The effects of browsing on growth, structure and physiological aspects of *Acacia grandicornuta* and *Combretum apiculatum*.

by

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Declaration

I, Charlotte Thandeka Mamashela, declare that the work contained in this thesis is my original work except where references have been made. This work neither has been submitted for nor will be submitted for an award in another University or Institution of higher learning.

Candidate's Signature	Date
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Abstract

Very little is known about plants growing in savannas, especially how woody plants respond to browsing and variations in resources. It is assumed that growth rate and concentrations of defenses are inversely related, but will be affected by resource availability. It can be postulated further that increased growth and photosynthesis would be observed concomitant with reduced tannin concentration when water and nutrients are abundant at the beginning of the wet season. Growth in terms of shoots length, thorn length and plant height would increase in terms of increasing photosynthetic rate on browsed plants compared to unbrowsed plants. Therefore, research was conducted to investigate the effects of browsing on *Acacia grandicornuta* and *Combretum apiculatum*.

Research was conducted at the Nkuhlu experimental exclosures (Kruger National Park) on these two tree species. The exclosures were designed so that there were three broad levels of browsing pressure: no mammal herbivores excluded, elephants and giraffes excluded and all mammal herbivores bigger than hares excluded, all of which incorporate the catena from sodic footslope to sandy crests. The focus was on heavily browsed plants between the heights of 0.7-1.7 m. Ten trees per species per treatment (full, partial and no exclosure) were sampled and height and stem circumference were measured in the early and late wet season. Five new shoots per tree were marked; these shoots were re-measured at intervals to monitor their growth. Leaves were harvested from other short trees of both species in all the exclosures. Leaves were dried, milled and analysed for phosphorus (P), nitrogen (N) and condensed tannins (CT). Photosynthetic measurements were also recorded at the same time from short individual trees of *C. apiculatum* from the full and non exclosure.

The results showed that there were no significant effects of the treatments and no significant growth on shoot and thorn length of *A. grandicornuta* in all the treatments

from October to December 2007. There was significant growth in the shoot lengths of *C. apiculatum* from October to December 2007 in all the treatments (full exclosure t = -4.65, df = 10.20 and P = 0.001, non exclosure t = - 4.67, df = 7.90 and P = 0.002 and partial exclosure t = - 8.86, df = 7.30 and $P < 0.001$).
There was a significant effect of the treatments on the shoot lengths of *C. apiculatum* during December 2007. There was no significant growth in heights and stem circumference of *A. grandicornuta* from September 2007 and March 2008 ($P > 0.05$). There was significant growth in the height of *C. apiculatum* in the full exclosure (t =2.33, df = 17.10 and P = 0.032) and the stem circumference of *C. apiculatum* was significantly smaller in the partial exclosure (t =3.71, df =8.00 and P = 0.006) in September 2007 compared to March 2008.

The pattern for both *A. grandicornuta* and *C. apiculatum* in all the treatments was a decreasing P concentration from October 2007 to March 2008. Nitrogen decreased in *A. grandicornuta* where mammals were excluded and also decreased in the non exclosure. In the partial exclosure there was no change in N concentration from October 2007 to March 2008. For *C. apiculatum*, N concentration decreased in all the treatments from October 2007 to March 2008. The CT concentration remained the same for *A. grandicornuta* in all the treatments and in all the months. The CT concentration of *C. apiculatum* in the full exclosure significantly increased (t = -7.08, df = 10.60 and $P <$ 0.001), in the non exclosure the CT concentration also increased (t = -5.34, df = 8.00 and P = 0.001). In the partial exclosure, the CT concentration remained the same for *C. apiculatum* from October 2007 to March 2008. The leaf removal of *A. grandicornuta* was not correlated with CT concentration and was not correlated for *C. apiculatum*.

The results from the photosynthesis data showed a steadily decrease in J_{max} and V_{cmax} from November 2007 to March 2008 where the herbivores were excluded. The plants in the non exclosure responded differently compared with the plants in the full exclosure. J_{max} and V_{cmax} decreased form November 2007 to February 2008 but increased again during March 2008 where herbivores were not excluded.

A nursery experiment was conducted to see the effects of browsing as affected by water availability. The interests were in finding out the responses of plants after browsing at different water levels. Potted seedlings of *C. apiculatum* were allowed to grow in the nursery and watered once after 3 days. The heights, basal circumferences, shoot lengths were measured and plants were given to goats to achieve a range of browsing intensities in September 2007 (0, 30, 60 and 90% intensity).

Plants were weighed before and after browsing to estimate the amount removed, and were separated into three groups that were watered after 3, 7 and 10 days for four months. After four months of growth, the plants were re-measured (heights, basal circumferences, shoot lengths), harvested, separated into morphological parts (roots, shoots and leaves), oven dried and each part weighed. Leaves were then analysed for P, N and CT.

Increased water availability resulted in increased leaf, shoot and root mass. The results from the combined data showed that water treatment had a significant effect on leaf mass ($t = -9.39$, df = 20.60 and $P < 0.001$) shoot mass($t = -6.18$, df = 34.40 and $P < 0.001$) and root mass ($t = -2.19$, df = 31.20 and $P = 0.036$). Defoliation did not have an effect on leaf and root mass within each water treatment but defoliation had a significant effect on shoot mass.

The data was combined and the results show that water treatment had a significant effects on heights ($t = -4.28$, df = 38.00 and $P < 0.001$) shoot length ($t = -8.02$, df = 29.10 and $P < 0.001$) and basal circumference of the seedlings after defoliation ($t = -7.02$, df = 29.00 and $P < 0.001$). Defoliation had a significant effect on height, shoot length and basal circumference of seedlings after defoliation ($P< 0.001$).

Water had a significant effect on P, N and CT concentration in the seedlings where P, N and CT concentrations increased with increasing water availability. The chemical concentrations between seedlings which were watered every 10 and 3 days, were significant for phosphorus ($t = -2.07$, df = 28.20 and $P = 0.047$) N concentration ($t = 2.74$, df = 35.90 and $P = 0.009$) and CT concentration ($t = -2.23$, df = 20.50 and $P = 0.037$). There was no effect of defoliation on P and N concentrations on the seedlings which were

defoliated at different intensities. Defoliation had an effect on CT concentration on seedlings which were watered after 7 and 3 days.

It was concluded that *A. grandicornuta* was not affected by treatments or season in terms of growth. The *C. apiculatum* trees which were exposed to browsing increase their growth rate in the form of shoots length and stem circumference. No growth in terms of height was detected in *C. apiculatum* trees in browsed plants because the plants were suppressed by browsing. No growth in *A. grandicornuta* was recorded in all the treatments.

The CT concentration for *A. grandicornuta* remained constant in all the treatments for all the seasons. The CT concentration in *C. apiculatum* increased under browsing, which was unexpected. Increased shoot length occurred when browsing occurred during the growth season when resources were abundant and browsing was inevitable. When growth increases, photosynthesis was expected to increase in *C. apiculatum* trees exposed to browsing. The photosynthetic rate in *C. apiculatum* exposed to browsing was higher than in unbrowsed *C. apiculatum* trees.

Heavily defoliated seedlings increased their growth in the form of height, stem diameter and shoot length as a defence strategy as expected. Seedlings with more resources produced more CT in their leaves as a form of defence mechanism which was expected. Seedling parts which allocated more biomass had more water access than seedlings which had less water.

Since very little is known about tree responses to browsing, this research will help improve knowledge and understanding of browse browser interactions in savannas. The knowledge gained from this research is useful for building models of browse-browser interactions in seasonal subtropical zones where browsers are abundant and have the potential to deplete vegetation resources and how to prevent this from happening.

CHAPTER ONE

General Introduction

1.1 Problem Statement

Very few studies have been conducted on woody plants growing in savannas or their response to browsing. Savannas are seasonal systems dominated by both grass and trees and are characterized by alternating wet and dry seasons. Savannas cover 65 % of the land surface of sub-Saharan Africa and > 50 % of southern Africa (Hempson *et al.* 2007). Savannas are very important because they provide food for millions of people and livestock, and are also important for pastoralism, game ranching and nature conservation (Scogings 2003). Savannas comprise woody plants and grass species which provide important nutrition for herbivores. Since many people and animals rely on savannas for food resources, they are likely to have an impact on savannas (Scogings and Mopipi 2008). Hence, these systems need to be managed so that they can still sustain people and animals which depend on them for food.

The Kruger National Park (KNP), in north-eastern South Africa, covers approximately 20 000 km^2 (350 km in length and on average 60 km wide), and falls within the semi-arid savanna biome, in the Lowveld region (Low and Rebelo 1998). The KNP supports about 30 species of large herbivores (> 5 kg), of which 11 species are browsers, including elephants, giraffe, kudu, eland and a high number of impala and all these mammals have an effect on vegetation of the park (Du Toit 2003). The KNP is a natural, managed system which offers a unique opportunity to do research on plant defences and plant responses to browsing. This will help develop alternative hypotheses for savannas and will give answers on how to better manage savanna systems in Africa through research. The KNP has experimental exclosures which were designed to explore the effects of elephants and other mammalian herbivores on one of the main vegetation types of the park which can be monitored long term. By monitoring and maintaining these exclosures, the effects of mammals on vegetation can be determined and provide management with knowledge on how to conserve and manage this vegetation.

1.2 Literature Review

1.2.1 Importance of African Savannas

Savannas are defined by the co-existance of trees and grasses in one system, in varying amounts. Many mammals occur in savannas and feed on the resources that the savanna system provides (Dangerfield and Modukanele 1996). Savannas can differ in structure and are differentiated into arid shrub lands, lightly wooded grasslands, deciduous woodlands and dry forests. The reason for such a variation in shapes of savannas has been a question to many researchers for many decades and various answers have been brought forward. Some scientists say the variation is from competition for resources such as nutrients, water and lights, while some say it's other factors such as fire and large herbivores. The results from recent studies show that water is an important factor in shaping the savannas (Hempson *et al.* 2007).

The savanna ecosystem of southern Africa provides an interesting opportunity to study plants and their interactions with herbivores, most importantly the browsers and their impact on the production of trees and how plants respond to herbivory. Regardless of their importance, very little research has been done hence little is known or understood about the savanna trees. Little is understood about how herbivores affect trees growing in African savannas or which plant species have the ability to respond positively to browsing pressure (Dangerfield and Modukanele 1996). Processes influencing the relative quantity of trees and grasses are important, and can cause a rapid change in the composition of a savanna. The principal factors shaping savanna ecosystems are water supply, nutrient availability, fire regimes, and herbivory (Scholes 1997).

1.2.2 Plant Defence Mechanisms

Herbivores consume plants to acquire nutrition and energy. Mammal browsers feed from plants which vary in chemical and physical characteristics that influence feeding behaviour of the herbivores (Stamp 2003). For example, defences may reduce intake rates (Scogings *et. al.* 2004). However, herbivores have evolved counter-measures that work as selective pressure on plants for additional defence (Stamp 2003).

Plants employ different defence mechanisms including physical responses (such as thorn production), chemical defences (increasing tannin concentrations), or growth strategies (growing too tall for the leaves to be eaten) (Rohner and Ward 1997). Factors which are important for the plant to be able to compensate for tissue loss or damage due to browsing are timing (time of the year when a plant is browsed), degree of damage (how much a plant has been browsed), competition (competing for resources with other plants) and nutrient availability (limited resources lead to more defences) (Rooke *et al.* 2004a).

Plants that grow slowly, in low-resource environments are expected to invest more in chemical defences against herbivores (Stamp 2003). In a low resource area, it is difficult to replace lost tissue, and high defence mechanisms help prevent herbivory. Plants that grow rapidly in high resource areas rely on rapid growth (growth strategies) to replace the tissue loss. The plants can escape from the browsing zone by growing too tall to be eaten (Rooke *et al.* 2004a). The dominance of physical and chemical defences depends on the amount of nutrient supply in the ecosystem. Nitrogen limited ecosystems have plants which are mostly defended by tannins, when N-based and structurally defended plants occur where there is more N in the ecosystem that is available to the plants (Craine *et al.* 2003).

Plant defences against herbivore utilization can also be explained in terms of tolerance or compensation. A plant’s ability to survive or endure the damage by and the ability to reduce the negative effects of herbivory on their growth and reproduction is defined as tolerance (Gadd *et al.* 2001).

Mechanisms of tolerance are linked with the essential growth rate and with morphological and physiological characters which allow the plant to replace biomass lost to herbivory. Compensation is when a plant increases its growth (height, shoot length or thorn length) after loss of tissue to herbivores. Compensation is not regarded as a real increase in plant growth but the results of relocating resources to restore tissue loss (Gadd *et al.* 2001). Induced defences are produced in response to browsing when the plant increases its fitness by reducing the herbivore performance and they are known to decrease the rate of herbivory (Stevens *et al.* 2007).

Highly defended plants are less impacted by browsing and these plants do not benefit from compensation. It has been indicated that compensation and tolerance can sometimes benefit the plants (Stevens *et al.* 2007). The costs of resistance and tolerance will also differ in response to environmental conditions such as soil nutrient availability, herbivore damage, and intra-or interspecific plant competition. (Fornoni *et al.* 2004).

1.2.3 Plant Responses to Browsing

Seedlings are more sensitive to browsing since they can easily be reached by most mammalian herbivores because of their low height. Seedlings are also very sensitive at the time when they are changing stages and they are known to resist browsing better than they can survive droughts (Scogings 2003). Herbivores tend to repeatedly go back to the same plants which they have browsed before. This phenomenon is known as re-browsing, meaning that a lower number of trees are browsed than would be expected from a random utilization by herbivores. This repeated browsing of certain trees often leads to the development of a feeding loop and possible ‘browsing lawn’ where a tree is kept short by herbivores (Makhabu and Skarpe 2006).

Apical dominance determines a plant's natural branching habit and its response to pruning. It produces orderly, controlled growth and gives plants their characteristic shape. When the terminal bud is gone, its apical dominance is eliminated and the growth of the plant is altered as other buds begin to grow (Cline 1997).

If the leading shoots are browsed or if the stem is somehow broken, the apical dominance is reduced, this leads to reduced plant height and more shoots grow at lower levels in the canopy, where mammals which feed from the ground can reach them (Makhubu and Skarpe 2006). When this happens, the number of meristems is reduced and this leads to fewer and larger shoots, sometimes with higher concentrations of nutrients and lower concentrations of carbon based defence compounds (Makhubu and Skarpe 2006). Frequent, heavy browsing does not stimulate shoot production, but infrequent browsing does because of additive effects. Some plants are believed to respond to herbivory by just replacing lost tissue over what has been lost to the herbivores (Stokke and Du Toit 2000).

Herbivores may damage the plant at any stage of their life and the damage will vary with life stage of the plant. Plants that are still growing are likely to be more affected by herbivory than old plants. Older plants are much bigger in size compared with herbivores. If bigger herbivores utilize a young plant, they are likely to kill the plants by browsing the entire plant. So it is important for a plant to have various defences against any utilization from herbivores. Plants can have many defences, which are called multiple defence mechanisms and are common in some plant species. The possession of several defence mechanisms may be very costly for a plant since investing in any defence is assumed to reduce the resources which may be used for growth and reproduction by a plant (Koricheva *et al.* 2004, Strauss *et al.* 2002).

Plants may only concentrate their defences in those structures or parts that are most likely to be eaten. Plant parts which are accessible and non-accessible to herbivores depend on the height of the trees. Parts which are lower down in the tree canopy can be more easily accessed compared to those higher up in the canopy (Ward and Young 2002). Young trees need more physical and chemical defences than older trees since they can be reached by most herbivores. Lower branches which can be accessed by herbivores have longer thorns than branches higher up in the tree (Gadd *et al.* 2001).

Plant parts which are more exposed to herbivores have various investments and produce stronger defences when they are needed by the plant (Ward and Young 2002). Responses to browsing depend on the timing of herbivory, the type and amount of herbivory, the availability of resources in the environment to support regrowth and the browsing history of the plant (Paige 1992, Gadd *et al.* 2001).

An important matter is the time in which browsing occurs and this is important because it determines the plant responses to browsing (Paige 1992, Gadd *et al.* 2001). Most savanna trees are deciduous with leaf herbivory usually occurring during the growth season. Shoot biting is common during the dry season and droughts (Rooke *et. al.* 2004b). Summer browsing usually leads to shoot growth during the growth season because the rate of recovery is quickest since resources are usually available. Winter browsing has no benefits to the plants because it does not generally stimulate any production of new shoots (Danell *et. al.* 1994). Increasing browsing is related to an increase in C-rich secondary metabolites in palatable species. Reduced concentration of the C-rich secondary metabolites is associated with increased nutrient concentrations (Canham *et al.* 1994, Danell *et al.* 1994).

1.2.4 Herbivore Effects on Plants and the Relationship Between them

The defences which are engaged by plants depend on the size of the herbivore which is likely to utilize those (Hanley *et al.* 2007). Differences in behaviour and the way herbivores feed lead to differences in nature, degree and other effects of damage applied to plants. Plants are utilized by both vertebrates (mammals) and invertebrates (insects) and they both have a particular way of feeding on each plant. Most invertebrates feed on plants by gradually removing small amounts of plant tissue over a prolonged period of time (Hanley *et al.* 2007).

As a result of this continuous damage, plants have to continue draining their resources constantly to defend themselves. This constant damage of little amounts of tissue loss has a large impact on the plants but these utilizations are specific and plants can focus their defences on specific vulnerable areas (Hanley *et al.* 2007).

Most importantly, these slow rates of damage by invertebrates permit the plant time to react with increased defences or increased growth if necessary. On the other hand, herbivory by vertebrates is often rapid and harsh because of their large size relative to that of plants they utilize. Vertebrates are bigger and less selective in their choice of plant material they eat than invertebrates (Hanley *et al.* 2007). Therefore, defences tend to be large because it is often difficult for the plant to defend specific parts of their structure against vertebrates (Hanley *et al.* 2007).

Herbivores consume large amounts of food because of their size and feed on large, vigorous, sprouting shoots. Herbivores can get large bite sizes with high nutrients from new shoots (Du Toit *et al.* 1990). For example, many large herbivores face problems when feeding because they are poorly adapted to dealing with small amounts of plant tissue. The feeding structure and requirements of a large herbivore means that their minimum plant intake is the large amount. For example, herbivores with smaller mouthparts are more suited for dealing with the challenging task of removing small leaves from lots of thorns or spines from the plants (Hanley *et al.* 2007).

When more plants are utilized, they improve their defences and herbivores also often find a way of dealing with and overcoming those defences. Therefore, there is an evolutionary relationship between plants and animals (Coley *et al.* 2005). Browsers in semi-arid regions where more plants are defended with thorns and spines tend to have long, flexible tongues, tough, leathery mouthparts and nicitating eye membranes as an adaptation for coping with browsing thorny plants. Browsing damages the plant but it is also very important to a plant because browsers are known to influence the future preference and performance of associated herbivores and therefore improve the fitness of the plant that is being eaten (Gowda 1997, Scogings 2003). This leads to a beneficial relationship between the plant and its herbivores through increased growth, increased structural defences and seed production (Gowda 1997).

1.2.5 Physical Defences

Some species can respond to herbivory by increasing structural defences. These include growing thorns or spines for protection. Structural defences are regarded as avoidance mechanisms and sometimes they are used for climbing but they evolved as a defence against herbivores (Rohner and Ward 1997). Increasing structural defences are very costly to a plant and they are irreversible. (Scogings 2003, Rohner and Ward 1997).

Spinescence is a collective term used to describe plant structural spines, thorns and prickles. A spine is defined as a sharp-pointed petiole, midrib, vein or stipule, thorns are woody, sharp-pointed branches, and prickles are any sharp-pointed outgrowth from the epidermis or cortex of an organ (Hanley *et al.* 2007). For many spinescent species, it has been said that thorns and spines have evolved as a mechanical defence against large browsing herbivores. Spines and thorns do not prevent the plant from being browsed but the tissue loss is decreased, bite sizes and intake rates are restricted, and the herbivores must select leaves rather than biting off the shoots from the plants (Rooke *et al.* 2004b).

Spinescence is also considered to be effective against large mammals rather than small invertebrates, due to the size associations among them. Vertebrate herbivores should use a stronger and more reliable selection on leaf spines than invertebrates herbivores (Grubb 1992). If spines are only effective against large herbivores, their presence can only mean that they reduce the rate of herbivory by impeding stripping motion and forcing the herbivores to feed around the defences at a slower rate (Myers and Bazely 1991, Wilson and Kerley 2003). *Acacia* species often possess thorns or spines which are often regarded as defence mechanisms (Rooke 2004b). Spines limit access to the plant and change the feeding behaviour of the herbivores. Plant parts that are accessible e.g. more defended than those which are not accessible (Gowda 1997). Spines are effective defences, their length is increased after browsing and this has been proven in many studies. Increasing spine or thorn length after browsing induced some changes in the shoot formation and development (Gadd *et al.* 2001).

These changes in the shoots are sometimes related to nutritional quality and other factors. Spinescent trees growing in areas with low numbers of mammal herbivores produce shorter spines than areas with high number of herbivores (Gadd *et al.* 2001). Other studies have shown an increase in spine or thorn length on trees which have been subjected to browsing compared with unbrowsed trees (Rooke *et al.* 2004b). Longer spines on the shoots which can be reached by browsers (lower branches) were also found to have an increased spine length or biomass compared with shoots out of reach and above browsing height (higher up in the canopy) (Young *et al.* 2003).

1.2.6 Chemical Defences

Chemical defences are important induced defences because they can be produced quickly and they are transported quickly from one part of the plant to another. Woody plants growing in semi arid areas which are often reached by mammalian herbivores are more defended by polyphenols (chemical defences) than those which can not be reached by browsers (Ward and Young 2002). Browsing for plants which are not chemically defended against herbivores is a major challenge to plants. Avoidance mechanisms involve chemical defences (the production of phenolics) that reduce herbivores from continuing to feed following an initial bite (Hanley and Lamont 2001).

The chemical characteristics of woody plants have been explained in terms of resource-driven hypothesis. This hypothesis predicts that the concentration of C-rich chemical defences (phenols), are negatively correlated with the growth rate of plants. Such chemical defences would be less concentrated in the plants during growth than during dormancy. These chemicals have the ability to reduce the nutritional quality of plants for herbivores which consume them (Scogings *et al.* 2004).

1.2.7 Chemical responses after herbivory

Many plants induce chemicals when they defend themselves from being utilized by herbivores and many chemicals are inducibly produced. This means that most of the chemical defences are likely to be produced after the plant has been utilized (Baldwin 1998).

Plants produce a large number of secondary metabolites which function as defensive chemicals that assist the plant to deal with utilization from herbivores. Many chemical defences are only produced when they are required especially during herbivory utilization (Kessler 2007). When plants are utilized by herbivores, their food quality and chemical defences are altered to defend the plant and allow it to resist herbivores (Baldwin 1998).

Chemical defences make the food to taste unpalatable for herbivores and reduce further utilization of plants (Baldwin 1998). When the costs of defences are high and levels of herbivory are unpredictable. Plants produce defences only when browsed and these are called induced defences. These defences help the plant not to waste energy on defences unless stimulated to produce by herbivores (Ward and Young 2002). These induced responses initiate some changes in plant quality after the plant has been damage or eaten by herbivores. These changes in the plant have an enormous effect on the subsequent herbivore that will consume the plant (Agrawal 1999).

Chemical defences increase the plant's ability to withstand and resist herbivory utilization and are beneficial to the plant (Baldwin 1998). More types of chemical defences mean more protection for the plant. The plant can withstand herbivory and can grow to be adult and reproduce further. The production of the chemical defences after herbivory can also be costly when they are not required by the plant because resources which should be used for growth and reproduction are used for defences. The use of extra resources creates difficulties for the plant since lots of the herbivores that eat the plant also pollinate the plant or disperse the seeds so reproduction is reduced (Baldwin 1998).

1.2.8 Importance of Condensed Tannins

Among the many chemical compounds considered as defensive traits, phenolic (tannins) have received significant attention (Rooke *et al.* 2004a). Tannins are polyphenolic compounds found in most plants and are thought to function as plant chemical defences against herbivory. Tannins are classified into two chemically different groups: hydrolysable tannins (gallotannins, HT) and condensed tannins (proanthocyanidins, CT) which are more common in forage legumes, trees and shrubs (Gedir *et al.* 2005).

