Quantifying *Acacia* defences: understanding the role of hooks, spines and architecture

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Abstract

The effectiveness of the structural defences of *Acacia*s vary within and between species as a result of differences in thorn type (hooks versus spines) and branching architecture. This study aims to develop methods for quantifying the effectiveness of defences, thereby allowing for comparisons between individuals and localities. Measurements of a range of thorn and branching parameters of eight *Acacia* species were used to calculate three indices (stripping, biting and branching) describing different components of *Acacia* structural defences. These were combined to form an overall defence index that was tested against the results of three feeding simulation tests as well as actual animal feeding rates obtained from trials using nyala held in bomas. The stripping, biting and branching indices were shown to provide a good measure of the different components of a structural defence, although the scaling of their contribution to an overall defence index is complicated by variation in the suite of browsers and the resources available at a locality. The indices developed in this study provide a useful tool for assessing the role of large mammal herbivory in different environments when attempting to understand variation in the life history strategies of *Acacia*s.

Introduction

Thorns are designed to limit the damage done to plants by herbivores. Just as there is a wide range in the shape and size of herbivores, there is also significant variation in the structural defences of plants. This variation in plant structural defences has been shown to affect different sets of herbivores in different ways (e.g. Cooper & Owen-Smith 1986, Milewski *et. al.* 1991, Bond *et. al.* 2004). Thorns seemingly offer no protection against insect herbivory, while their ability to defend against megaherbivores such as black rhino may also be limited (Owen-Smith & Danckwerts 1997). The benefits of structural defences in reducing herbivory damage have, however, been shown empirically for intermediate sized mammal herbivores such as impala and kudu (Cooper & Owen-Smith 1986). The browsing techniques of these two species are affected in different ways by variation in thorn design (Cooper & Owen-Smith 1986), and it is this finer scale of structural defence against herbivory which is the focus of this study.

The African savannas are one of the few remaining ecosystems where large mammal interactions with their natural environment can still be observed. These systems have evolved with top down controls such as fire and herbivory acting as integral components in shaping them (Bond *et. al.* 2003, Scholes *et. al.* 2003). The natural variation in the balance and intensity of fire and herbivory pressure creates a variable pattern of plant distribution and response across landscapes (Gowda 1996, Young et. al 2003). This effect can be quite marked within a species, as has been shown for the phenology and structural defence of *Acacia karroo* (Archibald & Bond 2003), and also between species (Owen Smith & Dankwerts 1997, Bond 1997).

A. karroo is a widely occurring tree species in southern Africa, with different varieties being typical of different environments. Archibald & Bond (2003) describe three growth forms from forest, mesic savanna and arid karoo-shrubland environments respectively. Individuals occurring in mesic savannas, where fire is often the most important top-down influence (Bond 1997), develop as single stemmed pole-forms designed for rapid height gain to escape the fire trap (Bond & van Wilgen 1996). In the karoo-shrublands, where fire is of little importance (Bond 1997), the increased importance of herbivory has resulted in *A. karroo* typically being multi-stemmed, shorter and having a distinctive cage-like architecture (Archibald & Bond 2003). These individuals also had the longest spines recorded across the three environments which, when coupled with the cage architecture, limit herbivore access to the inside of the plant and its soft new shoots.

The intraspecific variability in growth form of *A. karroo* illustrates the potential significance of the impact of herbivory on a species. Defences against herbivores are produced at a cost to the plant, which needs to be traded off against the benefits in terms of the reduction in herbivory (Loehle 1988, Gowda 1996). The interspecific variation in degree and form of structural defences observed in the genus *Acacia* suggest an array of potential life history strategies and limits to populations variously susceptible to and influenced by herbivory. Understanding how these different defences work and the degree of protection they afford individuals is central to this study.

Acacia structural defences are made up of thorns (hooks and/or spines) and the branching architecture that presents them. Two main *Acacia* thorn types exist – short, hooked thorns (hooks) and long straight thorns (spines). Spines are produced from leaf stipules, while hooks can develop from the outer layers of the branch or from leaf stipules (Coates Palgrave 2002). *A. tortilis* has modified some of its spines into short hooked thorns. The density, length, angle, robustness and colour of thorns are all parameters that can vary between species, within species and even within individuals (Midgley et. al 2001). Leaf length is also considered an important variable in *Acacia* defences, with individuals with smaller leaves being considered relatively better defended by the same defence than individuals with larger leaves (Milewski *et. al.* 1991, Gowda 1996). Architecture varies on the scale from the growth form of the whole tree to the specific localised branching pattern (Archibald & Bond 2003). This branching pattern can vary from creating a closed cage effect that effectively excludes herbivores to a loose, open pattern that allows herbivore access deeper into the tree (Archibald & Bond 2003).

Variation in *Acacia* structural defences affects the bite size (grams/bite) and bite rate (bites/minute) of herbivores, and thus their overall feeding rates (grams/minute; e.g. Cooper & Owen-Smith 1986, Milewski *et. al.* 1991, Belovsky *et. al.* 1991 and Gowda 1996). Cooper & Owen-Smith (1986) calculated feeding rates for kudu, impala and goats for plants with their thorns intact and also with their thorns removed. Hooks and spines had different effects on the different animals. Hooks were most effective in decreasing the bite rates of the smaller impala and goats by catching their lips and tongues as they picked off small clusters of leaves. The kudu took larger bites and were impeded most by spines which limited their bite size. The smaller animals were quite effective at manoeuvring between the spines.

