanisms of the nine *Acacia* species, a framework for interpreting responses to herbivory was developed. Lastly, the information gathered on the water-use efficiency of the different *Acacia* species presents valuable lessons towards interpreting the potential impacts of global change on the species composition and persistence within savanna ecosystems such as HUP.

5.1.1 Why is A. karroo invasive in HUP?

Is it the best N_2-fixer?

The $\delta\,^{15}N$ results of the study show that *A. karroo* is one of the best N_2-fixers of the *Acacia* species within the Umfolozi part of HUP. In Hluhluwe where the annual rainfall is higher and fire is prevalent, *A. karroo* does not seem to be at an advantage over the other plants growing in its vicinity as a result of being a N_2-fixer.Therefore, if *A. karroo* is invasive in HUP because of its ability to fix N_2, then it would be expected to be more invasive in the drier parts of HUP and to a lesser extent in Hluhluwe where it is wetter and fires are more prevalent. However, evidence from past studies (Bond *et al.*, 2001) shows that *A. karroo* is more dominant in Hluhluwe than it is in

Umfolozi where it is limited by physiological constraints (O' Connor, 1995). This suggests that being the best N_2-fixer is not the main driver of the invasiveness of this species.

Does it have the best resistance against herbivores?

A. karroo was found not to have the highest [N] and is thus not expected to be the preferred source of nutrition by herbivores over the other eight *Acacia* species occurring within HUP. This was also reflected by the not very strong or complex defence mechanisms of *A. karroo*, such as the low concentrations of tannins and the shorter and weaker spines). However, the results of this study support the argument by Bond *et al.* (2001) that A. *karroo* is preferred over *A. nilotica* by herbivores. The amount of tannins in the leaves of *A. nilotica* is much higher than that found in A. *karroo*. Furthermore, the very strong and curved spines of *A. nilotica*, which are considerably longer than its leaves, provide much better resistance and discourage herbivores from feeding than those of *A. karroo*. This shows that resistance to herbivory is not the driving factor responsible for the success of *A. karroo* as an invasive species.

Is it the most water-use efficient?

Of the nine *Acacia* species from HUP whose water-use efficiency was assessed, *A. grandicornuta* and not *A. karroo* utilised available water most efficiently. The exact mechanisms responsible for the variation in the water-use efficiency of the selected acacias proved to be more complex than anticipated (i.e. beyond the effects of just leaf size and rainfall variation). Generally however, the water-use efficiency of *A. karroo* was better in Umfolozi than in Hluhluwe. This indicates that if *A. karroo* is invasive because of its water-use efficiency, then it is expected to be more invasive in Umfolozi than in Hluhluwe. However, as already mentioned above O' Connor (1995) found that *A. karroo* in the eastern Cape region of South Africa did not establish when the rainfall was less than 500mm. This indicates that the ability for *A. karroo* to invade HUP is due to a combination of factors rather than a single one. Its ability to resprout vigorously after fires and the dominance of its juvenile stages over adults in HUP are some of the other factors involved that need to be explored in order to gain more insight as to what makes *A. karroo* such an aggressive invader.

5.1.2 Impacts on soil by N_2-fixing plants

There was no evidence of permanent soil effects from the presence of N_2-fixing plants such as the nine *Acacia* species in HUP. This was indicated by the similarity between acacia and reference plant soil $\delta\ ^{15}N$ in both Hluhluwe and Umfolozi. This suggests that N_2-fixation and its effects on the soil do not necessarily give legumes an advantage over non-fixing plants, and is not solely responsible for the invasive nature of *A. karroo* in HUP.

5.1.3 Framework for interpreting herbivory

Even though this study did not monitor and record any herbivore feeding behaviour, it provides a framework for the interpretation of herbivore feeding behaviour. For example, the information on the different defence strategies of the nine *Acacia* species can be used to explain why some species are better defended than others or why some species are preferred by herbivores (Bond *et al.*, 2001). This is illustrated above (section 5.1.1) where the preference of *A. karroo* by herbivores over *A. nilotica* can be explained by the degree of defence each of the species has, where *A. nilotica* is better defended both mechanically and chemically.

5.1.4 Global change and water-use efficiency impacts

Predicted changes in temperature and precipitation in response to global change effects, suggest that southern Africa will experience increasing aridity and prolonged spells of severe drought (Hulme, 1996b). Temperatures will be highest in the most arid parts of South Africa (e.g. Umfolozi), and a broad reduction of rainfall in summer rainfall regions such as Hluhluwe. This shows that water-use efficiency will greatly influence plant survival and distribution in the next 10 to 50 years. The most water-use efficient plants are those from the drier Umfolozi, this suggests that the distribution of these species will spread beyond Umfolozi into Hluhluwe. Hluhluwe plants will experience water limitations which they are not adapted to, and their distribution is thus likely to become fragmented possibly only surviving in isolated wet patches within Hluhluwe.

RADIAL DIFFUSION ASSAY FOR TANNINS

Reagents

(i) Buffer:

0.05M acetate containing 60µM ascorbic acid at a pH of 5.0. For 1 L of this buffer solution, dilute 2.85 mL glacial acetic acid to about 800mL, add 10.6 mg ascorbic acid, and adjust Ph to 5.0 with 2NaOH. The final volume to be brought to 1L with water.

(ii) Agarose:

Type I, low EEO, gel point 36 C (Sigma A-6013)

(iii) Bovine Serum Albumin:

Fraction V powder, 96-99% albumin (Sigma A-3350)

(iv) Well punch:

4.0mm punch (Biorad 170-4029)

(v) Disposable Petri Dishes:

Petri dishes of actual diameter about 8.5 cm (nominally 10 cm).
Washable and re-usable, possibly Fisher catalog # 8-757-13, 100 x 15mm.

(vi) Extras:

Parafilm
Water bath
Hot plate/stirrer
Refrigerator
30°C incubator

Preparation of plates

A 1% solution of agarose was prepared (1.0g agarose to 100.0mL of buffer solution) by heating the suspension of agarose to boiling while stirring. The solution was cooled to 45°C in a water bath and 0.1g of protein BSA (Bovine Serum Albumin) was added while the solution was gently stirred. The solution was dispensed in 9.5mL aliquots into the Petri dishes and allowed to cool and the agarose solidified on cooling. The plates were cooled on a level surface to obtain slabs of uniform thickness. These plates were then stored at 4°C to prevent bacterial growth.

Plant Extraction

Plant tissue (leaves) was extracted for an hour at room temperature with 50% aqueous methanol. To do this a solvent-to-tissue ratio of 5.0mL solvent per 100mg leaf sample size was used. The extracts were then applied directly to the diffusion gels (hardened agarose and buffer plates in Petri dishes) without any sample cleanup.

Assay

Uniform wells were punched into the plates, 1.5cm apart. Tannin-containing samples (plant extracts) were added to the wells with a Hamilton micro-syringe, in 8µL aliquots. Where tannin-containing samples were very dilute, several successive 8µL aliquots were added to a single well as the liquid was absorbed by the gel. After dispensing the tannin-containing plant extracts into the wells, the Petri dishes were covered and sealed with parafilm. The dishes were incubated at 30°C for 96 - 120 hours. The diameters of the rings formed on the gels were them measured for each ring. To minimised errors due to non-uniform ring development, two diameters at right angles to one another were measured for each ring. Tannin concentration was calculated from the square of the average of the two diameters using an appropriate calibration curve.

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