Tannins are estimated to be the fourth most abundant biochemical produced by plants in response to browsing and are important in ecosystem processes (Ferwerda *et al.* 2005). Condensed tannins (CT) are found in plants and are important in influencing herbivores as defences since they can precipitate proteins and reduce the activity of the digestive enzymes of herbivores (Rooke *et al.* 2004a).

Condensed tannins have a profound influence on the nutrition of herbivores and can cause a decrease in digestibility and voluntary intake, reduce live weight gain, reduce mineral absorption and they can damage the intestines or liver of mammalian herbivores (Gedir *et al.* 2005). The reason that the herbivores will still continue to feed on plants which contain high levels of CT is because they are not detrimental when ingested in moderate amounts (< 5 % dry matter) and may have some beneficial effects. CT can act as a natural detergent to reduce bloat; they also decrease dependence on synthetic anthelminthic compounds which control intestinal parasites (Gedir *et al.* 2005).

An increasing concentration in CT of the plants creates problems for mammal herbivore who feed on them. Plants and animals together have evolved ways to overcome those defences. Therefore, herbivores can handle a certain concentration of tannins. Browsers can produce salivary tannin-binding protein, which act as an adaptation to forage which is rich in proteins, hence allowing the herbivores to continue utilizing the plants with high concentrations of CT (Rooke *et al.* 2004a). If plants are being eaten, producing chemical defences is very costly to a plant and is thought to impose a selective penalty on plants but these are also thought to be traded off against improved herbivore defences (Ferwerda *et al.* 2005).

Chemical defences are the only means of protection for plants which have no spines and cannot afford to defend themselves from being browsed by mammal herbivores. Plants which are often utilized by herbivores are likely to have more chemical defences in their leaves than those which are not often browsed (Ferwerda *et al.* 2005).

But differences in CT concentration in plants have been related to leaf age, and other environmental factors such as soil property, temperature stress, light intensity and herbivory (Ferwerda *et al.* 2005). There is a negative correlation between feeding preferences of mammalian herbivores and concentrations of CT in different forage species and this suggest that intake by herbivores may be limited by the ability to detoxify CT. Herbivory and palatability in plants can be reduced when there are low concentrations of nitrogen or high concentrations of fiber (Rooke *et al.* 2004b).

When browsing intensity increases, the concentration of CT also increases, while an increase in nutrient concentration is found when CT decreases (Rohner and Ward 1997). Browsing benefits both herbivores and plants but plants tend to use more of their resources trying to defend parts which have been browsed instead of using those resources for growth or reproduction (Rohner and Ward 1997).

1.3 Research Aims

Very little is known about tree responses to browsing. Models which were developed in other studied systems may be used to test hypotheses about savanna tree responses to browsing**.** It is therefore assumed that the main resources for plants in semi-arid savannas are nutrients and water, which are greatest at the beginning of the wet season where elevated soil water drives a pulse of nitrogen mineralization (Scogings and Mopipi 2008). It can also be assumed that reducing resource availability affects plant growth before it affects net assimilation rate of photosynthesis (Herms and Mattson 1992), and when resources are abundant, re-growth after herbivory is not limited and excess carbon (C) does not accumulate as C-rich secondary metabilites (Stamp 2003).

Water, nutrients and herbivores, rather than light, are the major determinants of plant growth in African savannas and these factors affect plant growth and development (Scholes 1997, Skarpe 1992). At very low resource levels, when light is also low, both photosynthesis and growth are limited and the limited C may be used only for primary metabolism and maintenance respiration. As water and nitrogen (N) availability increase towards levels at which they no longer limit growth, allocation of photosynthate to defence becomes more costly in terms of lost competitive ability (Stamp 2003).

However, defences also become more costly (in terms of lost stress tolerance) as water and N availability decrease towards levels at which photosynthesis is limited. The outcome is that C-rich chemical defences are predicted to increase in plants when there is a moderate shortage of water or nutrients, decrease when there is an extreme shortage of water or nutrients (as well as a shortage of light) and likely to occur at intermediate levels when there is no shortage of nutrients or water (Stamp 2003).

This study was aimed at improving the existing knowledge and understanding of browse-browser interactions in savannas by doing field-based and nursery-based experiments which were aimed at:

- Determining the effect of browsing and seasonal differences on chemical defences (condensed tannins) of *Acacia grandicornuta* (*A. grandicornuta*) and *Combretum apiculatum* (*C. apiculatum*).
- Discovering the effects of browsing and seasonal changes on growth responses (height, stem diameter and shoot length) of *A. grandicornuta* and *C. apiculatum*.
- Establishing the effect of browsing on structural defences (thorns) of *A. grandicornuta*.
- Investigating the physiological changes (photosynthesis) of *C. apiculatum* at different seasons.
- Determining the effects browsing on growth (height, stem diameter and shoot length) in *C. apiculatum* watered and defoliated at different levels.
- Discovering the effects of browsing on chemical defences (condensed tannins) in *C. apiculatum* watered and defoliated at different levels.
- Determining resource allocation to different parts (leaves, shoot and roots) of *C. apiculatum* watered and defoliated at different levels.

It can be postulated further that increased growth and photosynthesis would be concomitant with reduced tannin concentration when water and nutrients are abundant at the beginning of the wet season. Growth in terms of shoots length, thorn length and plant height would increase in terms of increasing photosynthesis rate on browsed plants compared to unbrowsed plants. Resources like water are limiting in semi-arid systems, investigating the effects of browsing on resource allocation to different parts of the plant and discovering the growth responses in plants with less and more water is useful in proving that water is essential for plant growth and in defence mechanisms.

1.3.1 Expectations from the study

The following expectations were derived from the above mentioned hypothesis:

- The CT concentrations decrease under browsing for both species when shoot and thorn length increase.
- Growth (height, stem diameter and shoot length) increases in browsed plants of both species as a response to browsing.
- *Acacia grandicornuta* thorns become bigger where browsing takes place as a response to browsing.
- Photosynthesis of browsed plants increases compared with unbrowsed plants for *C. apiculatum*.
- Heavily defoliated plants increase their growth (height, stem diameter and shoot length) when more water is available.
- Heavily browsed plants allocate more condensed tannins to their leaves to avoid further browsing.
- Plants with more resources (water) recover more quickly from browsing to compensate for tissue loss.

The knowledge gained from this research will be useful for building models of browse-browser interaction in seasonal subtropical zones where browsers are abundant and have potential to deplete vegetation resource and how to prevent this from happening. The future development of robust models of browse-browser interactions in savannas benefit from improved knowledge of the ecology of trees growing in savannas. Game managers and conservation sectors benefit from the knowledge gained from the study and ideas of plant responses to browsing since very little is known.

1.4 Structure of the Thesis

Chapter 1: The first chapter contains the general introduction with the problem statement, literature review and research aims. The second chapter describes the field experiment with its own introduction, methods, results and discussion, while the third chapter describes the nursery experiment.

Chapters 2 and 3 are written as separate manuscripts intended for submission to peer-reviewed journals. Therefore, there may be some repetitions and overlap between each chapter. Chapter 2 is the field experiment, which was done in the Kruger National Park and only looks at the effects of browsing on growth, chemical and physiological aspects of *A. grandicornuta* and *C. apiculatum*. Then chapter 3 is a nursery experiment, which looks at the effects of browsing as affected by water availability.

Chapter 4: The final and fourth chapter presents the conclusions and recommendations made regarding both the nursery and field experiment.

CHAPTER TWO

Effects of browsing on growth, structure and physiological aspects of *Acacia grandicornuta* and *Combretum apiculatum.*

2.1 Introduction

Plants and herbivores comprise a very high percentage of organisms on the planet (> 50 %) and their interactions have led to the creation of an interesting variety of plant defences and plant responses to browsing (Coley *et al.* 2005). Plant defences have been the subject of interest for many decades and further research is still required to understand the ecological and evolutionary processes involved (Herms and Mattson 1992). Previous research has shown that plants are able to live and survive in areas with a high number of herbivores. Plants are browsed by different types of herbivores and can resist and recover from high herbivory effects. The tissues of many plants have the quality to reduce herbivory through low nitrogen concentrations, low moisture content, and toxin or chemical compounds (Hanley *et al.* 2007).

When plants are being browsed by herbivores, they defend themselves and different plants respond differently to herbivory. Plant responses to herbivory can involve chemical (e.g. tannins), physical (thorns) as well as growth responses (photosynthesis, plant height and shoot length) that reduce herbivory or help plants to recover after the impact of herbivory. These types of responses which are produced after the utilization by herbivores can be grouped into (a) plant parts which reduce the impact or the performance of herbivores (spines and thorns) (b) regrowth traits such as compensation for tissue loss which reduce the damage to the plant after herbivore damage has been done, and (c) phenological responses which reduce the accessibility of plant parts to herbivores when they are most harmful (Fornara and du Toit 2007).

The focus of this study was on growth (height and shoot length), structural (thorns), physiological (photosynthesis) and chemical responses (condensed tannins) of plants after they have been utilized by herbivores. This does not suggest that plants will not grow if they are not being eaten but after browsing or removal of the shoot, growth is stimulated through the reduction of the apical dominance (Paige 1992). One mechanism of plants to avoid herbivory is to grow tall and escape from herbivory. Plants grow too tall and raise their leaves to get sufficient sunlight but herbivory is one of the main and important reasons for such fast growth in plants which have been browsed (Paige 1992).

Structural defences such as thorns and spines are thought to be defence mechanisms because they are induced after herbivory utilization and are found in shoots which have been browsed before (Ward and Young 2002). This has been proven by looking at spine length in areas with and without herbivores and also by comparing shoots which are within browsing height (lower in the canopy) and those shoots which are out of reach from herbivores (higher up in the canopy). It was found that shoots which are higher up in the plant had short or no spines defending their shoots and those shoots which were within browsing height had higher biomass and longer spine length (Ward and Young 2002).

Attention has been focused on chemical defences which are produced by plants after herbivory. Chemical defences are compounds which are contained in some tissues of the plants and affect digestion in the gastro-intestinal tract. These chemical defences have a toxin effect after they are absorbed by herbivores (Craine *et al.* 2003). The chemical defences of interest are the condensed tannins (CT) which form part of the compounds which are nitrogen (N) free compounds. CT is an important defence mechanism for some plants since they reduce the availability of proteins and other nutrients and deactivate the digestive enzymes of herbivores. Herbivores prefer plants which are spineless and where they can take large shoot bites in less time (Young *et al.* 2003). The removal of plant biomass is costly to a plant and herbivory could be a major constraint on plant growth and plant defences use resources which can be used for growth and reproduction (Craine *et al.* 2003).

After browsing, growth is likely to occur and in order for the plant to grow, the rate of photosynthesis must increase. Plants which have been browsed are likely to have a higher photosynthetic rate than unbrowsed plants. This results in plants replacing lost tissue by compensation growth (Gonzales *et al.* 2008). Photosynthesis is an important factor when it comes to growth (height and shoot length) since growth is one of the major strategies that are employed by plants. As a result plants can survive and continue to grow in areas with herbivores because they have ways of interacting and coping with herbivory but still provide herbivores with sufficient food while trying to avoid them (Hanley *et al.* 2007).

This study was aimed at understanding how herbivores affect plants and how plants respond through growth, chemical, structural and physiological changes. The following predictions concerning plant responses to browsing were made (1) tree species decrease condensed tannins under browsing in the beginning of the growing season and increase condensed tannins at the end of the growing season (2) growth (height, stem diameter and shoot length) increases in browsed plants in the beginning of the growing season as a response to browsing and cease towards end of the growing season (3) *Acacia grandicornuta* thorns become bigger where browsing takes place as a response to browsing (4) photosynthesis increases in browsed plants compared with unbrowsed plants for *Combretum apiculatum*.

2.2 Materials and Methods

2.2.1 Study Area

The study was conducted in the Kruger National Park (KNP) which is roughly bisected in terms of geology and climate, where the western half is on granite geology and the eastern half is on basalt with the southern section being relatively wetter than the north section. The vegetation in the area is classified as sub-arid and arid wooded savannas. These different, contrasting geological and rainfall areas have been classified into land systems according to geological, geomorphologic and climatic features. The distribution of herbivores in the area is determined by the variation in these features, where the eastern part has higher herbivore densities, than the western part (Grant *et al.* 2006).

The Nkuhlu Exclosures, located between latitude 24 ° 58 'South, 31 ° 46 'East, and 30 kilometers (km) east of Skukuza, were designed to determine the effects of fire, elephants and other herbivores on the vegetation. They were erected in 2001 and include two exclosures (25-30 ha each). Most research to date has been restricted to opportunistic, short term studies of the effects of unplanned treatments in areas with unknown history of browsing. The Nkuhlu experimental exclosures are in the thicket of the Sabie River, consisting of dense woody vegetation characterized by *Acacia nigrescens* and *Combretum apiculatum* (Gertenbach 1983). The exclosures are designed so that there are three broad levels of browsing pressures: no mammal herbivores excluded (non exclosure), elephants, and giraffe excluded (partial exclosure) (Figure 2.1) and all mammal herbivores bigger than hares excluded (full exclosure) (Figure 2.2). The exclosures extend from the river up a catena. Apart from a riparian zone, there is distinct vegetation on the footslope compared to the midslope and crest (O'Keefe and Alard 2002)). For further details on landscape and catenal vegetation pattern, refer to Gertenbach (1983).

Acacia grandicornuta and *Euclea divinorum* are common in the 200-300 m wide zone of deep clay (sodic) soils on the footslope, while *Acacia exuvialis, C. apiculatum, Grewia flavescens* and *Dichrostachys cinerea* are common on the shallow sandy soils of the rest of the catena. Mammal herbivores, especially impala concentrate on the footslope. The result was that *A. grandicornuta* trees are permanently defoliated below 1.5 m. For 150 m upslope of the sodic vegetation, defoliation below 1.5 m was clearly evident, while for the rest of the catena very little defoliation by impala was evident. For further details on the exclosures, see http:// w ww.sanparks.org/parks/kruger/conservation/scientific/exclosures/exclosure-field-manual.pdf.

Figure 2.1: The partial exclosure which excludes giraffes and elephants.

Figure 2.2: The full exclosure which excludes all mammal herbivores.

The rainfall patterns during the time of sampling are illustrated in Figure 2.3 showing precipitation in Skukuza. Open bars show long term monthly mean calculated from data of 47 years of data and shaded bars are values for each month during July 2007 to June 2008. The graph shows that the year of sampling was a relatively dry year in comparison to the long term average (Figure 2.3). Long term average rainfall for Skukuza was 553 mm and the total rainfall for the study period was 453 mm. The maximum and minimum temperatures at Skukuza during the sampling period were 30.5 ºC and 14.5 ºC (South African Weather Service 2008).

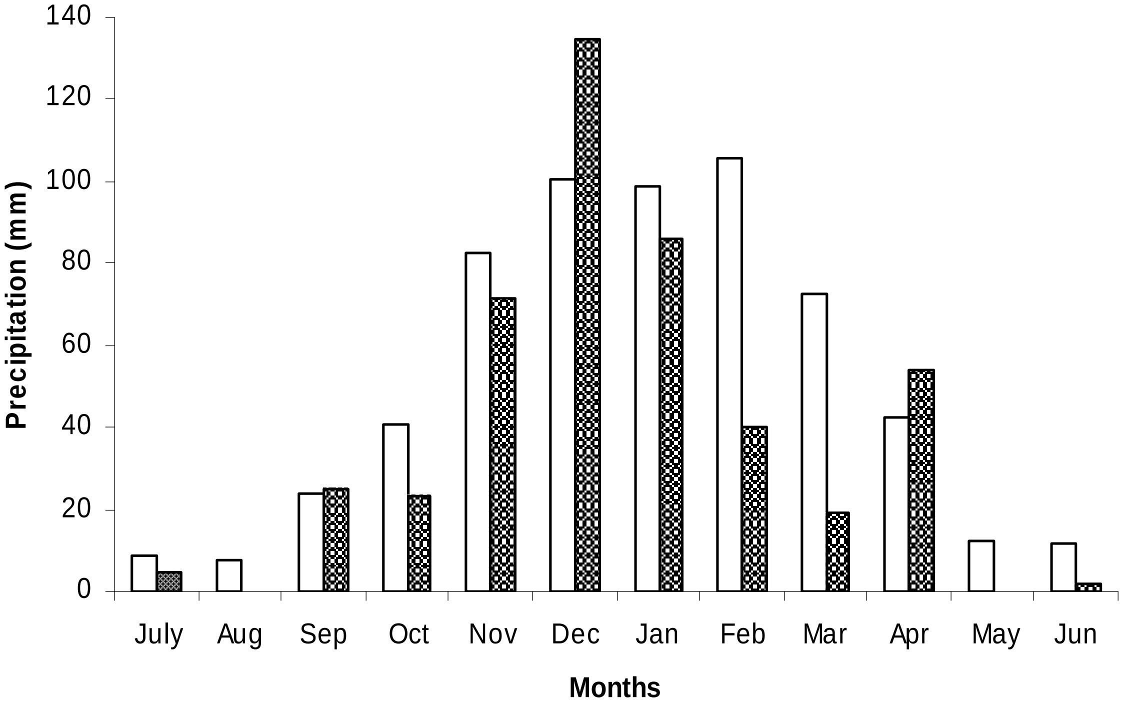

Figure 2.3: Precipitation in Skukuza, open bars show long term monthly mean calculated from 47 years of data and shaded bars are values for each month during sampling period (July 2007- June 2008).

2.2.2 Study Species

The two species (*Acacia grandicornuta* and *Combretum apiculatum*) were chosen because these were the most abundant trees and there were enough trees to obtain a sufficient sample size. The high number of these trees allowed intensive sampling for the purpose of the research. *Acacia grandicornuta* (Figure 2.4), also known as Horned Thorn is a medium sized, spinescent tree with small fine leaves. This plant occurs in drainage lines in gravelly open plains and riverine valleys and can form thicket on clay rich soils in dry areas. Leaves and pods of this species are browsed by game animals, while seeds are eaten by monkeys and baboons (Pooley 1993)

A. grandicornuta is one of the species that normally shows the impacts of Impala browsing and hence it is an important food source for Impala and is likely to show response to browsing. *A. grandicornuta* only grows on the foot slope of the study area. *Acacia* species form important components of bush encroachment and browse production in Africa, and are the most studied woody plants in savannas (Scogings and Mopipi 2008). Therefore, the future development of robust, management-oriented models of browse-browser interactions in savannas, whether for conservation, bush control or animal production, would benefit from improved knowledge of the ecology of *Acacia* trees (Scogings and Mopipi 2008).

Figure 2.4: An example of *A. grandicornuta* sampled in all the treatments between the heights 0.7- 1.7 m.

Combretum apiculatum (Figure 2.5), known as Red Bushwillow is a semi-deciduous, broad leaved, non spinescent tree that grows in the crest of the study area. This species often occurs on sandy to rocky soils in dry open woodland in areas of low rainfall to semi-arid conditions and prefers areas with less than 600 mm of rain per year. *C. apiculatum* is a useful fodder tree for domestic stock and game animals. The leaves are browsed by game animals including elephant, giraffe, eland, kudu, bushbuck, impala, klipspringer and steenbok and may show impact to browsing. The leaves are also browsed by cattle and are used medicinally. The tree is usually the dominant species in the crest (Venter and Venter 1996).

Figure 2.5: Broad leaved species *C. apiculatum*, showing its four wing shaped fruits.

2.2.3 Research Activities

If selection pressure by herbivores has led to the evolution of plant defences, assuming that defences are costly, they should be more abundant and effective in plant parts which are more accessible to herbivores. Herbivores feeding from the ground are more restricted in feeding height than insects which have access to all parts of the plant. Juvenile trees are more accessed by herbivores and are expected to be more vulnerable to browsing and are more defended than tall trees (Rooke *et al.* 2004a). During the time of sampling, it was discovered that there was uneven browsing. The lower half of the slope was more highly utilized by impala than the top of the crest. Impala dung was abundant near *A. grandicornuta* trees and these trees often had browse lines. The focus was on the high impact zone where most of the browsing took place. Short trees between the heights 0.7-1.7 meters (m) were sampled because they could be reached by all herbivores.

Estimation of (a) structural and chemical traits and (b) growth rates and physiological parameters of two species (*A. grandicornuta* and *C. apiculatum)* were measured during the beginning and middle of the wet season. The measurements were taken at the beginning of the wet season since more water and nutrients available for growth and less water and nutrients are available for growth in the middle of the wet season. This was done to detect the effects when they occur (e.g. late in summer) and not at another time (e.g. early summer). Forage quality may be affected later in the summer because resources are relatively limited for growth, but not photosynthesis. Growth rates and photosynthesis were estimated to describe physiological states of plants at times of sampling for chemical analysis.

2. 3 Data Collection

2.3.1 Growth Rate Measurements

A sampling strategy developed during a pilot study which forms part of a broader project that started in 2006 was used. Trees from *C. apiculatum* and *A. grandicornuta* were located inside and outside the exclosures by randomly selecting 30×30m cells from a grid on a LIDAR image of the experimental site (Figure 2.6). Samples of *C. apiculatum* were sampled in the crest and *A. grandicornuta* was sampled in the footslope. These random points were entered into a Global Positioning System (GPS) which was then used to navigate to the centre of each grid cell from where the closest short (0.7-1.7 m) trees of each species was marked. Trees that were either on termite mounds, shaded or deformed were excluded.

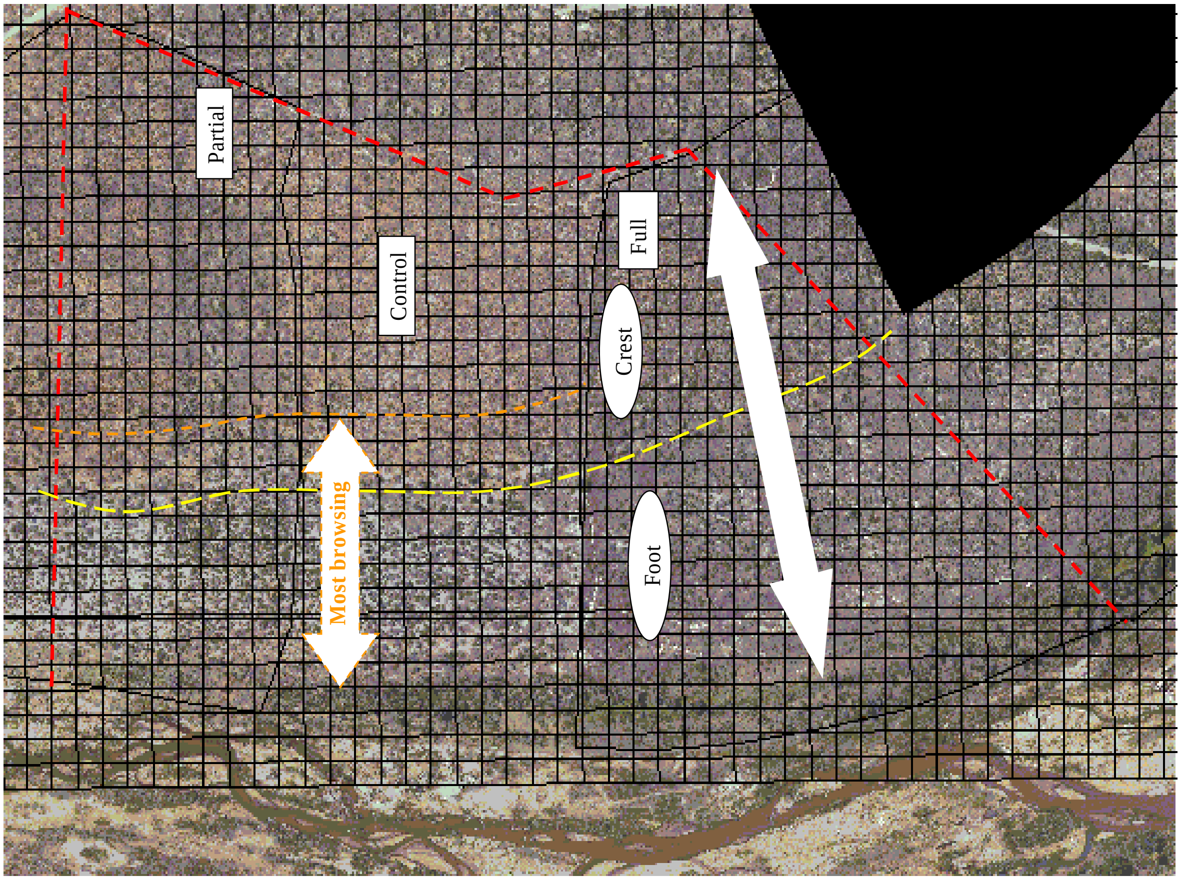

Figure 2.6: Arial View of the exclosures showing the grid cells which were randomly selected for all the measurements on the footslope and crest.

The heights and stem circumference at 10 centimeters (cm) of ten trees of each species per treatment (20 species per exclosure) were measured in the first week of September 2007 to make sure that the trees were within the range of 0.7-1.7 meters (m) (Table 2.1). These trees were used to measure growth on both species and thorn lengths for *A. grandicornuta* since it had thorns. Five new shoots per tree were marked using 'tippex' and a red and white plastic strip during the third week of October 2007 so the shoots could be easily identified to monitor their growth. Five shoots per species per treatment were measured in the beginning of the growing season (third week of October 2007) since this was 2-3 weeks after the first rain and high growth rate was assumed. Shoot length was measured using a caliper (Figure 2.7) twice in October 2007(third and fourth week), second week of November 2007, and first week of December 2007 (Table 2.1). No shoots were measured from *A. grandicornuta* and *C. apiculatum* in March 2008 since most were damaged. In the full exclosure, the shoots were browsed by insects so no measurements were done for both species.

Five longest thorns were randomly selected from the marked shoots of *A. grandicornuta* to see if the thorn length was affected by browsing. Thorns were measured in the second week of November 2007 and in the first week of December 2007 (Table 2.1). No thorn lengths were recorded in 2008 since no shoots could be found for *A. grandicornuta* because all the shoots were browsed. A caliper was also used to measure thorn lengths in all the treatments. The height and stem circumference at 10 cm of all the trees in all the treatments were re-measured in the third week of March 2008 to identify any growth (Table 2.1).

Figure 2.7: A caliper was used to measure growth of all the marked shoots for both species in all the treatments.

2.3.2. Photosynthesis Measurements

A/Ci curve (net CO_2 assimilation rate versus calculated internal CO_2 concentrations, *Ci*) analysis is a useful tool and is used to estimate leaf photosynthesis under different conditions. *A/Ci* response curves are straightforward to conduct, quick, and easy to interpret. The *A/Ci* curves have been successfully used around the world to estimate and calculate the rate of photosynthesis and some assumptions have been made regarding the curves. It has been assumed that the rate of carboxylation is limited by one of the three different processes: (i) the total activity and kinetics of Rubisco (Wc), (ii) the time taken for ribulose-1,5-bisphosphate regeneration (Wj), and (iii) availability of occasionally, triose phosphate (Manter and Kerrigan 2004).

A/Ci curves are used to estimate leaf photosynthesis and the curves require the *Ci* value and this was when the *A/Ci* curves change between the Rubisco and electron transport (*Ci-t*). The assumptions that have been made from this are that the common values of *Ci-t* which are used for the analysis will range between 20-25 Pa and the Wc process will take place at the lowest *Ci* value (Manter and Kerrigan 2004). Another assumption was that in the *A*/*Ci* curve analysis, *Ci* is nearly equal to that of the catalytic site of Rubisco (*Cc*). Using *A*/*Ci* curves to estimate photosynthesis rate can also have some weaknesses and the results could be accurate and sometimes they may not be accurate (Manter and Kerrigan 2004).

Photosynthesis was measured for sub-samples of 12 plants around the same time that shoot measurements were recorded. The measurements were taken on six short trees from both the non and full exclosure from November 2007 to March 2008. Random points were located with the GPS and the GPS was used to navigate to the center of each cell where the closest undamaged short tree was measured. Only *C. apiculatum* was used for photosynthesis measurements since it is a broad leaved plant. Photosynthetic measurements were done only in the full and non exclosure to compare the differences in the photosynthetic rate of trees which were browsed (non exclosure) and not browsed (full exclosure). By comparing the two treatments, we can compare rates of growth by comparing photosynthesis in browsed and non browsed plants.

Photosynthetic measurements were only recorded on sunny days because light influence the rate of photosynthesis. Photosynthesis measurements were taken before 09:00am because after 09:00am it was too hot and the stomata were closed therefore affecting the rate of photosynthesis. Three young, fully expanded leaves from undamaged trees were used to measure photosynthesis in both treatments. Measurements of *A*/*Ci* response curves were performed from the 13-14 November 2007 and 28-30 November 2007 in both treatments. The photosynthesis measurements were repeated again from the 6-13 February 2008 (Table 2.1).

The last measurements were done on 20 and 21 March 2008 in the full and non exclosure using a Li-Cor 6400 portable photosynthesis system (Figure 2.8). Methods on how to download and analyse the data are defined in Appendix 1.

Figure 2.8: Li-Cor 6400 portable photosynthesis system was used to estimate photosynthesis on seedling of *C. apiculatum* in the full and non exclosure.

The gas exchange system was zeroed daily using anhydrous calcium carbonate (Drierite) to remove water and soda lime (Sofno lime granules) was used to remove CO_2 from the air entering the cuvette. The CO_2 cartridge was changed every day. Leaf temperatures were set at 30 ºC for all measurements. Leaves were illuminated using a red-blue light source attached to the gas-exchange system and photosynthetically active photon flux density (*Q)* was maintained at 1200µmol m^{-2} s^{-1}.

The flow rate was set at 400 µmol s^{-1}. The leaf area was set according to the size of the leaves of the tree, between 2 and 6, and the stomata ratio was set at 0.5 for all the measurements. Measurements of assimilation were made starting at 400 µmol mol^{-1} CO_2 surrounding the leaf, decreasing stepwise to 50 µmol mol^{-1} , returning to 400 µmol mol^{-1}, and increasing stepwise to 800 µmol mol^{-1} CO_2 and measurements were recorded after equilibration to a steady state. Leakages of CO_2 into and out of the empty cuvette were determined at each reference concentration value used to correct measured leaf fluxes with the equation provided in the Li-Cor operator's manual.

After the Li-Cor 6400 portable photosynthesis system was set accordingly, the leaf was chosen and clamped. Each leaf was recorded for 15 minutes and each tree was recorded for 45 minutes meaning that 3 leaves were used from one tree. The Li-Cor 6400 portable photosynthesis system was set so that it could record all the measurements and these values were downloaded. The *A/Ci* curves were used to estimate V_{cmax} (maximum Rubisco activity), J_{max} (maximum electron transport capacity), based on the equations of Farquhar *et al*. (1980). Values for *A* and *Ci* were calculated and were used to solve for V_{cmax} and J_{max} using the equations of Farquhar *et al.* (1980).

2.3.3 Chemical Traits

Leaves were sampled from 197 plants for chemical analysis during October 2007 to March 2008 by randomly locating points with the GPS. Leaves were collected from *A. grandicornuta* in the footslope and *C. apiculatum* in the crest. Leaves were collected from 53 plants during the third and fourth week of October 2007, 41 plants in the second week of November 2007, 53 plants in the first week of February 2008, and 50 plants during the third week of March 2008 in all the treatments for both *A. grandicornuta* and *C. apiculatum*) (Table 2.1). No samples of *A. grandicornuta* were collected in November 2007 because no suitable trees were found at most of the random points. The total number of trees sampled inside and outside the exclosures for each species is described in Table 2.2. The trees which were used for leaf sampling in March 2008 were the trees used for shoot and thorn measurements.

Table 2.1: Data collection for all measurements in all the treatments from September 2007- March 2008.

Measurement	Month	Weeks	No. of days
Shoots	October 2007	Week 3-4	4 days
	November 2007	Week 2	1 day
	December 2007	Week 1	1 day
Heights	September 2007	Week 1	1 days
	March 2008	Week 3	3 days
Stem circumference	September 2007	Week 1	1 days
	March 2008	Week 3	3 days
Thorns	November 2007	Week 2	1 day
	December 2007	Week 1	1 day
Leaf collection	October 2007	Week 3-4	2 days
	November 2007	Week 2	4 days
	February 2008	Week 1	1 day
	March 2008	Week 3	3 days
Photosynthesis	November 2007	Week 2 and 4	5 days
	February 2008	Week 1-2	8 days
	March 2008	Week 3	2 days

Table 2.2: The total number of trees sampled inside and outside the exclosures from October 2007 to March 2008 for chemical analysis.

Exclosure	Species	October 07	November 07	February 08	March 08
Full exclosure	*C. apiculatum*	10	10	10	10
	A. grandicornuta	10	9	8	9
Non exclosure	*C. apiculatum*	9	8	9	8
	A. grandicornuta	8		8	10
Partial exclosure	*C. apiculatum*	6	6	8	5
	A. grandicornuta	10	8	10	8

Browsing impact was recorded on each plant before the leaf samples were taken by looking at how much had been browsed from each plant and by recording recent leaf removal from each tree, as a proportion (%) of the total volume of the tree. Browsing impact was recorded to see how much that particular tree had been browsed and this was only done in the partial and non exclosure because that was where browsing occurred. The GPS was also used to navigate to the centre of each random cell where the shortest tree was used to collect leaves from both species in all treatments. The short trees were sampled by randomly taking 30-50 leaves (5g in dry weight) from each tree. Controlling for the effects of canopy position and time of a day, fully expanded, mature leaves were removed from the northern (sunny) side of each plant's canopy.

Foliage of similar age was collected and never more than once from any tree. Care was taken not to remove too much material from the trees. The leaves were removed by picking up leaves from each tree and putting the leaves into small brown paper bags. The leaves were oven-dried at 60 ºC for 24 hours and the leaves were then stored in small plastic bags. Dried leaf samples were milled using 0.5 mm screen and analyzed for nitrogen (N), phosphorus (P) and condensed tannins (CT). Kjeldahl-N was determined according to Smith (1980). Murphy and Riley (1962) Technique was used to determine P, and condensed tannins were determined by the acid-butanol proanthocyanidin assay (Hagerman, 1995), with sorghum tannin as the standard (Appendix 2).

2.4 Data analysis

The experimental design was not replicated and this constrained statistical tests or the use of Analysis of variance (ANOVA) (Underwood 1997). The experimental design was not replicated because of logistical and technical constrains. The design of the field experiment was already set up by South African National Parks (SANParks) and it covers a large area so replication was impossible at that scale. ANOVA was therefore not done. The only option was descriptive statistics. Shoot and thorn lengths were averaged per plant and the values per plant were used to estimate means and standard errors. The P, N and CT concentrations were averaged per plant and the values were also used to estimate means and standard errors per plant. The same was done for photosynthetic parameters. The average photosynthetic rate was measured on a number of leaves per plant. The average photosynthetic rates per plant were used to estimate means and standard errors in all the plants in the treatments.

Average shoot lengths for each species were plotted against different months (October, November, and December) for all different treatments to see shoot growth. Mean thorn length for *A. grandicornuta* was plotted against November and December to see if the thorn length increased. The mean height and stem circumference recorded in October 2007 and re-measured in March 2008 in all the treatments were plotted by taking the means of the two months and these were plotted against different treatments (full, partial and non exclosure). Monthly mean P (%), N (%) and CT (%) and standard errors of each species per month for all treatments were calculated and plotted against each other to identify the monthly variation between each treatment. The correlation between leaf removal and CT content of both trees in the partial and non exclosure in March 2008 was analysed. This month was chosen because this was when more leaf removal was recorded in both species. CT concentration was plotted against leaf removal (%) to see if these two are related.

Photosynthesis was measured in the full and non exclosure only since the objective was to see if there were differences in plants which were browsed and plants which were not browsed. Mean J_{max} (maximum electron transport capacity) and V_{cmax} (maximum carboxylation rate of Rubisco) were plotted against different months (Nov, Feb and March) in the full and non exclosure. Photosynthetic measurements were not analysed due to insufficient data, not enough trees were measured so the data restricted any tests. Means were compared by looking at the graphs and comparing differences without any statistical analysis.

Systat 10 was used to analyse the data and to see if treatments were significantly different or not. The data were first checked for normality and if it was not normally distributed, the data were log transformed. Differences were considered significant if $P < 0.05$. Correlation analysis was used to check if there was any relationship between the leaf removal and CT concentration. T-tests were conducted to test differences between two treatments or months considering the data was balanced and looking at the size of the error bars. The t-test was done in those variables which had error bars not overlapping with each other since these were likely to be different from each other. The t-test was conducted on shoot lengths for both *A. grandicornuta* and *C. apiculatum* to see if there was significant growth from October to December 2007 within each treatment.

The differences in shoot length during the month of December 2007 for *C. apiculatum* was analysed using a t-test to prove the effect of the treatments. December was chosen since the shoots during this months seemed to be affected by the treatments and the shoot lengths were different from each other. The t-tests were done to see if there was significant growth in terms of heights and stem circumference from September 2007 and March 2008 for both species. The tests were also done to see variations in P, N and CT concentrations from October 2007 to March 2008 for both species in all the treatments by comparing the months. The t-tests were done to check the effects of the treatments on the P, N and CT concentrations for both species by comparing different treatments.

For *A. grandicornuta*, the effects of the treatments were analysed by comparing the P, N and CT concentrations during October 2007 in all the treatments. October was chosen because there seemed to be difference in P, N and CT concentrations between the treatments.

2.5 Results

2.5.1 Shoot and Thorn Length

There was no significant growth in shoot length of *A. grandicornuta* in all the treatments from October to December 2007 *(P*> 0.05). There was no effect of the treatments on shoot lengths of *A. grandicornuta* (*P*> 0.05) (Figure 2.9). The thorn length of *A. grandicornuta* was not significantly different in November 2007 and December 2007 (*P*> 0.05*)*. Both months and treatment had no effect on growth of thorn length of *A. grandicornuta* (*P*> 0.05) (Figure 2.10).

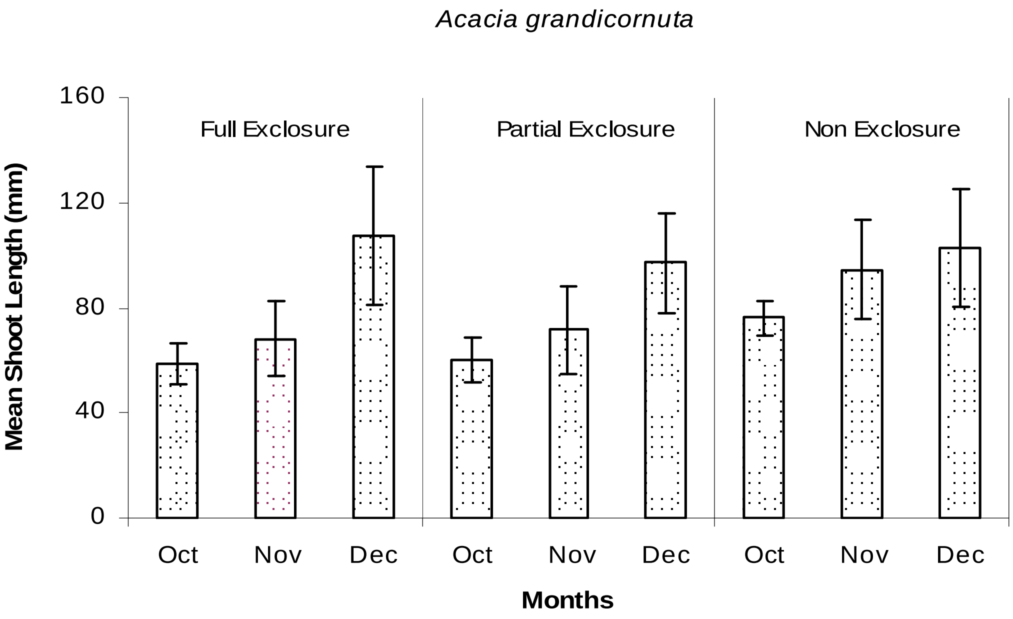

Figure 2.9: Mean Shoot Length (mm) of *A. grandicornuta* in different treatments (full, partial and non exclosure) in 2007. Error bars represent ±1 Standard Error (n=8-10).

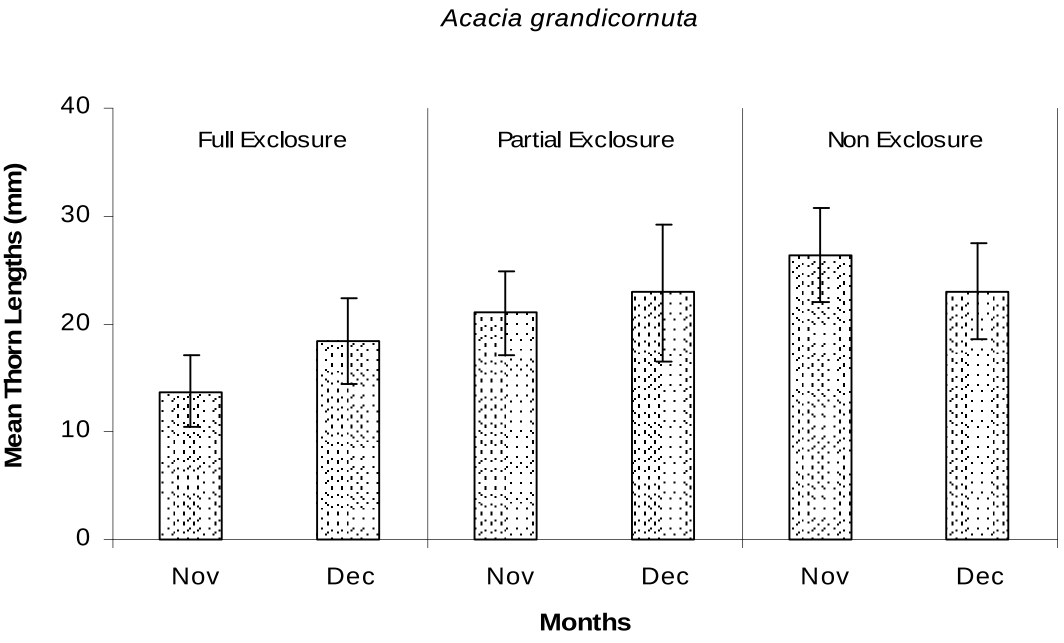

Figure 2.10: Mean Thorn Length (mm) of *A. grandicornuta* in all the treatments (full, partial and non exclosure) in 2007. Error bars represent ±1 Standard Error (n=6-10).

There was significant growth in the shoot lengths of *C. apiculatum* from October to December 2007 in all the treatments ($P < 0.05$). In the full exclosure, the shoots of *C. apiculatum* in October 2007 were significantly shorter than the shoots measured in December 2007 (t = -4.65, df = 10.20 and P = 0.001). In the non exclosure the shoot length of *C. apiculatum* in October 2007 were significantly shorter than the shoots measured in December 2007 (t = - 4.67, df = 7.90 and P = 0.002). In the partial exclosure the shoot length of *C. apiculatum* measured during October 2007 were significantly shorter than the shoots measured in December 2007 (t = - 8.86, df = 7.30 and $P < 0.001$) (Figure 2.11). There was a significant effect of the treatments on the shoot lengths of *C. apiculatum* during December 2007 ($P < 0.05$). In the full exclosure, the shoot lengths of *C. apiculatum* were significantly shorter than the shoots in the partial exclosure during December 2007 (t = - 3.99, df = 5.70 and P = 0.008). In the non exclosure, shoot lengths for *C. apiculatum* during December 2007 were significantly shorter that the shoots measured in the partial exclosure (t = -3.08, df = 6.80 and P = 0.019) (Figure 2.11).

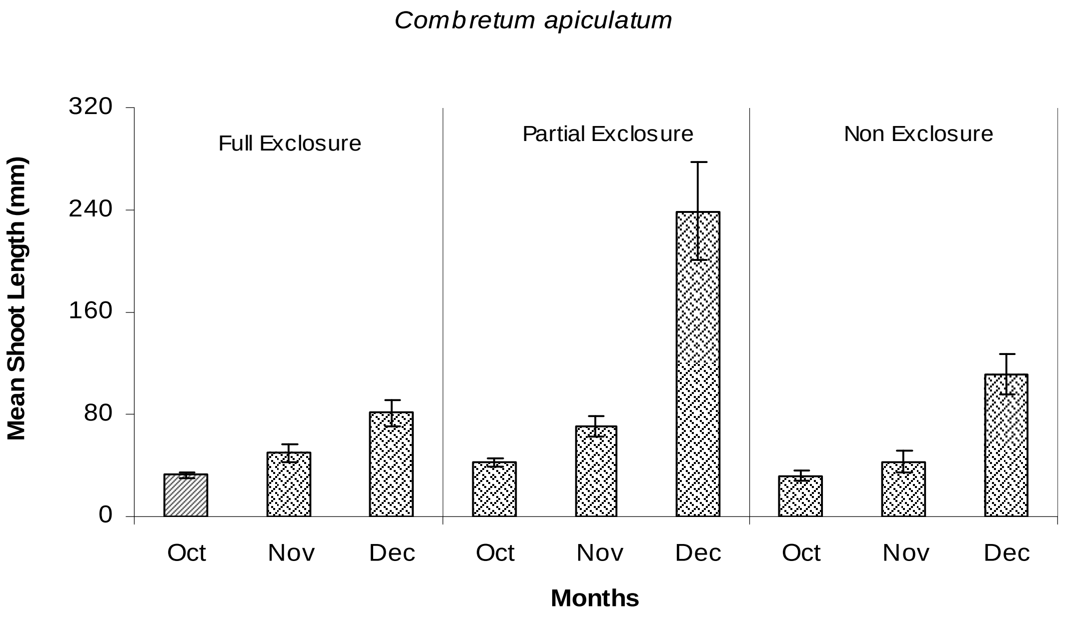

Figure 2.11: Mean Shoot Length (mm) of *C. apiculatum* in different exclosures (full, partial and non exclosure) in 2007. Error bars represent ±1 Standard Error (n=6-10).

2.5.2 Growth Measurements

2.5.2.1 Heights

There was no significant growth in heights of *A. grandicornuta* from September 2007 to March 2008 (*P*> 0.05). There was no effect of the treatment on the height of *A. grandicornuta* since there were no differences in all the treatments (*P*> 0.05). No growth was detected from all the treatments (Figure 2.12).

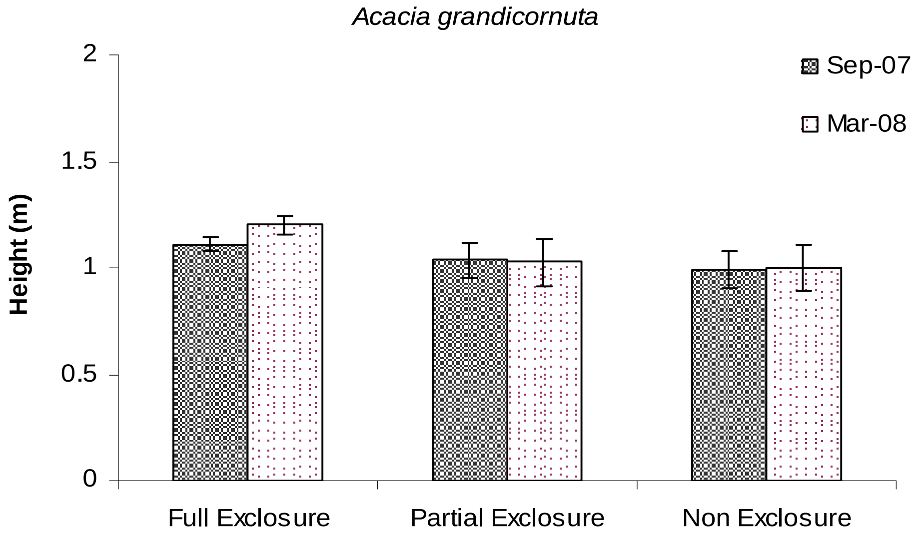

Figure 2.12: The heights of *A. grandicornuta* were measured to monitor their growth during the beginning (September) and end of the growing season (March). Error bars represent ±1 Standard Error (n=10).

There was significant growth in the height of *C. apiculatum* in the full exclosure from September 2007 and March 2008 (t =2.33, df = 17.10 and P = 0.032). No significant growth was detected in other treatments in terms of height (P> 0.05). There were no effects of the treatments in heights of *C. apiculatum* (P> 0.05) since there were no apparent differences in heights of *C. apiculatum* in different treatments (Figure 2.13).

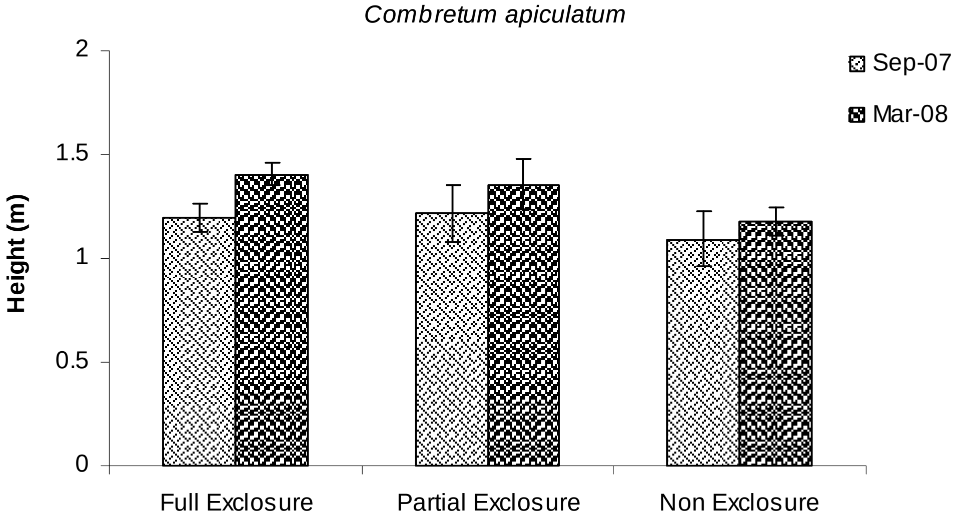

Figure 2.13: The heights of *C. apiculatum* were measured to monitor their growth during the beginning (September) and end of the growing season (March). Error bars represent ±1 Standard Error (n=10).

2.5.2.2 Stem Circumference

There were no apparent effects of the treatment for both species and no significant differences in stem circumference for *A. grandicornuta* in September 2007 and March 2008 (P> 0.05) (Figure 2.14).

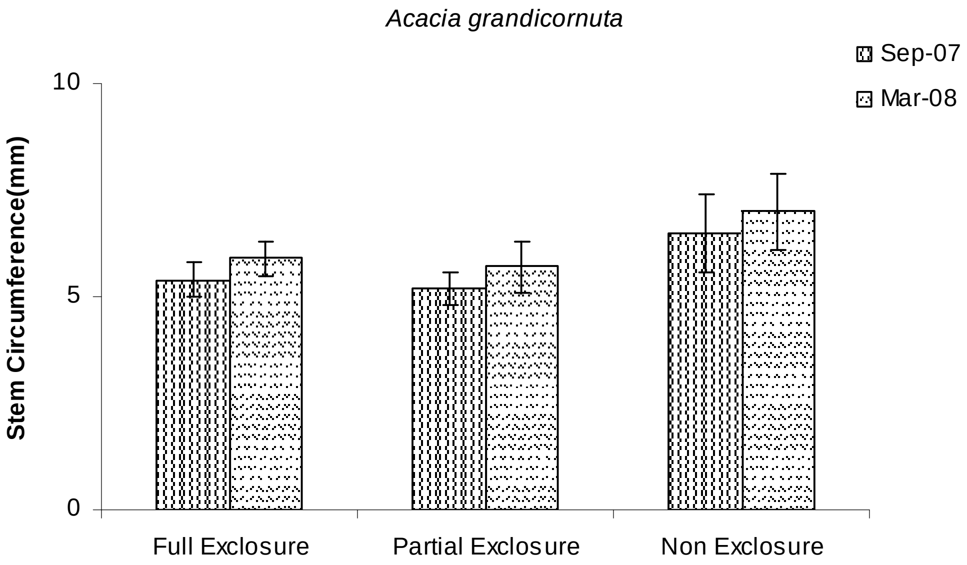

Figure 2.14: The stem circumference of *A. grandicornuta* was measured to monitor their growth during the beginning (September) and end of the growing season (March). Error bars represent ±1 Standard Error (n=10).

Stem circumference data for March 2008 was not normally distributed and were log transformed. The stem circumference of *C. apiculatum* during September 2007 was significantly smaller than the stem circumference measured in March 2008 in the partial exclosure (t =3.71, df =8.00 and P = 0.006) (Figure 2.15). There was no significant difference in stem circumferences in other treatments (P> 0.05).

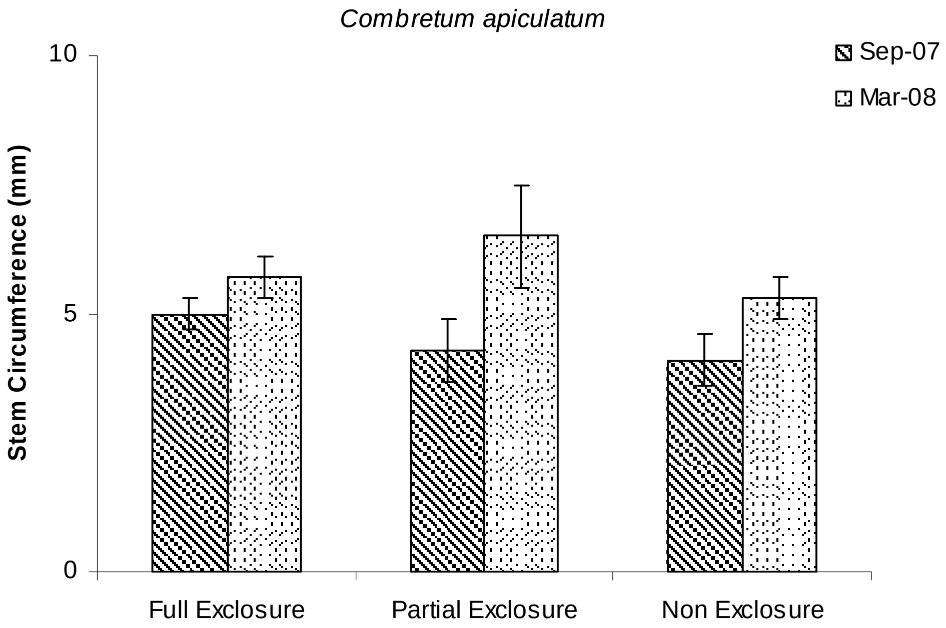

Figure 2.15: The stem circumference of *C. apiculatum* was measured to monitor their growth during the beginning (September) and end of the growing season (March). Error bars represent ±1 Standard Error (n=10).

2.5.3 Chemical concentrations in the leaves

Comparative data on the chemical components of the two species are presented in Table 2.3.

2.5.3.1 Phosphorus concentration in the leaves

Phosphorus concentration in the full exclosure for *A. grandicornuta* significantly decreased from October 2007 to March 2008 (t = -7.14, df = 12.80 and $P < 0.001$) (Figure 2.16). In the non exclosure P concentration significantly decreased from October 2007 to March 2008 for *A. grandicornuta* (t = -4.48, df = 11.30 and P = 0.001). In the partial exclosure the P concentration significantly decreased as well for *A. grandicornuta* (t = -6.70, df = 12.80 and $P < 0.001$) (Figure 2.16). The pattern for *A. grandicornuta* in all the treatments was a decreasing P concentration from October 2007 to March 2008 (Figure 2.16).

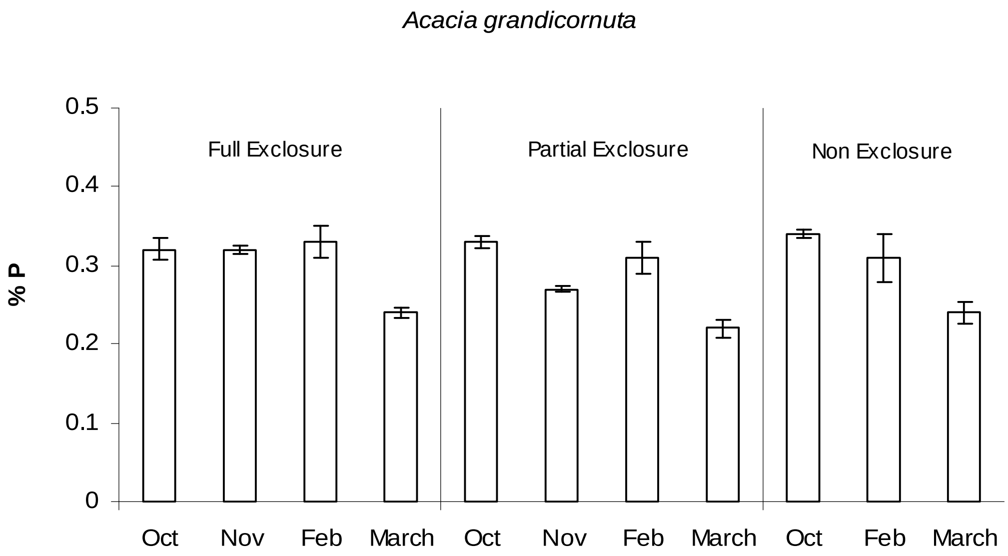

Figure 2.16: Monthly mean of P concentration (%) in *A. grandicornuta* leaves in all the treatments (full, partial and non exclosure). Error bars represent ±1 Standard Error (n=8-10).

The concentration of P for *C. apiculatum* in the full exclosure significantly decreased from October 2007 to March 2008 (t = -8.58, df = 17.60 and P < 0.001). The *C. apiculatum* P concentration in the non exclosure decreased from October 2007 to March 2008 (t = -5.32, df = 11.70 and P < 0.001) (Figure 2.17). In the partial exclosure the P concentration for *C. apiculatum* also decreased from October 2007 to March 2009 (t = -3.62, df = 7.0 and P = 0.008). The general trend for *C. apiculatum* in all the treatments was a decreasing P concentration from October to March (Figure 2.17).

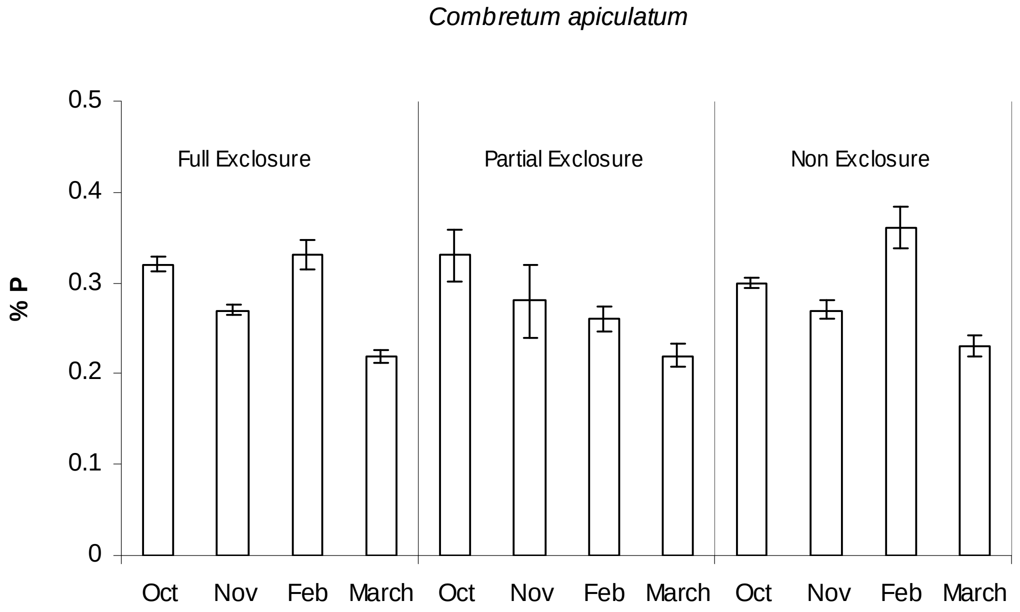

Figure 2.17: Monthly mean of P concentration (%) in *C. apiculatum* leaves in all the treatments (full, partial and non exclosure). Error bars represent ±1 Standard Error (n=5-10).

2.5.3.2 Nitrogen concentration in the leaves

The N concentration for *A. grandicornuta* in the full exclosure decreased significantly from October 2007 to March 2008 (t = -5.80, df = 16.10 and $P < 0.001$) (Figure 2.18). In the non exclosure the N concentration also decreased from October 2007 to March 2008 for *A. grandicornuta* (t = -3.87, df = 15.30 and P = 0.001) (Figure 2.18). In the partial exclosure no change in the N concentration was detected for *A. grandicornuta*, hence N remained the same (Figure 2.18). There was an effect of the treatments on N concentration for *A. grandicornuta* during October 2007. In the full exclosure N concentration during October 2007 was significantly higher than the N concentration in the partial exclosure (t = 3.79, df = 17.40 and P = 0.001) (Figure 2.18). The partial exclosure during October 2007 for *A. grandicornuta* had lower N concentrations than the non exclosure (t = 4.21, df = 13.80 and P = 0.001) (Figure 2.18).

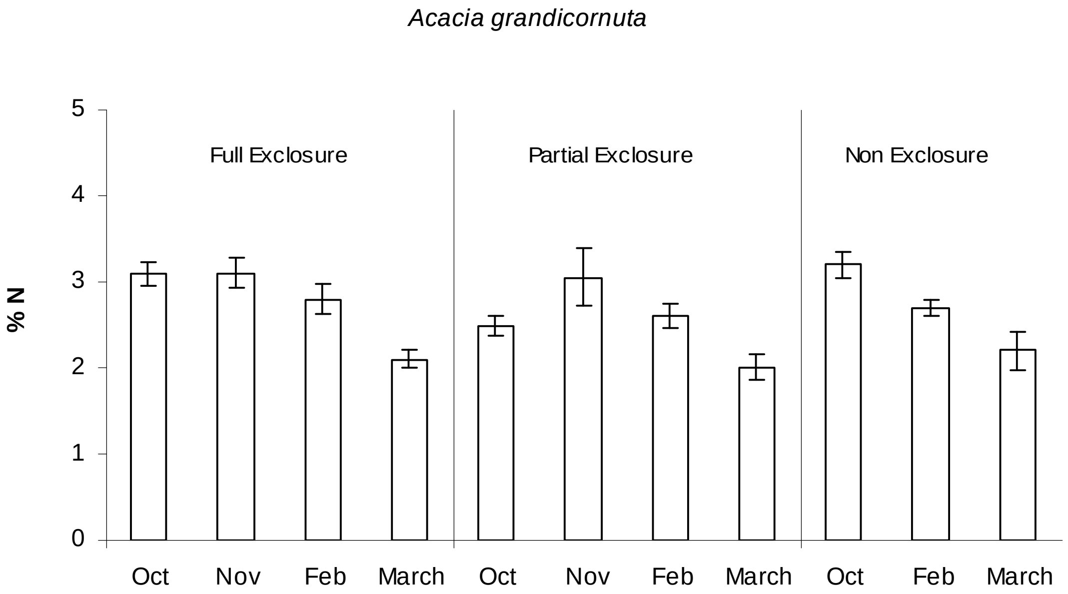

Figure 2.18: Monthly mean of N concentration (%) in *A. grandicornuta* leaves in all the treatments (full, partial and non exclosure). Error bars represent ±1 Standard Error (n=8-10).

In the full exclosure, N concentration for *C. apiculatum* decreased significantly from October 2007 to March 2008 (t = -7.96, df = 11.10 and $P < 0.001$) (Figure 2.19). The N concentration in the non exclosure for *C. apiculatum* decrease significantly from October 2007 to March 2008 (t = -6.78, df = 15.00 and $P < 0.001$) (Figure 2.19). In the partial exclosure N concentration for *C. apiculatum* decreased significantly from October 2007 to March 2008 (t = -7.27, df = 7.10 and $P < 0.001$) (Figure 2.19). The general pattern for *C. apiculatum* in all the treatments was a decreasing N concentration from October to March (Figure 2.19). There was no effect of the exclosures that was detected in both species since there were no differences in P and N concentrations in all the treatments ($P > 0.05$). There was an effect of the months since there were differences in P and N concentration from October to March in all the treatments ($P > 0.05$) (Figure 2.19).

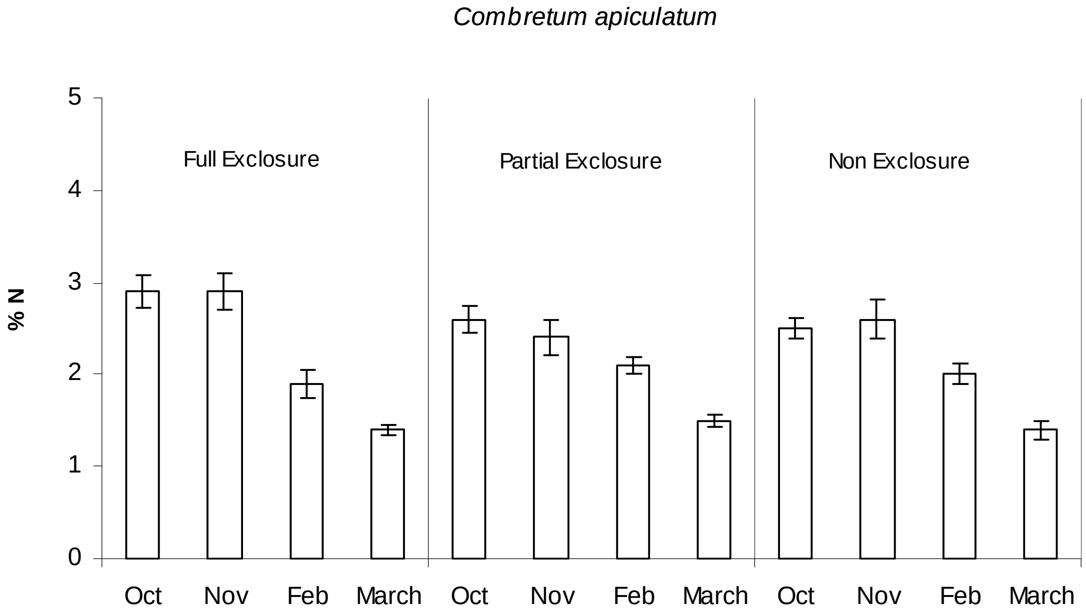

Figure 2.19: Monthly mean of N concentration (%) in *C. apiculatum* leaves in all the treatments (full, partial and non exclosure). Error bars represent ±1 Standard Error (n=5-10).

2.5.3.3 Condensed Tannin concentration in the leaves

The CT concentration remained the same for *A. grandicornuta* in all the treatments and in all the months ($P > 0.05$) (Figure 2.20). The CT concentration of *C. apiculatum* in the full exclosure increased significantly from October 2007 to March 2008 ($t = -7.08$, df = 10.60 and $P < 0.001$) (Figure 2.21). In the non exclosure the CT concentration for *C. apiculatum* significantly increased from October 2007 to March 2008 ($t = -5.34$, df = 8.00 and $P = 0.001$) (Figure 2.21). In the partial exclosure, there were no differences in CT concentration for *C. apiculatum* from October 2007 to March 2008.

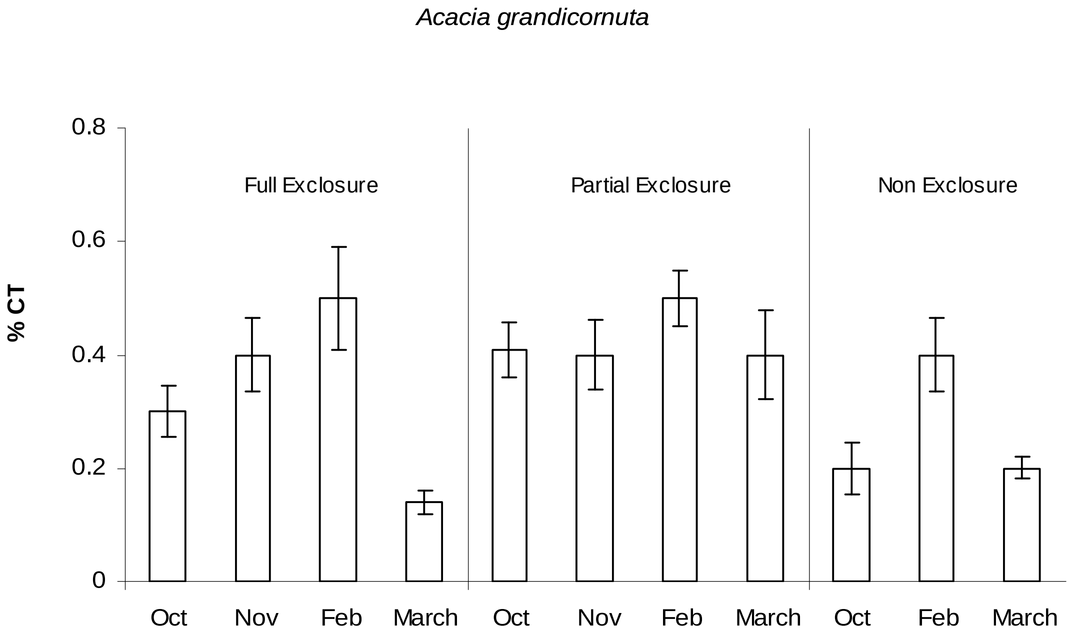

Figure 2.20: Monthly mean of CT concentration (%) in *A. grandicornuta* leaves in all the treatments (full, partial and non exclosure). Error bars represent ±1 Standard Error (n=8-10).

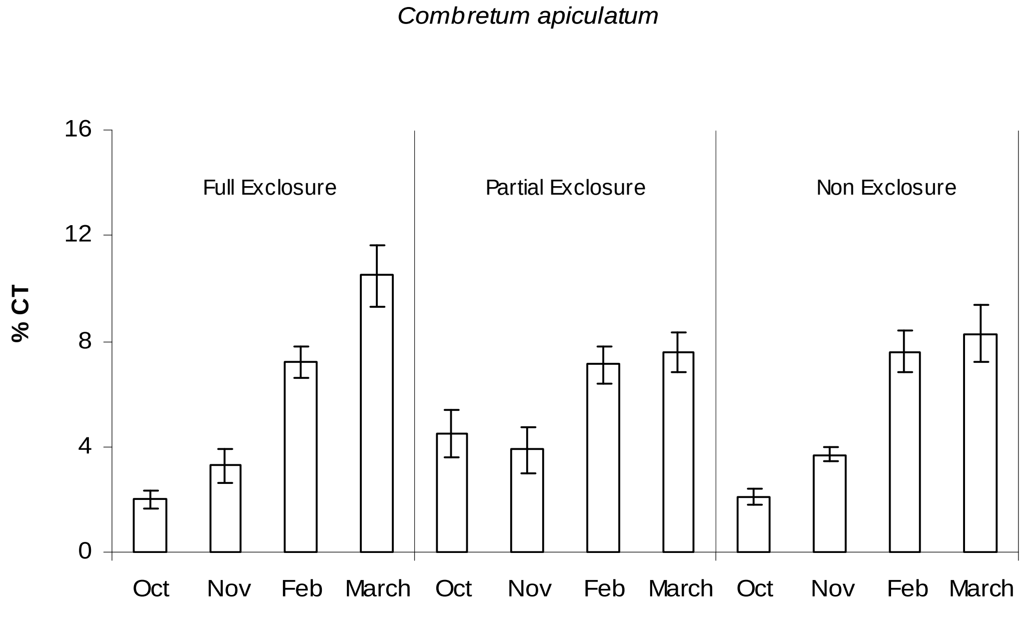

Figure 2.21: Monthly mean of CT concentration (%) in *C. apiculatum* leaves in all the treatments (full, partial and non exclosure). Error bars represent ±1 Standard Error (n=5-10).

Table 2.3: Chemical concentrations in leaves collected from all the exclosures during October 2007 to March 2008.

			P concentration		**N concentration**		**CT concentration**	
Species	**Exclosure**	**n**	**Mean**	**Standard Error**	**Mean**	**Standard Error**	**Mean**	**Standard Error**
C. apiculatum	Full exclosure	40	0.29	±0.009	2.25	±0.146	6.38	±0.678
	Non exclosure	34	0.30	±0.013	2.11	±0.134	5.81	±0.610
	Partial Exclosure	25	0.30	±0.024	2.19	±0.124	5.80	±0.823
A. grandicornuta	Full exclosure	36	0.28	±0.012	2.79	±0.145	0.32	±0.055
	Non exclosure	26	0.29	±0.017	2.65	±0.157	0.29	±0.044
	Partial Exclosure	36	0.30	±0.011	2.63	±0.188	0.39	±0.060

2.5.4 Relationship between leaf removal and CT in the partial and non exclosure

The leaf removal of *A. grandicornuta* was not correlated with CT concentration where ($n = 16$, $r = -0.195$ and $P = 0.469$) for the non and partial exclosure. The results show that as leaf removal increase the CT concentration was unchanged (Figure 2.22).

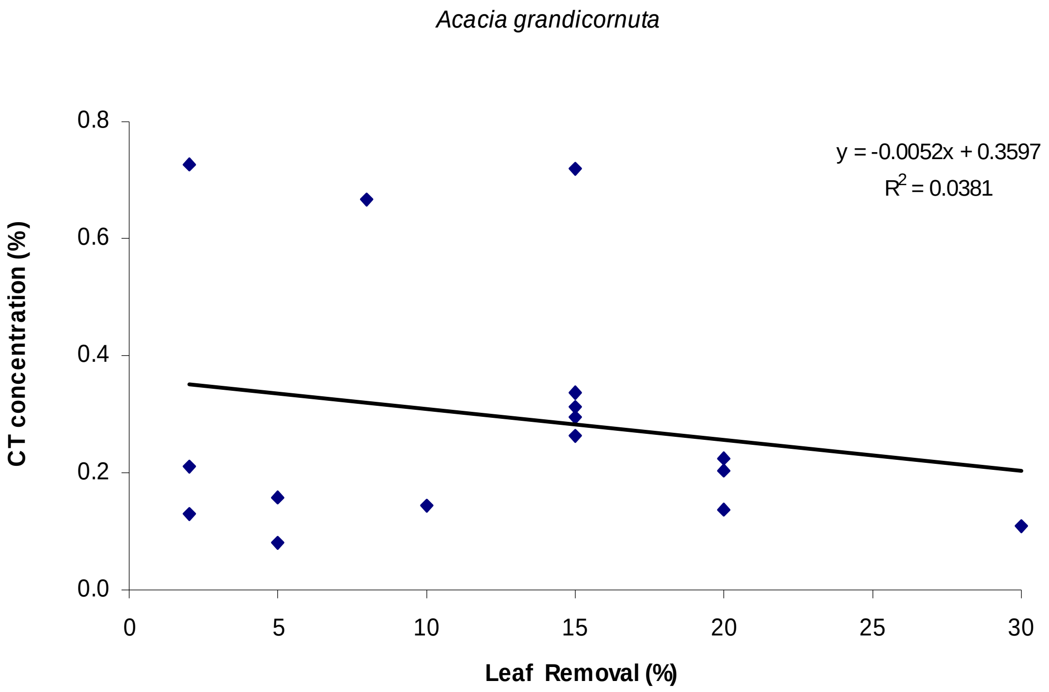

Figure 2.22: The correlation between leaf removal (%) on CT concentration (%) for *A. grandicornuta* in different months in the partial and non exclosure (n=8-10).

The leaf removal for *C. apiculatum* was not correlated with CT concentration where (*n* = 13, *r* = 0.098 and *P* = 0.817) for the non and partial exclosure. The results show that as leaf removal increases, the CT concentration for *C. apiculatum* was unaffected (Figure 2.23).

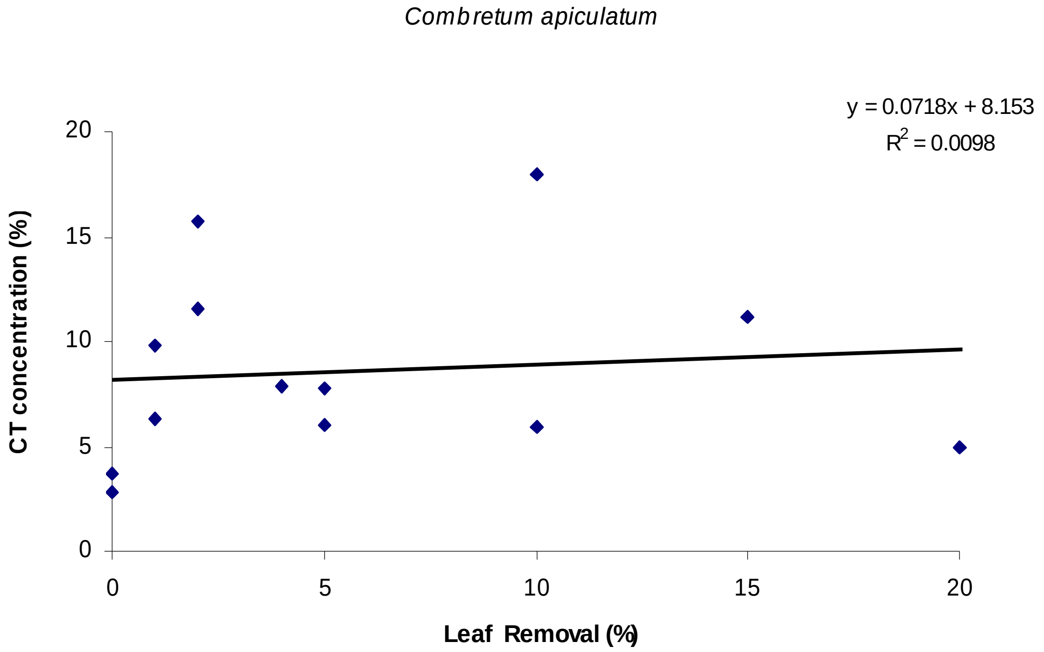

Figure 2.23: The correlation between the leaf removal (%) and CT concentration (%) for *C. apiculatum* in different months in the partial and non exclosure (n=6-10).

2.5.5 Photosynthetic Measurements

The results from the photosynthesis data of *C. apiculatum* showed a steady decrease in J_{max} from November 2007 to March 2008 where the herbivores were excluded. J_{max} in the full exclosure was highest in November 2007 compared with other months and lowest in March 2008 (Figure 2.24). The plants in the non exclosure responded differently compared with the plants in the full exclosure. J_{max} decreased from November 2007 to February 2008 but increased again during March 2008 where herbivores were not excluded. The plants in the non exclosure increased their photosynthetic rate in March 2008 and J_{max} was higher in November 2007 than in March 2008 (Figure 2.24).

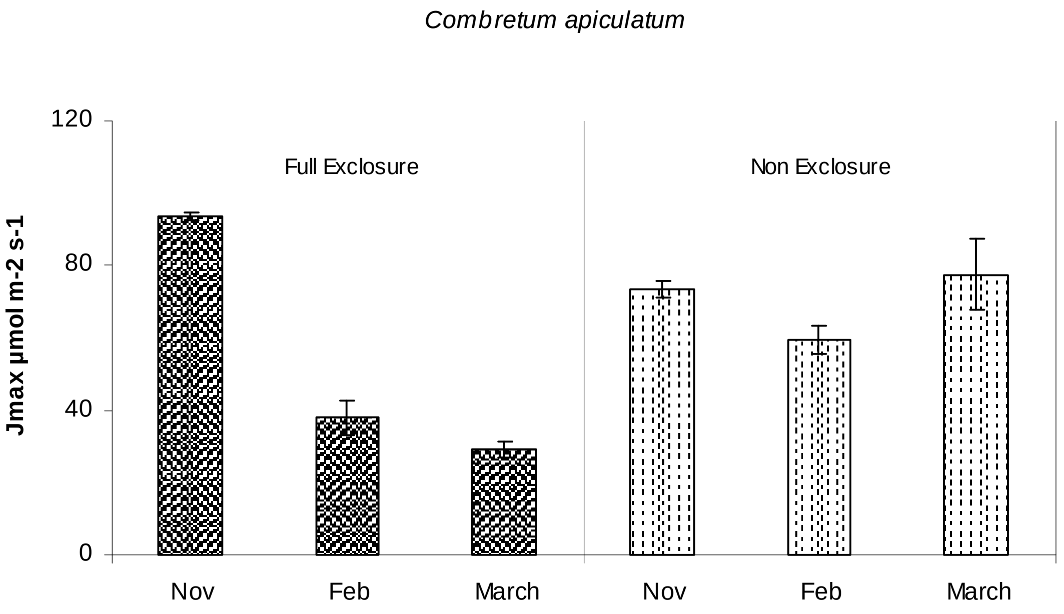

Figure 2.24: Monthly mean of photosynthetic parameters of *C. apiculatum* for J_{max} (maximum electron transport capacity) in different months taken in the non and full exclosure. Error bars represent ±1 Standard Error (n=2-6).

The results showed a decrease in the photosynthetic rate from November 2007 to March 2008 in the full exclosure for V_{cmax} (Figure 2.25). The similar pattern was followed by V_{cmax} where photosynthesis steadily decreased in the full exclosure from November until March. The *C. apiculatum* in the non exclosure responded differently where V_{cmax} was the highest in November and decreased in February and increased again in March (Figure 2.25). In the full exclosure V_{cmax} and J_{max} appeared to be higher in November and lower in March. From November to February V_{cmax} and J_{max} decreased but increased again in March in the non exclosure. The t-tests were not done since data were not balanced. Both treatments had an effect on photosynthetic rate where the full and non exclosure were different from each other (Figure 2.24 & 2.25).

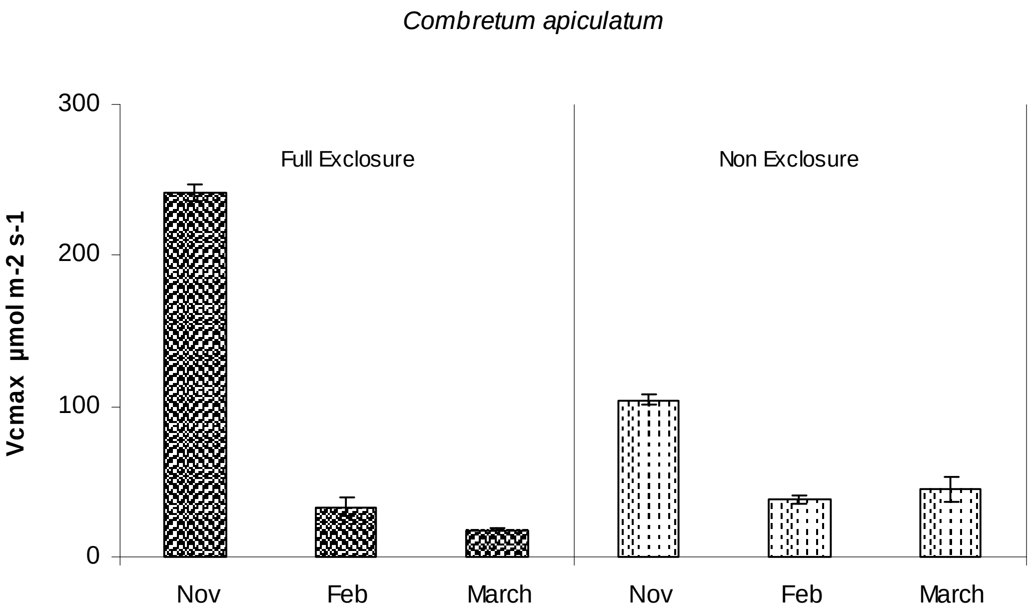

Figure 2.25: Monthly mean of photosynthetic parameters of *C. apiculatum* for V_{cmax} (maximum carboxylation rate of Rubisco) in different months taken in the non and full exclosure. Error bars represent ±1 Standard Error (n=2-6).

2.6 Discussion

2.6.1 Effects of browsing on growth

The unexpected results from the data showed no difference in the growth of shoot length in *A. grandicornuta* for both browsed (non exclosure) and unbrowsed plants (full exclosure). The results did not agree with our predictions. Shoot browsing did not usually take place in *A. grandicornuta*; rather leaf picking was more evident in some of the short trees during the time of sampling because it was difficult for mammals to bite the shoots. This resulted in the shoots of *A. grandicornuta* not being browsed often and not showing re-growth. One possible reason for this was that the removal of leaves led to a loss of plant resources and therefore growth of shoots was reduced. Damage through herbivory removes sinks and resources that are stored by the plants in the leaves, thus modifying allocation patterns (Stowe *et. al.* 2000). During the growing season, photoassimilates are accumulated and transferred to the stem and roots where they are stored for growth the following spring (Quiring and McKinnon 1999). Leaf removal during the growing season would therefore result in an overall decrease in resources. A similar response has been observed for other species such as *Acacia tortilis, Grewia flavescens* and *Dichrostachys cinerea* (Bryant *et al.*1991) where browsing decreased shoot growth. The same responses have been reported by Bergstrom and Danell (1995) for birch trees following leaf removal. Both browsed and unbrowsed trees did not increase shoots lengths.

Contrary to the predictions, the field data showed no significant increase in the thorn length of *A. grandicornuta* in the browsed treatments for all the months. The results agree with the outcomes of Cooper *et al.* (2003), where they reported that browsed *Acacia* species, spine length remained the same after browsing. The study done by Young *et al.* (2003) reported a continued slow and steady reduction of spine length where herbivores were excluded. The study reported that the spine lengths were low in total exclusion plots and larger in plots that allowed herbivores and plots with megaherbivores (giraffe and elephant) (Young *et al.* 2003). The study also showed that simulated large mammal browsing induced greater spine length on trees that had reduced spine length after several years of herbivory exclusion (Young *et al.* 2003).

It may be postulated that the thorns of *A. grandicornuta* did not grow longer under browsing because shoots were apparently unaffected by browsing. Thorn length increase was an induced response to herbivory and thorns help to protect the plants for further herbivory (Rooke *et al.* 2004b). The possible reason for no increase in thorn length could be because *A. grandicornuta* grows new shoots on the nodes so leaf picking did not induce thorn length increase. Gowda *et al.* (2003) reported in their results that production of long spines greatly increase when shoot growth increase at high nutrient availability. For the above reason no shoot growth resulted and no thorn length was increased. These results do not support the hypothesis. Previous studies have reported an increase in spine length on *Acacia* trees subjected to browsing compared to unbrowsed trees (Rohner and Ward 1997, Young *et al.* 2003).

As predicted, the shoot length of *C. apiculatum* increased in all the treatments. The partial exclosure had the highest shoot length compared to the other treatments. During the sampling period, impala and other herbivores were observed browsing the trees in the partial exclosure. Thus it may be inferred that browsing triggered shoot growth. Plants are expected to respond to herbivory by regrowth, in this case shoot length increased (Rooke and Bergstrom 2007). Increasing shoot length as a response to herbivory has been reported in other woody plants (Alados *et al.* 1996). Hrabar *et al* (2009) reported that pruning by elephant resulted in the production of longer shoots. It has also been reported that severe loss of tissue during the growth season (September to December) may induce a fast response through refoliation which enables the trees to replace lost tissue during the same seasons of impact (Rooke and Bergstrom 2007).

Lost tissue in *C. apiculatum* was replaced as a response to herbivory through regrowth or increasing shoot length. Trees replace lost tissue by growing new shoots and growing fast to escape herbivory (Herms and Mattson 1992). If the shoots are consumed or broken, the apical dominance is usually removed and resources are redirected towards lateral meristems (Stokke and du Toit 2000, Makhabu 2005).

When the apical dominance is removed, part of the photosynthetic material of a plant is removed, more water and nutrients are available for the remaining photosynthetic material resulting in an increasing shoot length (Hrabar *et al.* 2009). Shoot length in trees which were exposed to browsing increased under heavy browsing. The ability of the plants to re-grow could be because resources were probably abundant during this period (growing season) so trees could easily replace all the lost tissue through regrowth and increasing shoot length after herbivory. The total regrowth of a plant depends on the amount of resources available and the ability to allocate these resources after browsing (Obesa 1993, Trumble *et al.* 1993).

The results from this study showed no significant growth in the form of increased height for both species under browsing. It was clear that both species were severely browsed by herbivores leading to no growth in plant height. Intense browsing resulted in a significant decrease in growth in the form of tree heights (Turker *et al.* 2004). Similar patterns from other studies also indicated a reduction of tree growth after browsing (Carus 2004 and Kanat *et al.* 2005). Carus (2004) showed that stands of pines subjected to heavy browsing showed radial and height growth decrease. Kanat *et al.* (2005) found that browsing caused a significant decrease in the radial growth of pines after heavy defoliation. Hjalten *et al.* (1993) found that the growth of ***Betula pubescens*** saplings was significantly reduced under heavy browsing. From the finding in the study by Carus (2009) it was reported that intense browsing can lead to corresponding decrease in tree growth (heights and stem circumference).

From the data collected in this study, it was clear that *A. grandicornuta* was severely browsed through leaf picking by Impala. Since many of the resources are stored in the leaves, browsing may have removed a large proportion of the stored resources leading to limited growth in height or stem circumference of *A. grandicornuta*. It was also confirmed in the study by Rooke and Bergstrom (2007) that the removal of leaves signified a loss of resources leading to the reduction of growth in tree height.

The height of *C. apiculatum* increased where all the herbivores were excluded since no browsing was taking place. The height of *C. apiculatum* was reduced but the stem circumference increased where browsing took place. The results were confirmed by Rooke *et al.* (2004b) where they found increased shoot diameter after browsing. The *C. apiculatum* trees compensated for browsing by increasing radial growth. The *C. apiculatum* trees invested in growing taller in the full exclosure but in the partial exclosure, it grew a wider stem. This response by *C. apiculatum* can be due to re-browsing, where a plant is repeatedly browsed thus the development of a feeding loop (Makhabu and Skarpe 2006) and likely a 'browsing lawn' where the tree is kept short at a particular height, with bigger stem hence leading to no growth in height but stem circumference increases (Owen-Smith 2003).

The results from the data do not support the entire hypothesis but only support one aspect where *C. apiculatum* increased the stem circumference under browsing. The possible difference in responses of both species could be different soil types, defoliation and growth forms. The *A. grandicornuta* trees were browsed by leaf picking because they have thorns and the trees were sampled on the footslope. The *C. apiculatum* trees were browsed by shoot biting because they have no thorns and the trees were sampled in the crest site.

2.6.2. Effects of browsing on P and N

From the results of the study, both nitrogen and phosphorus were highest at the start of the growing season (October) as they were required for growth. Similar results were reported by Scogings *et al.* (2004) where the N and P content of all the plant species that were studied increased when the plants were growing in summer. Nitrogen and phosphorus are important requirements for plant growth and are limiting factors for plant growth (Van Patten 2005, Martinez-Sanchez 2006). The nutrient concentration in the leaves decreased in March 2008, possibly because it was lost through browsing. Herbivory may cause nutrient stress in plants because many of the nutrients are stored in leaves and therefore their removal leads to loss of nutrients (Kosola *et al.* 2001).

Contrary to this study Rooke and Bergstrom (2007) reported higher nitrogen content in *C. apiculatum* leaves which were browsed compared to unbrowsed trees. Bryant *et al.* (1991) reported that leaves of slow-growing species had increased levels of nitrogen after browsing. Danell *et al.* (1994) reported an increased level of nitrogen in birch trees the year after summer browsing. There were no differences in both N and P concentrations in browsed or unbrowsed trees from the present study.

From the growth results, it was confirmed that more growth occurred during October therefore more P and N were available in the soil for plant growth (Van Patten 2005, Martinez-Sanchez 2006). Plants absorb N and P from the soil easily during the wet season (Scholes 1997). The decreasing N and P concentrations in March can be related to less rainfall and thus less growth during this time of the year. When the dry season was approaching, the N and P concentrations were decreasing since it was assumed that no growth was occurring during March. The decreased nutrient concentrations in March 2008 can also be related to resorption where leaves were senescing and losing nutrients as the dry season was approaching. These results confirm the results found by Bryant *et al.* (1991), where they discovered a decrease in P and N concentrations in matured leaves.

2.6.3 Effects of browsing on CT

In the present study, the condensed tannin (CT) concentrations of *A. grandicornuta* were not different in browsed or unbrowsed plants from October 2007 to March 2008. In the study by Ferwerda (2005) no significant induced CT response in Mopane plants after browsing was reported. Artificial pruning (Gowda 1997) and browsing by wild herbivores (Du Toit *et al.* 1990) had no effects on the production of phenolic compounds (condensed tannins) in the leaves of *Acacia tortilis*. Ferwerda *et al.* (2005) confirmed that physical damage to the plants by removal of leaves did not have an effect on CT concentration in the remaining leaves. According to Boitumelo (2000) browsed plants generally have high condensed tannins compared with unbrowsed plants. This was because tannins form part of anti-herbivory strategy that is used by plants to defend their leaves.

The possible reason for no significant differences in this study may be linked to the possibility that CT was not a useful chemical defence for *A. grandicornuta* because thorns are used as form of defences. Less energy was invested in physical defences rather than chemical defences (Gowda and Palo 2003). The production of the chemical compounds e.g. CT in other species under high browsing pressure may not be a response to browsing (Rohner and Ward 1997).

For *C. apiculatum*, the CT increased from October to March in the full and non exclosure. When comparing browsed and unbrowsed trees, there were no significant differences in the amount of CT concentrations of *C. apiculatum* trees. Provenza and Malechek (1983) reported that the plant parts which were more exposed to herbivores had higher concentrations of CT than parts which are not easily accessed by herbivores. The higher concentrations of CT were found in the leaves of treated trees relative to untreated trees (Wessels *et al.* 2007). It was possible that under browsing in the non exclosure, more CTs were produced in the leaves as a defence mechanism to avoid further browsing (Stamp 2003). Contrary to this study, Bryant *et al.* (1991) reported a decreased level of CT after severe browsing on six woody species.

When no browsing took place, an increase in CT concentration from October to March in *C. apiculatum* was probably because of leaves maturing. These results support the findings of Bryant *et al.* (1991), who found an increase in CT concentrations as leaves matured. However, the results contradict the findings of Koukoura and Nastis (1992) who found decreased CT content as the leaves mature. Another study reported the concentration of secondary metabolites (CT) were highest in immature leaves and then decline in concentration when leaves expand (Herms and Mattson 1992).

The CT concentration in browsed *A. grandicornuta* was expected to decrease under browsing. The results showed no significant differences in browsed and unbrowsed CT concentration. The leaves and shoots of *A. grandicornuta* are not easily accessed by herbivores unlike *C. apiculatum*. The two species responded differently in terms of chemicals possibly because their morphology and physiology are different.

The differences in chemical concentration of plants have been related to potential leaf age and other environmental factors such as soil properties, defence mechanisms, temperature stress, light intensity and herbivory (Kraus *et al*. 2003, Osier and Lindroth 2001). The two species may have different CT concentrations because they are found on different soils. *C. apiculatum* was sampled on the crest and *A. grandicornuta* was sampled on the footslope. The footslope soil is made up of deep clay, while the crest has shallow sandy soils (Gertenbach 1983, O'Keefe and Alard 2002). Ozturk *et al.* (2006) reported in their study that different species and sites had a significant effect on CT contents of tree leaves.

No relationship between leaf removal and CT concentration was found in both species. The results show that when more leaves were removed from both species, the CT concentration was not affected. This holds true for *A. grandicornuta* where leaf removal did not affect the CT concentration. These results do not support *C. apiculatum* where under browsing, there was an increase in the production of CT as defence mechanisms to prevent further utilization compared to where no browsing took place (Stamp 2003).

2.6.4 Effects of browsing on photosynthetic rate

Plants which were exposed to herbivores had higher photosynthetic rate compared with unbrowsed plants as expected. Similar results were established by Huttunen (2008) where an increased photosynthetic rate was found in plants that had experienced tissue loss. Ozaki *et al.* (2004) reported that the removal of leaves increased photosynthetic rates in seedlings of *Pinus radiata*. Comparable patterns were found by Gonzales *et al.* (2008), where photosynthesis increased in damaged plants in comparison to undamaged plants. Teague (1989) also established that defoliation increased the rate of photosynthesis in plants. Damaged plants had higher photosynthetic rates (Thomson and Cunningham 2003). An increase in photosynthetic rate after herbivory (compensatory photosynthesis) involves the increase in photosynthetic rate of leaves that regrow after browsing (Ozaki *et al.* 2004).

The pattern which was observed from this study was that plants in the full exclosure gradually decreased the rate of photosynthesis from November towards March. The decreasing rate of photosynthesis was probably a result of decreasing growth rate of plants in the full exclosure, which coincided with a decrease in P and N concentrations in the full exclosure towards March for *C. apiculatum*. The decrease in growth could also be related to less water availability towards the early dry season and a decrease in available nutrients (March). The leaves were losing both P and N thus the leaves were senescing and therefore a decreasing photosynthesis rate in the full exclosure.

These results contradict those which were obtained in the non exclosure where the plants were browsed by large mammals. Photosynthetic rates were high during November most likely because more rain, P and N were still available for growth. Photosynthetic rate decreased in February where P and N were decreasing for *C. apiculatum* in the non exclosure. Several recent studies have emphasized the generality and importance of leaf age and this could be related to the decrease in photosynthetic rate towards February (Niinemets 2002).

The results from the study support the hypothesis that browsing has an effect on photosynthetic rate which increased in plants that were exposed to herbivores during February and March. Increased photosynthesis following browsing is the most recognized mechanism of tolerance in plants (Strauss and Agrawal 1999, Tiffin 2000). The increased photosynthetic rate in March was perhaps as a result of plants being browsed because all herbivores had access to the non exclosure. Tissue removal through browsing often leads to faster regrowth and increasing photosynthetic rate (Agrawal 2000). Plants are capable of replacing lost tissue through compensation depending on carbohydrates source-sinks stored in the plants (Kosola *et al.* 2001). The high demand placed on remaining leaves after browsing induce leaves to fix larger amounts of carbon and translocate photosynthate to growing parts at a fast rate on damaged plants (Kaitaniemi and Honkanen 1996, Thomson and Cunningham 2003). Plants replaced the lost tissue and therefore photosynthetic rate increased during March in the plants that were browsed.

CHAPTER THREE

Effects of browsing on resource allocation in *Combretum apiculatum* seedlings as affected by water availability**.**

3.1 Introduction

While much interest has been focused on interactions between herbivores and plant defences, less attention has been paid to understanding the plant response to browsing in water stressed plants and how they defend themselves. Defences are very costly and when resources are limited, its difficult for plants to allocate resources to defences and to growth at the same time. Environmental factors which are understood to influence the cost and availability of resources to anti-herbivore defences are climate, nutrients, seasonal variation, reproduction and importantly water availability (Briggz and Schultz 1990). Nutrients and water stress apart from the age of the plant, has been said to reduce shoot growth more than photosynthesis and are expected to lead to an accumulation of chemical compounds in the leaves (Estiarte *et al.* 1994).

Water is a driver determinant in plant growth in savannas as water is essential for growth of woody plant species. Water is more limiting than nutrients in savannas while nutrients are more limiting than water in forests (Scholes 1997). Woody species form an important food resource for many wild and domestic herbivores. Among domestic herbivores, goats and sometimes cattle rely on browse especially during the dry season when the food is scarce (Scogings *et al.* 2004). During the growing season, more food becomes available to herbivores in the form of shoots and leaves when plants start to grow and resources are allocated to functions such as growth, storage, defence and reproduction. This result in many changes such as carbon/nutrient balance, storage capacity and access to water and nutrients, growth, photosynthesis and other activities. These changes in plants affect the quality of plants as food for herbivores and are determined by protein, carbohydrates and secondary metabolite production. This implies that the changes in the functions of the plants like growth or reproduction during plant growth could promote changes in allocation of resources to plant defence (Boege and Marquis 2006).

Plants are subjected to a variety of stress factors which affect both their development and survival. In savanna environments where low nutrient availability and water scarcity are common, herbivory is the main factor that may limit optimal plant growth. In response to these limitations, plants have evolved strategies to deal with stress and herbivores. Plant defensive strategies are determined by the availability of resources (nutrients and water) to the plant. When trees experience herbivory, a temporary deficiency in nutrients is experienced, which may limit growth and reproduction (Katjiua and Ward 2006).

Mammal herbivores select plants which are less spinescent and where they can obtain most nutrients in a minimum time (Young *et al.* 2003). Plants which are not defended by spines or thorns can defend themselves by re-growing or by producing some chemical compounds in their leaves to avoid future browsing (Bazley *et al.*1991, Gowda 1997). Plants growing in areas with inadequate amounts of nutrients or water defend themselves differently from those plants which grow in areas of abundant nutrients and water (Fornara and Du Toit 2007).

This study focused on the growth (height and shoot length) and chemical defences (condensed tannins) when plants were browsed and watered at different levels. This part of the study has the following objectives:

- To determine the effects of browsing on growth (height, stem diameter and shoot length) in plants which are watered and defoliated at different levels.
- To determine the effects of browsing on chemical defenses (condensed tannins) in plants which are watered and defoliated at different levels.
- To determine resource allocation in different parts of the plants (leaves, shoot and roots) which are watered and defoliated at different levels.

The following hypotheses were derived from the above mentioned questions:

- Heavily defoliated plants will increase their growth (height, stem diameter and shoot length) when more water was available.
- Heavily browsed plants will allocate more condensed tannins to their leaves to avoid further browsing.
- Plants with more resources (water) recover more quickly from browsing by allocating more to leaf and shoot mass.

3.2 Methods

3.2.1 Study Area

The nursery experiment was conducted from June 2007 – February 2008 at the University of Zululand which is located near the town of Empangeni. The University of Zululand is situated KwaDlangezwa, 19 km south of Empangeni in the province of KwaZulu Natal in South Africa. The University of Zululand is located 56 kilometers west of the town of Richards Bay and is 80 m above sea level. This area joins the Indian Ocean which has warm Mozambique current and hence the rain occurs throughout the year, where the maximum rainfall is experienced between January and March, and the annual precipitation is said to be 1228 mm (Mpanza 2008). Winter rainfall is often associated with frontal weather from the south or may result from the influx of moist air from the east associated with the ridging Indian Ocean anticyclone. The average annual evaporation is ± 1400 mm (DWAF, 2000). The temperatures in summer reach a maximum of 36º C and 13.2º C in winter (South African Weather Service, 2004). The vegetation of this area has been classified as a Coastal Forest and Thornveld by Acock (1988). The experiment was conducted where sufficient light could enter the nursery, but rain was prevented from entering by a clear roof.

3.2.2 Plant Species

The Combretaceae consists of 18 genera, the largest of which is *Combretum*, with about 370 species (McGaw *et al.* 2001). *Combretum apiculatum* is a broadleaved, deciduous, non-spinescent tree. As it was stated in the previous study, *C. apiculatum* is an impotant food tree for domestic stock and game animals. The leaves are browsed by game and domestic animals, therefore it is an important food plant for livestock (Malan and Swinny 1993). The tree usually occurs in bushveld, often at low altitude. Seedlings of *C. apiculatum* were used because they are easy to grow under nursery conditions and grow fast. This species was chosen because very little research has been done on responses to browsing or resource allocation.

3.3 Data Collection

One hundred seedlings of *C. apiculatum* were obtained from the Skukuza nursery in June 2007 and were taken to the University of Zululand nursery. The seedlings were transplanted into new pots which were 25 centimeter in length. Loamy clay soil was mixed into each pot with Neutrog Bounce Back Pelletised organic slow release fertilizer (30g/kg N, 15g/kg P and 21g/kg K). The seedlings were transplanted into larger pots so that they could grow better and be maintained in the pots. The plants were allowed to grow for 4 months. The plants were given water once every 3 days (one litre per pot and the seedlings were weeded weekly.

After 4 months (September 2007) the plants were measured and the measurements were recorded to monitor the growth of the seedlings: heights using a 2 meter (m) metal rod (measured at two different heights, from the top of the pot to the top of the plant and from the nursery floor to the top of the plant), basal circumference using a tape measure at 10 cm, length of side shoots using a tape measure by taking the length of the longest shoot. Each pot was assigned a random number. Pots were randomized to substantially reduce the chance of bias which could arise if the seedlings in one treatment were different from those on another treatment and the seedlings were randomized for all the treatments.

From the 100 seedlings, 60 plants were randomly arranged in three sets of 20 plants per water treatment. The first set of 20 seedlings were given one l of water every ten days (low water), the second set of 20 seedlings were watered every seven days (medium water) and the third set of 20 seedlings were watered every three days (high water). The excess water was not stopped from running out. If excess water ran out, it was assumed that it was the same amount in all the pots. Each pot was randomly assigned to one of four defoliation treatments within each water treatment (Figure 3.1). Defoliation was done by goats to achieve target levels of 0%, 30%, 60% or 90% of biomass removed. Another 30 seedlings were divided into two groups, one group with 15 seedlings was used for regression models and another 15 seedlings were used for the conditioning of the goats and the remaining seedlings were kept as spares.

Figure 3.1: Seedlings of *C. apiculatum* were assigned a random number and were grouped into three sets for water treatments.

Goats were used instead of clipping to achieve real browsing. Goats were obtained from Owen-Sithole College of Agriculture. Castrated males of 2-3 years old were housed in pens in a shed (Figure 3.2) and were given pellets and hay at maintenance levels for 5 days before the actual experiment. The goats were also given water before the experiment after they were given pellets and hay.

Figure 3.2: Goats housed in pens and feeding on hay and pellets before the experiment.

Before the experiment was conducted in September 2007, the goats were conditioned to the plants since they had never eaten *C. apiculatum;* therefore the process of allowing them to taste it was very important. Fifteen seedlings were used for this process and each plant was randomly assigned a defoliation intensity from 30-90%. Each plant was weighed in its pot before it was given to the goat. Goats were taken individually out from their pens into a bigger pen and a plant was put in front of the goat (Figure 3.3). The goat was allowed to feed on the plant until four observers agree that the target intensity was reached.

The goat was stopped and removed from the bigger pen and the plant was then re-weighed (to 0.1 g). Each stem was cut off from the base and weighed. The actual proportion (%) of mass removed from the plant was estimated and compared with the target proportion.

In this way the observers were able to calibrate their ability to visually estimate the defoliation intensity. After the goats had been conditioned, the 45 plants assigned to defoliation levels > 0% were subjected to the same procedure.

Figure 3.3: Goats were used to browse each plant rather than simulated browsing.

After all the plants had been defoliated according to each defoliation intensity, the plants were taken back to the nursery. Each group of plants was watered according to its water treatment for 4 months. After the period of 4 months (October 2007 - February 2008), the measurements of height, basal circumference, number of new shoots and shoot length were repeated in February 2008 to see how the plants responded to defoliation. Thereafter, seedlings were harvested, separated into morphological parts (leaves, stems and roots) and were dried in the oven at 60ºC for 48 hours. Each plant part was weighed and leaf samples were milled to pass through a 0.5mm screen thereafter the samples were analyzed for nitrogen (N), phosphorus (P) and condensed tannins (CT).

Nitrogen was analysed using the Kjeldahl AOAC method (Smith, 1980), P was analysed using the method of Murphy and Riley (1962). The CT was analysed using the acid-butanol proanthocyanidin assay (Hagerman, 1995), with sorghum tannin as the standard. The procedure followed for determining P, N and CT is explained in Appendix 2.

3.4. Data analysis

The experimental design was not replicated and this constrained statistical tests or the use of Analysis of variance (ANOVA) (Underwood 1997). The experimental design was not replicated because of logistical and technical constraints. The nursery experiment could not be replicated because the number of seedlings available for use was insufficient for replication. ANOVA was therefore not done and the only option was descriptive statistics. The means of leaf, shoot and root mass of each defoliation intensity (0, 30, 60 and 90%) were determined for each water treatment with their standard errors to see how seedlings responded to different defoliation intensities and water treatments.

The means of height and basal circumference of each treatment were plotted with standard errors to assess how plants responded to defoliation at different intensities and given different water levels. Mean numbers of new shoots and standard errors for all seedlings watered at different levels and defoliated at different intensities were assessed to observe how many shoots were present after defoliation. The height, basal circumference and shoot length of seedlings were measured before and after browsing. The means of height, basal circumference and shoot lengths were plotted against different defoliation intensities for different water treatments and standard errors to observe how plants responded to browsing.

Means of phosphorus (P) and nitrogen (N) concentration (%) of each defoliation intensity and different water treatments were presented in Table 3.2 and 3.3 with standard errors to identify the distinction between each defoliation intensity and within each water treatment. Means of condensed tannin concentrations (%) within each water treatment for different defoliation intensity were plotted against each other for different water treatments to see how water affected the content of condensed tannins at different defoliation intensities.

Systat 10 was used to test the data and to see if means were significantly different or not. All the data were first checked for normality and if they were not normally distributed, the data were log transformed. Differences were considered significant if $P < 0.05$. The t-test was done to compare means of treatments that had error bars not overlapping with each other since these were likely to be different from each other. T-tests were also done to compare leaf, shoot and root mass of all the plants in all treatments which were defoliated at 0% and 90% to observe the effect of water on the leaf mass. Overlapping error bars were not significantly different.

The data were grouped by water treatments or by defoliation intensities depending on the variable tested. To check for the effects of the water treatments on leaves, shoots and roots, all the defoliation intensities within each water treatment were combined. Plants watered every 10 and 3 days were compared with each other so that the differences could be identified between high and low water treatments. The same was done for height, shoot length, basal circumference and the chemicals (P, N and CT concentrations). Two different defoliation intensities within each water treatment were compared to determine the effects of defoliation.

3.5 Results

3.5.1 Effects of browsing and different water levels

3.5.1.1 Effects of browsing on leaf mass

The results from the combined data show that water treatment had a significant effect on leaf mass, which increased with increasing water availability (t = -9.39, df = 20.60 and $P < 0.001$) (Figure 3.4). Leaf mass for plants which were watered every 10 days was significantly lower than that of plants which were watered every 3 days. Differences in leaf mass between all the water treatments in plants which were not defoliated were all significantly different from each other. In seedlings which were not defoliated, leaf mass in plants which were watered every 10 days was significantly lower than those which were watered every 7 days (t = -3.74, df = 5.50 and P = 0.011) (Figure 3.4). In seedlings which were not defoliated, leaf mass in plants which were watered every 10 days was significantly lower than those which were watered every 3 days (t = -4.64, df = 4.40 and P = 0.008). In seedlings which were not defoliated, leaf mass in plants which were watered every 7 days was significantly lower than those which were watered every 3 days (t = -2.44, df = 5.80 and P = 0.041) (Figure 3.4).

Defoliation did not have an effect on leaf mass ($P > 0.05$) because there was no pattern in all the plants watered every 10, 7 and 3 days. The leaf masses were similar within water treatments and no differences within each water treatment in terms of defoliation intensities. The leaf mass of seedlings which were not defoliated was similar to seedlings which were defoliated at 30, 60 and 90% in all the different treatments (Figure 3.4).

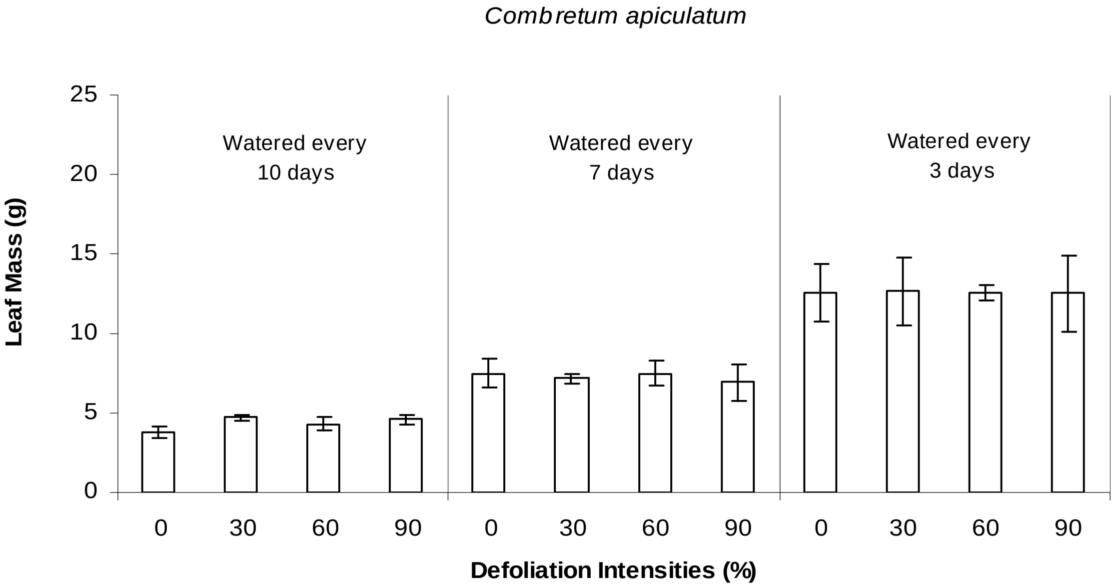

Figure 3.4: Leaf Mass (g) of *C. apiculatum* defoliated at different intensities and watered every 10, 7 and 3 days for four months. Error bars represent ± Standard Error (n=5).

2.5.1.2 Effects of browsing on shoot mass

The shoot data were not normally distributed so they were log transformed. The results show that water treatments had a significant effect on shoot mass (t = -6.18, df = 34.40 and $P < 0.001$) (Figure 3.5). Shoot mass for plants which were watered every 3 days were higher than those watered every 10 days. Shoot mass increase with an increasing water availability. Water had an effect on shoot mass since shoot mass increased with increasing water availability, plants watered every 10 days had lowest shoot mass, followed by seedlings which were watered every 7 days, plants that were watered every 3 days had the highest shoot mass (Figure 3.5). Differences in shoot mass between all the water treatments in plants which were not defoliated were all significantly different from each other (Figure 3.5).

In seedlings which were not defoliated, shoot mass in plants which were watered every 10 days was significantly lower than those which were watered every 7 days (t = -2.58, df = 7.90 and P = 0.033) (Figure 3.5). In seedlings which were not defoliated, shoot mass in plants watered every 10 days was significantly lower than those which were watered every 3 days (t = -3.21, df = 4.20 and P = 0.031) (Figure 3.5). In seedlings which were not defoliated, shoot mass in plants watered every 7 days was significantly lower than those which were watered every 3 days (t = -2.64, df = 4.20 and P = 0.030). Defoliation did not have an effect on shoot mass (P>0.05) since there was no pattern in all the plants watered every ten, seven and three days (Figure 3.5).

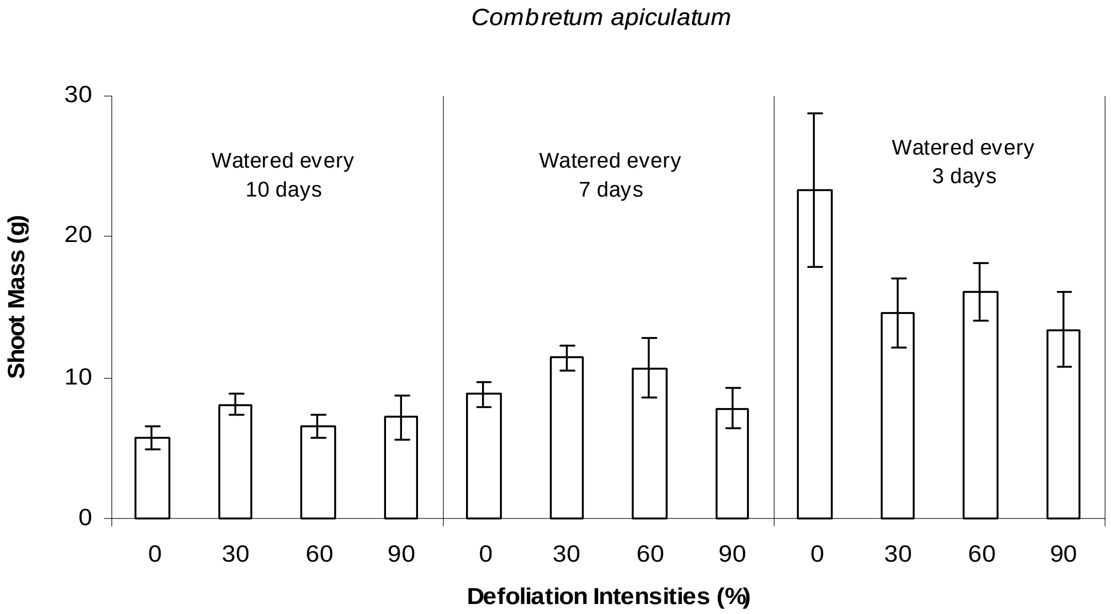

Figure 3.5: Shoot Mass (g) of *C. apiculatum* defoliated at different intensities and watered every 10, 7 and 3 days for four months. Error bars represent ± Standard Error (n=5).

2.5.1.3 Effects of browsing on root mass

Root mass in seedlings which were watered every 10 days was lower than that of those which were watered every 3 days (t = -2.19, df = 31.20 and P = 0.036) (Figure 3.6). Root mass increase with an increasing water availability.

Water had a significant effect on root mass. Defoliation did not have an effect on root mass within all the water treatments (*P*>0.05). The root masses were not significantly different in all the different defoliation intensities within all the water treatments (Figure 3.6).

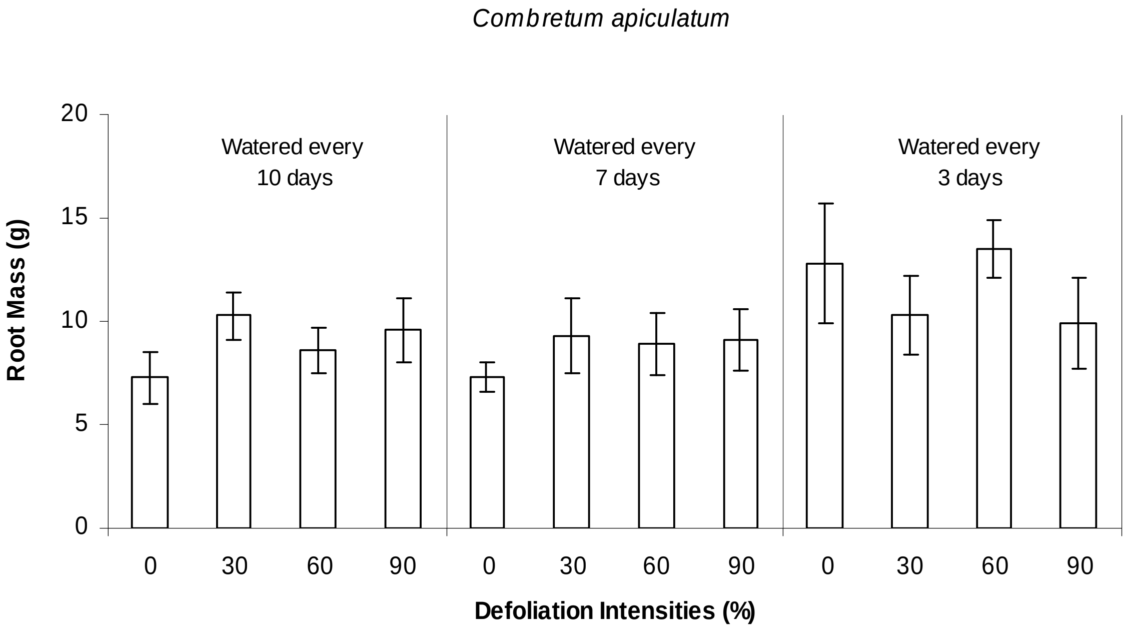

Figure 3.6: Root Mass (g) of *C. apiculatum* defoliated at different intensities and watered every 10, 7 and 3 days for four months. Error bars represent ± Standard Error (n=5).

3.5.2. Number of New Shoots

Both water and defoliation did not have an effect on the number of new shoots (*P*> 0.05). The number of new shoots did not increase with increasing water availability or defoliation intensities. The number of shoots was not significantly different in all the different water treatments and the number of new shoots in different defoliation intensities was not different (Table 3.1).

Table 3.1: Mean ± SE of new shoots in *C. apiculatum* following defoliation at various intensities where plants were watered every 10, 7 and 3 days for four months after defoliation (n=5).

Defoliation Intensities (%)	Water Levels	Mean new shoots	Standard Errors
0	10 days	21	±2.345
30	10 days	23	±1.924
60	10 days	11	±3.536
90	10 days	15	±3.558
0	7 days	18	±1.631
30	7 days	22	±4.802
60	7 days	25	±3.733
90	7 days	11	±3.723
0	3 days	14	±3.625
30	3 days	21	±3.723
60	3 days	16	±3.370
90	3 days	20	±4.167

3.5.3 Growth Measurements after defoliation

3.5.3.1 Plant Height

The data showed that water treatment had a significant effect on heights of the seedlings after defoliation (t = -4.28, df = 38.00 and $P < 0.001$) (Figure 3.7). In seedlings which were defoliated at 30 % and watered every 10 days, the height before defoliation was significantly higher than the height after defoliation (t =3.81, df= 4.00 and P = 0.019) (Figure 3.7). In seedlings which were not defoliated and watered every 3 days, the height before defoliation was significantly lower than the height after defoliation (t = -2.92, df = 4.00 and P = 0.043) (Figure 3.7).

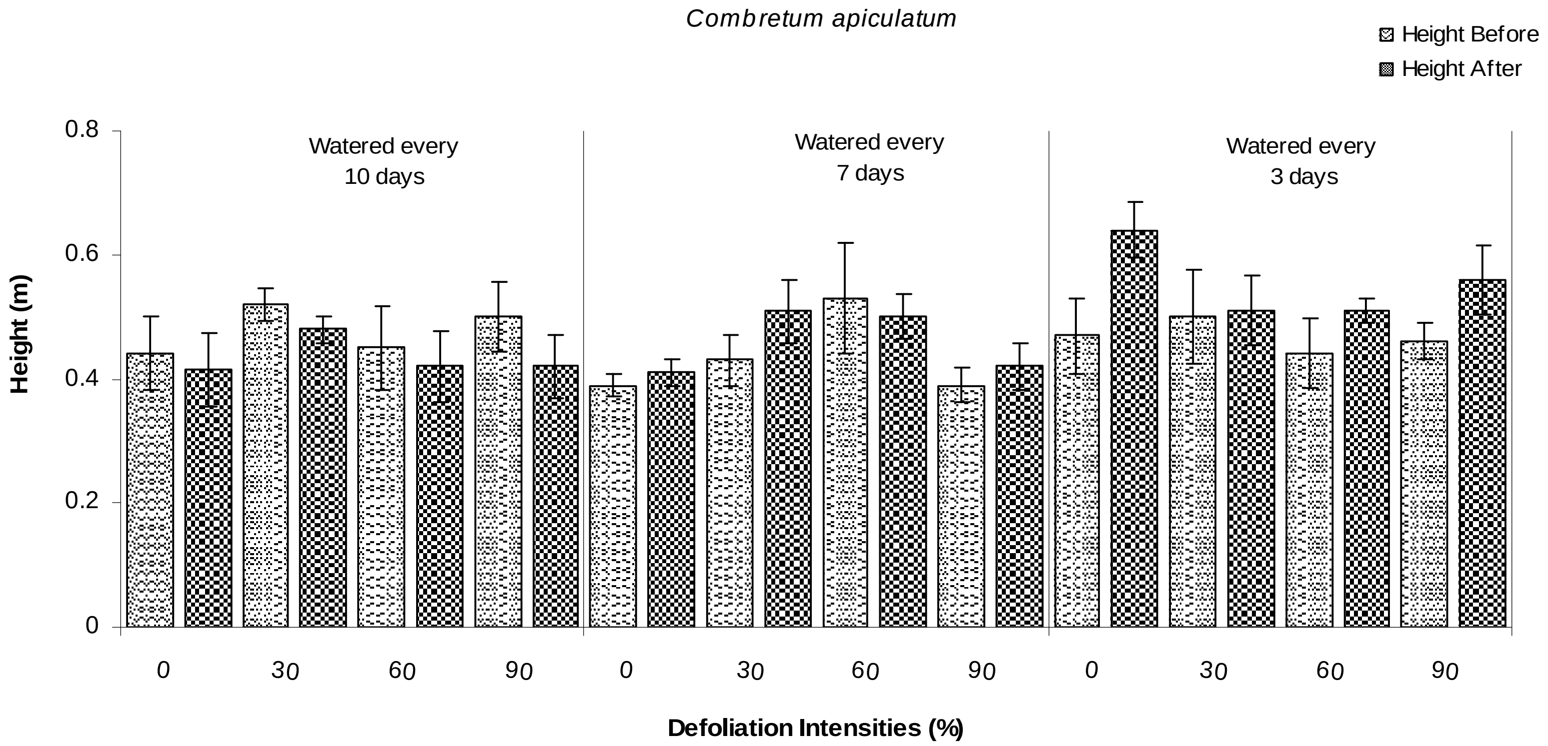

Figure 3.7: Heights (m) of *C. apiculatum* taken before and after defoliation at different intensities and different water treatments. Error bars represent ± Standard Error (n=5).

3.5.3.2 Shoot Length

The results from the combined data show that water treatment had a significant effect on shoot length of the seedlings after defoliation (t = -8.02, df = 29.10 and P < 0.001) (Figure 3.8). Water had a significant effect on shoot length since shoot length increased with increasing water availability. Defoliation had a significant effect in the shoot length of seedlings which were watered every 10 days and defoliated at 30% intensity. The shoot length before defoliation was significantly higher than the shoot length after defoliation in seedlings which were watered every 10 days and defoliated at 30% intensity (t = 5.34, df = 4.00 and P = 0.006) (Figure 3.8). There was significant growth in seedlings which were not defoliated and watered every 7 and 3 days. The shoot length of seedlings which were not defoliated and watered every 7 days significantly increased (t = -12.7, df = 4.00 and P < 0.0001). The shoot length of seedlings which were not defoliated and watered every 3 days significantly increased (t = -6.45, df = 4.00 and P = 0.003) (Figure 3.8).

In seedlings which were watered every 3 days, defoliation had significant effect in the shoot length of seedling which was defoliated at 30% and 90% intensity (Figure 3.8). The shoot length of seedlings after defoliation was significantly higher than the shoot length after in seedlings which were watered every 3 days and defoliated at 30% intensity (t = -3.05, df = 4.00 and P = 0.038) (Figure 3.8). The shoot length of seedlings before defoliation was significantly lower than the shoot length of seedlings after defoliation in seedlings watered every 3 days and defoliated at 90% intensity (t = - 10.79, df = 4.00 and, P < 0.001) (Figure 3.8).

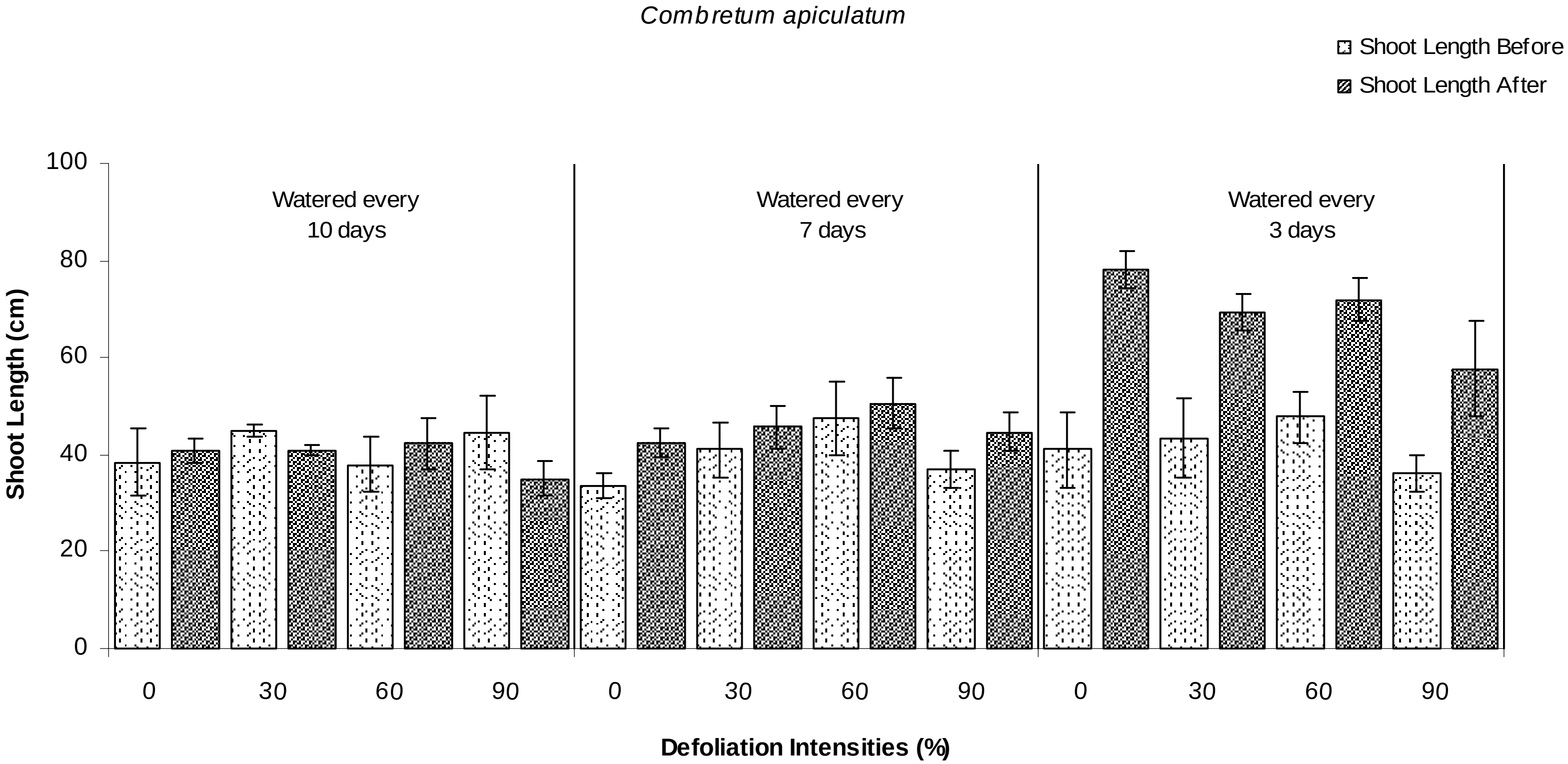

Figure 3.8: Shoot Length (cm) of *C. apiculatum* taken before and after defoliation at different intensities and different water treatments. Error bars represent ± Standard Error (n=5).

3.5.3.3 Basal Circumference

Water had an effect on basal circumferences because there was an increase in basal circumference with increasing water availability. The data for the basal circumference of seedlings before and after defoliation were not normally distributed so it was log transformed. The results from the combined data show that water treatment had a significant effects on basal circumference of the seedlings after defoliation (t = -7.02, df = 29.00 and $P < 0.001$) (Figure 3.9).

Defoliation had an effect in the basal circumference of seedlings which were watered every 7 days and defoliated at 60% intensity. The basal circumference of seedlings which were not defoliated and watered every 7 days significantly increased (t = -6.02, df = 4.00 and P = 0.004). The basal circumference of seedlings which were not defoliated and watered every 3 days significantly increased (t = -12.06, df = 4.00 and $P < 0.001$) (Figure 3.9). In seedlings which were watered every 7 days and defoliated at 60% intensity, the basal circumference before defoliation was significantly lower than the basal circumference after defoliation (t = -3.42, df = 4.00 and P = 0.027) (Figure 3.9).

In seedlings watered every 3 days, defoliation had a significant effect on basal circumference of all the seedlings which were defoliated in all the different intensities (30%, 60% and 90%). The basal circumference after defoliation for the seedlings which were defoliated at 30% intensity was significantly higher than the basal circumference before defoliation (t = -23.89, df = 4.00 and $P < 0.001$) (Figure 3.9). The basal circumference of seedlings before defoliation was significantly lower than the basal circumference after defoliation in seedlings which were defoliated at 60% intensity (t = -5.14, df= 4.00, and P = 0.007) (Figure 3.9). In seedlings which were defoliated at 90% intensity, the basal circumference before defoliation was significantly higher than the basal circumference after defoliation (t = -4.23, df = 4.00 and P = 0.013) (Figure 3.9).

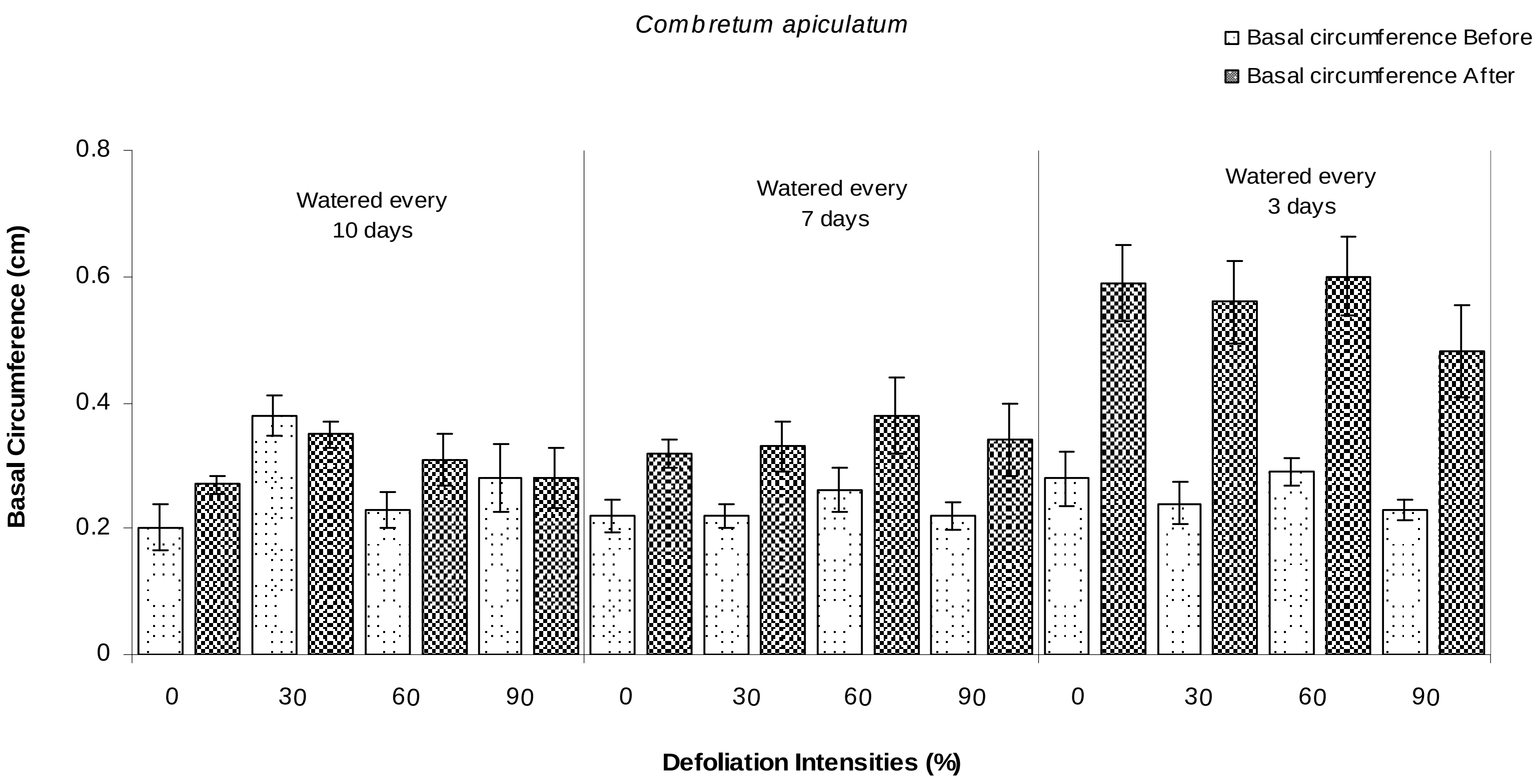

Figure 3.9: Basal Circumference (cm) of *C. apiculatum* measured before and after defoliation at different intensities and different water treatments. Error bars represent ± Standard Error (n=5).

3.5.4 Phosphorus, Nitrogen and Condensed Tannin concentrations in the leaves

3.5.4.1 Phosphorus concentration in the leaves

Water had a significant effect on phosphorus (P) concentration in the seedlings (Table 3.2). The results from the combined data show that the P concentration in seedlings which were watered every 10 days was significantly lower than the P concentration of seedlings which watered every 3 days ($t = -2.07$, df = 28.20 and $P = 0.047$) (Table 3.2). Defoliation did not have an effect on seedlings in all the water treatments ($P > 0.05$).

Table 3.2: Mean ± SE of phosphorus (P) concentration (%) following different defoliation intensities in *C. apiculatum* seedlings watered every 10, 7 and 3 days (n=5).

Defoliation Intensities (%)	**Water Levels**	**Mean P (%)**	**Standard Errors**
0	10 days	0.28	±0.010
30	10 days	0.26	±0.009
60	10 days	0.24	±0.007
90	10 days	0.25	±0.006
0	7 days	0.25	±0.008
30	7 days	0.28	±0.020
60	7 days	0.26	±0.006
90	7 days	0.24	±0.003
0	3 days	0.30	±0.030
30	3 days	0.30	±0.010
60	3 days	0.26	±0.030
90	3 days	0.27	±0.020

3.5.4.2 Nitrogen concentration in the leaves

Water had a significant effect in nitrogen (N) concentration in the seedlings (Table 3.3). The results from the combined data show that the N concentration in seedlings which were watered every 10 days was significantly lower than the N concentration of seedlings watered every 3 days (t = 2.74, df = 35.90 and P = 0.009) (Table 3.3). Defoliation did not have an effect on seedlings in all the water treatments (P> 0.05).

Table 3.3: Mean ±SE of nitrogen (N) concentration (%) following different browsing intensities in *C. apiculatum* seedlings watered every 10, 7 and 3 days (n=5).

Defoliation Intensities (%)	Water Levels	Mean N (%)	Standard Errors
0	10 days	2.32	±0.211
30	10 days	2.47	±0.257
60	10 days	2.22	±0.164
90	10 days	2.25	±0.051
0	7 days	2.21	±0.246
30	7 days	1.95	±0.255
60	7 days	1.94	±0.283
90	7 days	2.43	±0.171
0	3 days	2.19	±0.215
30	3 days	1.95	±0.166
60	3 days	1.71	±0.273
90	3 days	1.82	±0.255

3.5.4.3 Condensed Tannin concentration in the leaves

The data for condensed tannins (CT) concentration were not normal so it was log transformed. Water had a significant effect on CT concentration in the seedlings since CT concentration increased with increasing water availability.

The results from the combined data showed that the CT concentration in seedlings watered every 10 days was significantly lower than the N concentration of seedlings which watered every 3 days (*t* = -2.23, df = 20.50 and *P* = 0.037) (Figure 3.10). Defoliation had an effect in seedlings watered every 7 days, where CT concentration significantly decreased from 0% to 60% defoliation intensity (*t* = -3.38, df = 7.40 and *P* = 0.011) (Figure 3.10). The CT concentration in seedlings watered every 7 days and defoliated at 60% was lower than the CT concentration of seedlings which were defoliated at 90% intensity (*t* = 3.25, df = 5.60 and *P* = 0.031) (Figure 3.10).

The CT concentration of seedlings watered after 7 days increased from 60% to 90% defoliation (Figure 3.10). The CT concentration of seedlings watered every 3 days and not defoliated was significantly lower than the CT concentration of seedlings which were defoliated at 60% (*t* = 3.35, df = 3.10 and *P* = 0.042) (Figure 3.10). CT concentration significantly increased from 0% intensity to 60% intensity in seedlings which were watered after 3 days.

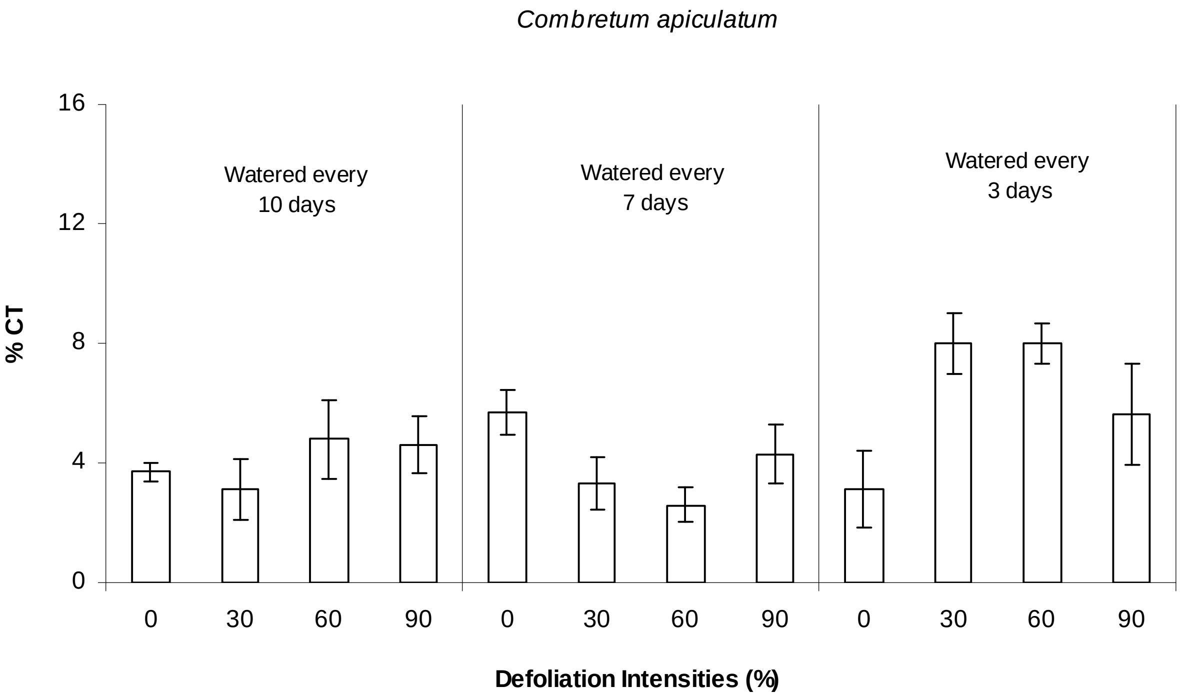

Figure 3.10: Condensed Tannins (CT) concentration (%) following different defoliation intensities in *C. apiculatum* seedlings watered every 10, 7 and 3 days. Error bars represent ± 1 Standard Errors (n=5).

3.6 Discussion

3.6.1 Effects of Browsing and Different Water Levels

The results from this study showed that leaf and shoot mass increased with increasing water availability. Seedlings with less water had lower leaf and shoot mass compared with seedlings with more water. Plants develop adaptive mechanisms when they are subjected to moisture stress. These strategies help to maintain their physiological processes under water stress conditions (Kramer and Boyer 1995, Nilsen and Orcutt 1996). The mechanism employed by seedlings in this study could be a reduced leaf surface to limit water loss, decreased leaf size and accelerated leaf senescence (Aref and El-Juhany 1999).

These findings coincide with the results of Aref and El-Juhany (2008) where severe water stress reduced the total leaf area and consequently reduced leaf dry weight by 65%. Reductions were also found in stem dry weight as a result of water stress (Aref and El-Juhany 2008). Decreased leaf size through decreased mass help prevent water loss but it also reduces the surface area for photosynthesis resulting in decreased growth rate (Kozlowski *et al.* 1991, Aref and El-Jihany 2008). The growth of roots decreased as a response to water stress and lower nutrients were available when less water was available. Aref and El-Juhany (2008) reported a decreased root dry weight due to water stress. Contrasting to these results, Khalil and Grace (1992) concluded that root growth increased under water stress. The results from this study do not support the findings from other studies as root mass increased with more water availability.

Resources must have been allocated to the roots when the seedlings had more water to ensure more water uptake. Different results were reported in the study by Osonubi *et al.* (1992) where it was found that *Faidherbia albida* tolerated water stress by producing long roots and *Acacia nilotica* responded by developing larger roots to be able to withdraw water from the soil.

The degree of growth following tissue loss depends on resource availability and the ability of plants to allocate the recourses after browsing (Obeso 1993, Trumble *et al.* 1993). The carbohydrates for replacing lost tissue are usually stored in big, existing leaves (Omari et al. 2003) or reserved in roots or other parts of the plant (Bowen and Pate 1993, Canadell and Lopez-Soria 1998). In the present study, seedlings which had more water weighed more than seedlings which were water stressed. More resources were available in the soils and they were allocated to regrowth in shoots and leaves after defoliation so more weight resulted in all the plant parts (Fang *et al.* 2006).

Leaf, shoot and root weights were not affected by defoliation since there were no significant differences in plant parts within different defoliation intensities. Shoot browsing resulted in less resources allocated to regrowth, resulting in a decreased overall shoot and leaf mass (Stowe *et al.* 2000, Hrabar 2006). Seedlings which were not defoliated (0%) had significant differences in different water treatments but no differences compared to other defoliation intensities. This does not agree with the hypothesis since no responses to defoliation was detected. The possible explanation was that tissue loss after defoliation had lowered the resources that were reserved in the seedlings therefore no significant differences in defoliation intensities were recorded (Huttunen 2008).

3.6.2 Growth Measurements

In this study, increasing water availability increased seedling heights, shoot length and basal circumference. The low water availability resulted in short seedlings with short shoots and a small basal circumference. Similar to these results, a study by Aref and El-Juhany (1998 and 1999) confirmed that the mean height and diameter of *Acacia* seedlings was reduced in water stressed plants. Other studies have observed reductions in plant height and basal circumference as a result of water stress (Omari 1994, Awodola 1991). When more water was available in the soil, nutrients can be easily absorbed by the roots and allocated to regrowth in the form of increased height, shoot length and basal circumference (Thomson *et al.* 2003, Fang *et al.* 2006).

The data from this study showed an increased height, shoot length and basal circumference as a response to defoliation. Seedlings increase growth rate as a defense strategy to escape herbivory (Agrawal 2000). Plants are expected to respond to browsing by regrowth (Rooke and Bergstrom 2007). Rooke *et al.* (2004a) found similar results where shoot length and shoot biomass increased after browsing. Other reports by Fang *et al.* (2006) showed high regrowth capacity to compensate for shoot loss in *Caragana korshinskii.* After defoliation or clipping, shoots compensate for tissue loss by growing long shoots, which is a form of tolerance (Du Toit *et al.* 1990, Riba 1998). Plants employ different strategies to tolerate tissue loss. These strategies include replace lost tissue by growing new shoots and growing too tall to escape herbivory (Herms and Mattson 1992).

When defoliation occurs in seedlings, the leaves and shoots are removed. When water and nutrients are available in the soil, resources are redirected towards the lateral meristems of the remaining tissues, leading to increased growth after defoliation (Hrabar 2006, Stokke and Du Toit 2000, Makhabu 2005). When enough water was available in the soil, plants can absorb enough nutrients for growth. An increasing height and basal circumference after defoliation occurred in seedlings which had more resources to recover from the initial damage (Fang *et al.* 2006).

Induced responses such as re-growth (mass compensatory growth) reduce the effect of herbivory by increasing plant height and basal circumference when browsing has already occurred (Agrawal 2000). The results from seedlings height, shoot length and basal circumference support the hypothesis since both water and defoliation had an effect on growth of seedlings. There was no significant difference in the number of new shoots in all the water treatments. These results do not support the hypothesis and were not expected.

3.6.3 Phosphorus, Nitrogen and Condensed Tannin concentrations in the leaves

From the results obtained in this study, phosphorus and nitrogen contents increased when more soil water was available. Soil nutrients in this case P and N have been recognized as important components of plants especially during growth (Gindaba 2006). More nutrients become available in the soil when more water is available (Scholes 1997). The N and P amount in the leaves indicate the nutrient availability in the soil (Martinez-Sanchez 2006). When a plant suffers from N and P deficiency, the plants become stunted and may lead to death depending on the extent of deficiency (Gindaba 2006). In the study by Gindaba (2006), soil P had less effect on the growth rate of plants in all the species compared to N. In this study, there were no significant differences in defoliated and not defoliated seedlings. Contrasting to these results, Kosola *et al.* (2001) reported a significantly decreased rate of nitrogen uptake in defoliated plants. Bryant (2003) found a high N content of leaves from browsed plants. Similar to this study, Parry (2000) found that leaf nitrogen levels in defoliated trees were not significantly different from the controls.

Corresponding with these results, the research by Lovett and Tobiessen (1993), found that defoliation did not affect N uptakes by red oak seedlings. The different response to defoliation in nutrient uptake can be due to differences in types and intensities of defoliation in different studies (Kosola *et al.* 2001).

The present study showed increased condensed tannin concentration when more water was available. Contradicting this study, others studies have shown that plants growing in less water environments maintain a high level of chemical defences against herbivore damage (Osier and Lindroth 2001). More water was available in the soil for growth, more CTs were produced as a form of defense on leaves to avoid herbivory (Baldwin 1998). Seedlings which had less water could not allocate more recourse to chemical defences; as a result less CTs were produced. Defoliated seedlings allocated more resources to produce CTs and to avoid being browsed (Fang *et al.* 2006).

Plants respond to defoliation by increasing their defensive traits and increasing CT production in leaves (Fang *et al.* 2006). Defoliation had an effect on seedlings which had moderate water levels. The CTs were produced in response to defoliation. Bryant (2003) reported a low tannin concentration on browsed leaves. Contrasting to this, Furstenburg and van Hoven (1994) found increased tannin contents of dietary foliage after browsing. Ferwerda *et al.* (2005) reported no differences in foliar CTs concentration on young *Colophospermum mopane* after browsing.

These results support the hypotheses because both defoliation and water treatments had an effect on CT concentration. CT concentration increased with increasing water availability. Seedlings which were heavily browsed allocated more CT in their leaves to avoid being browsed again (Stamp 2003).

CHAPTER FOUR

4.1 General Conclusions

From the results of the study, it was predicted that growth and thorn length will increase for *Acacia grandicornuta* under browsing. But contrary to the hypothesis, the results showed no overall regrowth in the shoot length, stem diameter and thorn length of *A. grandicornuta* under browsing, which was unexpected. Many studies have reported that browsing African *Acacia* trees can stimulate shoot production (Pellew 1983, Dangerfield and Modukanele 1996, Gowda 1997, Oba 1998). However, it was predicted that *Combretum apiculatum* will increase growth under browsing. The results showed that *C. apiculatum* responded to browsing by increased growth after browsing as predicted (Rooke and Bergstroem 2007).

An increase in growth after browsing in *C. apiculatum* may have resulted in increased photosynthetic rates. Growth in the form of increased photosynthetic rates could have been enhanced in *C. apiculatum* as more N and P were available during the growing season. This results in *C. apiculatum* plant replacing lost tissue by compensation growth (Gonzales *et al.* 2008). Nevertheless, browsing had no effects on condensed tannins (CTs) of *A. grandicornuta* as this species presumably relies on thorns for defence (Gowda and Palo 2003). Contrary to *A. grandicornuta*, the increased CT concentration of *C. apiculatum* where browsing took places proposed that this species employ CT to defend its leaves against herbivores. The hypothesis placed for *C. apiculatum* were supported by the results since there was an increase in growth (shoot length and height), and increased photosynthesis under browsing.

It was clear that both species responded differently to browsing because they were sampled in different areas (O'Keefe and Alard 2002). The thorny species *A. grandicornuta* responded to browsing by no growth and no decreased CTs. The broad leaved *C. apiculatum* responded to browsing by increased shoot length, increased CTs and increased photosynthetic rate. Intense browsing in both species resulted in significant decrease in growth in the form of tree heights (Turker *et al.* 2004).

It was predicted that both species will increase growth by increasing height and stem circumference under browsing. Both species responded to browsing by not increasing their heights (Makhabu and Skarpe 2006). But *C. apiculatum* increased the stem circumference under browsing. It can be presumed that less defended plants have the potential to be depleted by herbivores. It can also be postulated that repeated browsing resulted in short plants which cannot reach the adult stage and cannot reproduce. This can cause problems for the park because both species are a food source for many herbivores (Venter and Venter 1996, Pooley 1993). If not enough food is available for herbivores, mortality can be the end results and can affect many animals in the food chain.

Since very little is known about tree responses to browsing, this research will help improve knowledge and understanding of browse browser interactions in savannas. The knowledge gained from this research is useful for building models of browse-browser interaction in seasonal subtropical zones where browsers are abundant and have potential to deplete vegetation resources. The future development of robust models of browse-browser interactions in savannas benefit from improved knowledge of the ecology of trees growing in savannas. Game managers and conservation sectors benefit from the knowledge gained from the study and ideas of plant responses to browsing since very little is known.

From the hypothesis placed for nursery experiment, it was predicted that heavy defoliated seedlings will increase growth when more water was available. The results from the nursery experiment did not supported the predictions as defoliation had no effects in growth rate of seedlings. It was predicted that heavy browsing will results in seedlings allocating more CT to their leaves as a defence mechanism. Seedlings which had less water available could not produce CTs to defend their leaves from browsers. Less defended species have a high chance of being repeatedly browsed and may be depleted by herbivores. It was also predicted that seedlings with more water availability will recover more quickly from browsing to compensate tissue loss. From the results of the study, water availability increased growth of seedlings after defoliation. It can be concluded that less water availability resulted in no growth and hence no reproduction

By knowing which plants species cannot grow under intense browsing, managers can take action and protect these plant species from browsers so they can grow, reproduce and provide food for herbivores.

4.2 Recommendations for future research

Further research is still needed to understand why *A. grandicornuta* did not respond to browsing by increasing growth and CT concentrations? Another question that needs to be addressed is why plant heights for both species did not increase where browsing took place as predicted? The results obtained from this study proved that different species respond differently to browsing. If the same study has to be done again, it is recommended that an equal number of plants should be sampled in all the treatments because there were problems with analysis since the data were unbalanced. The measurements must be monitored regularly and in the long term, maybe for two years to have an indication on how the seasons affect growth, chemicals and the rate of photosynthesis. More exclosures must be constructed so the experiment can be replicated and allow more robust analyses.

When doing nursery experiments for the future, the amount removed by the goats must be the correct amount that is targeted. The number of seedlings must be increased to three times so that the experiment can be properly replicated. The correct model must be developed to calculate the amount removed by the goat instead of just estimating the amount because this can lead to over or under estimation of the results. The challenges faced from the study were the unexpected results where some of the predictions were not supported by the results. Another challenge was with the data where no ANOVA could be done as the results were not replicated.

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Appendix 1

PHOTOSYNTHESIS MEASUREMENTS METHODS

Mechanistic *A/Ci* curve analysis

The following information is based on the manual of photosyn programme which is a window software used for analysis of photosynthesis. The photosyn program was used to make estimates of four parameters that describe some photosynthetic characteristics of leaves. It is used for the analysis of *A/Ci* (CO_2 assimilation rate vs internal leaf CO_2 concentration) curves which were produced by using IRGAs to measure the photosynthetic activity over a range of concentrations of CO_2. The parameters leaf respiration (Resp), Vc_{max}, J_{max} and triose phosphate utilisation (TPU) were calculated by a minimisation routine that produces parameter values that represent the best fit for the data.

Summary

The program used the model proposed by Farquhar et al. (1980), as subsequently modified by Harley *et al.* (1992). These authors recognised that the A/C_i response consists of three phases. The first of these phases is the initial response below C_i concentrations of approximately 20 Pa; here, ribulose bisphosphate (RuBP) was saturated, and Rubisco activity limits carboxylation. The slower rise of the curve beyond its inflection point represents the second phase. The higher C_i levels present within this phase result in the limiting factor being the supply of RuBP. This model was used to provide estimations of the maximum rate of carboxylation by Rubisco (Vc_{max}), the PAR-saturated rate of electron transport (J_{max}) and the rate of triose phosphate utilisation (TPU) which indicates the availability of inorganic P for the Calvin cycle . In the calculation of these parameters according to the model, the following equation was used to express the relationship between assimilation rate and internal CO_2.

This reliesed on the concept that it was a minimum of any of the three factors; Rubisco activity (W_c), RuBP regeneration (W_j) and regeneration of inorganic phosphate (W_p) which limits CO_2 assimilation. That was:

$$A = \left(1 - \frac{0.5\,O}{t\ C_i}\right) \times \min(\mathrm{Wc}, \mathrm{Wj}, \mathrm{Wp})\ \text{ - }\ \mathrm{R}_{\mathrm{day}}\ .$$

R_{day} refers to the release of CO_2 in the light by processes other than photorespiration and may be estimated using the modelling equations below or by a number of experimental procedures . When the rate of carboxylation was solely limited by the activity of Rubisco, carboxylation can be described by the equation:

$$Wc = \frac{Vc_{\max}.Ci}{\left[Ci + Kc(1 + O / Ko)\right]}$$

where K_c and K_o respectively were the Michaelis-Menten constants of Rubisco for CO_2 and O_2 and O was the concentration of oxygen in the stoma [Pa]. The conditions of this limitation were imposed by low C_i levels (<20 Pa) and high irradiance (>1500 µmol photons m^{-2} s^{-1}). When electron transport limits photosynthesis by the regeneration of RuBP, carboxylation rate were expressed by the following equation;

$$W_j = \frac{J.C_i}{4(C_i + O / t\,)}$$

Tau (τ. represents the specificity factor for Rubisco . The factor 4 represents the fact that four electrons will generate sufficient ATP and NADPH to regenerate RuBP. The J, the potential rate of electron transport, were calculated using the empirical expression relationship (Harley *et al*. 1992)

$$J = a\,.\,I \div \sqrt{1 + (\frac{a\,.\,I}{J_{\max}})^2}$$

Where α was the efficiency of light conversion and J_{max} was the light saturated rate of electron transport and I was the incident radiation. Carboxylation limited by the regeneration of inorganic P can be described by the expression,

$$W_p = 3(TPU) + \frac{0.5 \times Vo \times O}{C_i \times t}$$

Where V_o represented the rate of oxygenation of Rubisco. Using the Farquhar *et al.* (1980) model, based on these equations, valuable information of the biochemical limitation to photosynthesis was obtained by applying the model to the basic A/C_i curve obtained experimentally. The program used an iterative procedure to make estimations of Vc_{max}, J_{max} and TPU from the A/C_i curves obtained through gas analysis (Harley *et al.* 1992, Wullschleger 1993). Initial estimates were obtained using the first half of the data to estimate Vc_{max} and R_{day}. This data was then incorporated into the model to estimate values for J_{max} and TPU. After initial estimates were obtained the program models all three curves together and minimizes the sum of squares (Nelder-Mead simplex method).

Instead of fitting the function W = min (Wc, Wj, Wp) some authors prefer to specify that one of Wc, Wj, Wp will apply in particular ranges of C; for specified limits c1 and c2 the program then fitted the function.

$$A = \left(1 - \frac{0.5\,O}{t\ C_i}\right) \times W\ -\ \mathrm{R}_{\mathrm{day}}\,.$$

where
$W = Wc \quad (0 < Ci <= c1)$
$W = Wj \quad (c1 < Ci <= c2)$
$W = Wp \quad (c2 < Ci)$

This facility was permitted by allowing users to specify limits c1 and c2 under Parameters. Users also chose to model any of the three curves in different combinations, depending on their data sets. For instance, it should be noted that TPU evaluation (Wp) should only be attempted on those curves that show a TPU limitation, indicated by a saturation of the curve (Wullschleger 1993). Various studies have shown that these parameters obtained from the A/C_i curves were modified by treatments such as elevated CO_2 concentrations (Harley *et al.* 1992) and nitrogen limitation . Important within these calculations were the values of a range of parameters that described the kinetics of Rubisco and the influence of temperature on these parameters.

They have modelled this influence using the data and approach of Harley ***et al.*** (1992) and Wullschleger (1993), permitting the calculation of Kc, Ko and τ. However they had also included the ability to alter these values if users have their own measurements of these parameters, and this was achieved either by altering the default parameters from the menu (which may be saved), or by entering Kc, Ko and τ directly for use by the modelling functions. Similarly the oxygen concentration and α (alpha) may be altered from the default values.

Operation

Data for the *A/C_i* curve to be analysed was entered into the grid provided, beginning with the lowest values in row one. Data was copied in from a Windows based spreadsheet using the Windows Clipboard and Edit, Paste data. *C_i* data was converted into Pa (approx. = ppm *0.10) and assimilation data as µmol CO_2 m^{-2} s^{-1}. Values for the light level and leaf temperature were entered into the boxes provided. The parameters that described biochemical characteristics of leaf components were included in a table and were edited as required. The default values were those used by Harley ***et al.*** (1992) and Wullschleger (1993) in their analysis of cotton and a wide range of C_3 plants. These values were edited and saved for future work.

Alternatively one could select to enter Kc, Ko and τ directly, in which case the default parameters were ignored. Initial est. was selected next to determine a set of initial values for the calculated parameters. These values were important to allow the minimization routine to operate. Select Fit curves and the application will minimize the parameters to produce a minimum sum of squares for the difference between experimental and model data. One may select between different curves to be used in the minimization routines, e.g. only Wc and Wj if appropriate for the data. For some data it may be necessary to manually enter the initial values to allow the minimization routine to operate successfully.

The ability to fix each of the parameters is included and then minimization with some parameters fixed is allowed. This permits modelling of the parameters if one of the parameters has been estimated from an alternative experimental method. The program will estimate Resp, Vc_{max}, J_{max} and TPU. Use the examples to demonstrate the operation.

A graph showing the data and the model functions was produced. For some data it may be necessary to specify the limits to be used for the estimation of each curve. This is permitted by selecting the checkbox Specify limits for equations and setting the limits (as Pa values along the x axis) in the Parameters option. Alter these values then select Initial est. and Fit curves again.

Appendix 2

PROCEDURE FOLLOWED FOR CHEMICAL ANALYSIS

Nitrogen

Digestion

The following information is based on method used by Smith (1980) to analyse nitrogen. Dry plant material (leaves) were grinded finely into a 0.1 mm mesh, 0.1 g aliquots of the material was measured out into thick-walled boiling tubes marked with permanent black pen. Standards were also prepared at the same time and they went through the same digestion process along with the samples. Zero was set as a blank where 4.5 ml of distilled water was added, for the standards a N stock solution [(1 mg N mL^{-1}): 4.7170 g $(NH_4)_2SO_4$ in 1 L distilled water]was added on each tube, 0.4 ml, 0.8 ml, 1.5 ml, 3.0 ml and 4.5 ml. 4.5 ml of acidified salicylic acid [(34 g salicylic acid L^{-1}): 34 g salicylic acid per liter of H_2SO_4] was added in each tube (samples, standards and a blank). The tubes were placed in a cold digestion block and heated at 150 ºC overnight to drive off water, these were digested further by using the following time and temperature sequence: 220 ºC for 1 hour, 250 ºC for 1 hour, 280 ºC for 1 hour, 300 ºC for 1 hour, 350 ºC for 2-4 hours until the digest were clear. If there was lots of dark or pink material on the inside of the tube, they were allowed to cool to 150 ºC and carefully rolled, the remaining liquid in the tubes around the walls of the tube to wash this residue back down into the acid solution.

The samples were re-heated in the above sequence using 30 minutes intervals at each of the lower temperatures and 1-2 hours at the highest temperature until the digest were clear again. The tubes were allowed to cool down, 5-10 ml of distilled water added into each tube, each digest was then made up to 50 ml by adding more distilled water to make it up to volume, mixed thoroughly, solution transferred to a 50 ml measuring cylinder (Smith 1980).

Reagents

0.12 % EDTA-Na_2: 2.4 g EDTA-Na_2 dissolved in 2 L distilled water.

Reagent A: equal volumes of (i) and (ii) were mixed.

- (i) 0.5 % sodium nitroprusside: 0.5 g nitroprusside in 100 ml distilled water, this solution was made fresh every day.
- (ii)10 % phenol in 95% ethanol: 100 g phenol dissolved in 1 L of 95 % ethanol. Stock solution was kept in the dark.

Reagent B: four parts of (iii) with one part of (iv) were mixed.

- (iii) alkaline P buffer: 6.93 g Na_2HPO_4 in 500 ml and 20.65 g NaOH in 300 ml of distilled water. Dissolved separately making sure the total volume does not exceed 1 L. The two solutions were mixed together and made up to 1 L by adding 200 ml distilled water. The Stock solution was stored.
- (iv) 1.5 % sodium hydrochlorite: 10 ml concentrated (15 %) sodium hypochlorite was added to 50 ml distilled water. The solution must be made fresh everyday because it loose its potency with time.

Colour Determination of Kjeldahl Digest Solution

0.5 g ml aliquots of digest solution was taken out by a pipette into 50 ml volumetric flasks, the sample was shaken before the aliquot was taken out, 25 ml (0.12 %) EDTA-Na_2 was added into each tube depending on how many samples will be run, followed by 2 ml of reagent A then 4 ml of reagent B, this was then diluted with 18.5 ml of distilled water. The standards were run at the same time with the samples and in the same way as the other samples, same amounts of aliquots 0.5 ml were taken out from the standard digest solutions into the 50 ml volumetric flaks by a pipette, 25 ml EDTA- $Na_{2,}$ reagent A followed by B, then this was diluted with 18, 5 ml of distilled water. The flasks were thoroughly mixed and sealed with parafilm. This was allowed to sit for an hour for the blue colour to develop. The machine was then zeroed with a blank, absorbency read at 635 nm (Smith, 1980).

Phosphorus

Reagents

The following information is based on method used by Murphy and Riley (1962) to analyse phosphorus (P). The mixture was made up fresh each day, only a pale yellow colour was used. All the reagents axcept for ascorbic acid was made up in advance, clearly labelled and stored.

1. 2.5 M H_2SO_4: 140 ml H_2SO_4 + 860 H_2O
2. Ascorbic acid: weighed out and dissolve in distilled water just prior to making M & R solution: 5.28 g dissolve in 300 ml distilled water.
3. Amonium molybdate $(NH_4)_6\ MO_7)_{24}$. $4H_2O$:20 g dissolved into 500 ml distilled water, this was then stored in a dark bottle.
4. Antimony Potassium tartrate: 0.5486g dissolved in 200 ml of distilled water.

Volume of Reagents

Depending on the number of samples, 1 liter of M & R was made for 80 – 110 samples. 500 ml of H_2SO_4 was taken out to a 1000 ml beaker (1 L), 300 ml of ascorbic acid added into the beaker, 150 ml of ammonium molybdate stock was added, 50 ml of Antimony Potassium was then finally added to make up 1 L of M & R solutionThe standards were also runned at the same time as the other samples. The stock solution was prepared by weighing out 0.4394 g KH_2PO_4 dissolving it into 1 L. (100 µg P ml-1), before the samples were run, 10 ml were diluted with distilled water to make up 500 ml (2 µg P ml-1). Zero was set as a blank; 47 ml of distilled water was added to make up 50 ml.

The standards were run as follows: into 2.5 ml of stock solution, 39.5 ml of distilled water was added, 5.0 l of stock was diluted with 37 ml of distilled water, 7.5 ml was diluted with 34.5 ml of distilled water, 10 ml received 32 ml of distilled water, and 15 ml diluted with 27 ml of distilled water. 8ml of M & R was added to all of the standards.

Murphy and Riley Colour Development

The samples were prepared by adding 37 ml of distilled water into 50 ml volumetric flasks, 5 ml aliquots of digest solution was taken out by a pipette into 50 ml volumetric flasks and 8 ml of M & R solution was then added to make up 50 ml. The flasks were thoroughly mixed and sealed with parafilm. This was allowed to sit for an hour for the blue colour to develop. The machine was then zeroed with a blank, absorbency read at 882 nm (Murphy and Riley, 1962).

Acid Butanol Assay Proanthocyanidins (Condensed Tannins)

Extraction

The following information is based on method used by Hagerman (1995) to extract CT and analyzing them. Dry plant material was weighed out, 0.04 g of ***C. apiculatum*** and 0.1 g of *A. grandicornuta* were weighed out into small vessels, 3 ml of 70 % acetone was added into each vessel and the cap was put on making sure it was tight. The vessels were sonicated for 30 minutes at 4 º C, crushed ice was put to minimize the temperature. After 30 minutes they were transferred into a centrifuge for 10 minutes at 3000 rotations per minute (rpm).

Reagents

Acid butanol: 950 mL of n-butanol was mixed with 50 ml concentrated HCl.

Iron reagent : 2 % Ferric ammonium sulfate was added into 2 M HCl. 20 ml of Concentrated HCl was brought up to 100 ml with distilled water which made 2 M HCl. 0.5 g $FeNH_4(SO_4)2 \times 12\ H_2O$ was dissolved in 25 ml of 2 M HCl, this reagent was then stored in dark bottle.

Colour Determination

In 13 x 100 mm screw cap culture tubes, 6 ml of the acid butanol reagent was added using a pipette, 0.1 ml of the aliquot of the sample was added with three replicates for each sample, 0.2 ml of the iron reagent was added, and the sample was vortexed with a vortex mixer.

Tubes were capped loosely and put in a boiling water bath for 50 minutes. The tubes were cooled and the absorbance read at 550 nm. The standards were run at the same time using 1 mg ml-1 Sorghum tannin. The stock solution (4mg STml-1) was prepared by weighing out 20 mg ST in 5 ml of 70 % acetone.

Five standards were made where the concentration of 0.5 was prepared by diluting 0.25 ml of the stock with 1.75 ml 70 % acetone, 1.0 concentration was prepared by diluting 0.5 ml of the stock with 1.5 ml of acetone, 2.0 concentration: 1 ml of stock solution was added into 1 ml of 70 % acetone, 3.0 concentration: 1.5 ml of stock solution was diluted with 0.5 ml of 70 % acetone and the concentration of 4 was not diluted, the stock solution was added as it was. The tubes were vortexed, capped loosely and put in a boiling water bath for 50 minutes. The tubes were cooled and the absorbance read at 550 nm. The standard curve was used to calculate the % condensed tannin for all the samples (Hagerman, 1995).