Milewski *et. al.* (1991) assessed variation in the structural defences of *A. tortilis* within and above the reach of giraffe along a gradient of giraffe browsing pressure. Spines were noted to be significantly shorter above the reach of giraffe. Within the reach of giraffe, spine length increased while leaf length decreased in response to an increase in giraffe browsing pressure. Gowda (1996) looked at the effect of variation in the defence of *Acacia tortilis* on goat herbivory. He describes two forms of goat browsing: pruning, in which branches and leaves are removed, and picking, where only leaves are removed. Increases in the density of spines lead to a shift in goat feeding from pruning to picking, with the result of an overall decline in the feeding rate. This shift resulted in a higher leaf loss being associated with increased spine density, but with the benefit of greater protection being afforded to the branches. It may thus be conjectured that while an increase in spine length increases protection for leaves (Milewski *et. al.* 1991), an increase in spine density protects branches (Gowda 1996).

Midgley *et. al.* (2001) assess *Acacia* structural defences widely across the genus, and propose a 'stems not leaves' hypothesis for the functioning of spines. This is based on an examination of the relationship between leaf length and spine length that reveals leaves to almost always be much longer than spines. They also point out that the generally wide angle between spines (>90°) results in the leaves growing midway between them being relatively undefended. The fact that the large majority of spines are white is argued to make them relatively easier for colour-blind herbivores to avoid when picking off leaves, and it is thus suggested that this is rather to act as a long distance visual deterrent to potential browsers. Hooked thorns quite clearly must operate in an entirely different manner as a structural defence. Midgley *et. al.* (2001) suggest that hooks may be designed to prevent leaf stripping, a role which could act in conjunction with the slowing of the biting rate observed by Cooper & Owen-Smith (1986).

The ability to quantify *Acacia* defences against different browsers allows for comparison between individuals, localities and species. This is an important tool for understanding variation in *Acacia* life history strategies, as amongst others, the relative importance of top down herbivory controls can be assessed. In order to effectively quantify *Acacia* defences, a holistic approach that incorporates the specific design and function of hooks and spines separately, and which then couples this with plant architecture, is essential. The aim of this study is to design methods for quantifying the various aspects of *Acacia* defences, which then can be calibrated using actual herbivory rates.

Methods

Study sites

Field work was conducted in two sessions, the first in the far north of the Kruger National Park during March 2004 and the second in the Hluhluwe-Umfolozi Game Reserve during July 2004 (Figure 1). Data for four species of *Acacia* were collected from each locality. In the Kruger National Park, *A. erubescens* and *A. tortilis* were collected from the Pafuri region near the Thulamela Heritage Site, where they occurred on the rocky hill slopes and valley bottom respectively. *A. robusta* and *A. nigrescens* were collected from the plains around the Punda Maria rest camp. *A. davyi* was collected from the foot slopes below Hilltop camp in Hluhluwe, while *A. caffra* and *A. burkei* were collected in the hilly Ledube area further south in Hluhluwe. *A. grandicornuta* was collected close to the Umfolozi River in the Umbondwe area of the Umfolozi Game Reserve.

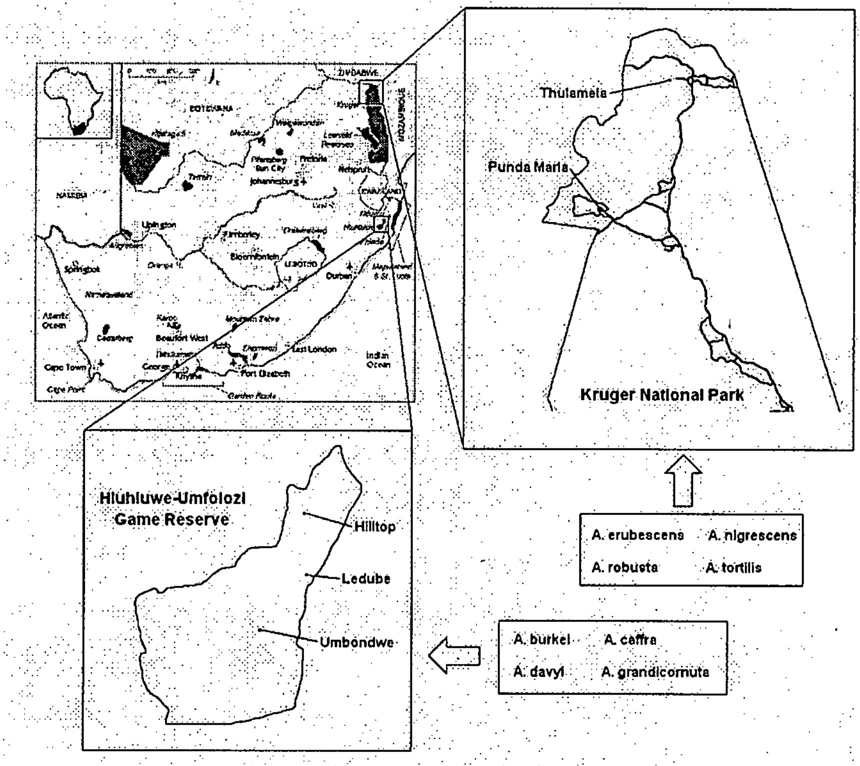

Figure 1: Location of the study sites from which the *Acacia* species indicated in the boxes were collected. Fieldwork in the Kruger National Park was carried out during March 2004 and in the Hluhluwe-Umfolozi Game Reserve during July 2004.

The Pafuri and Punda Maria regions of the Kruger National Park receive approximately 430mm and 540mm of rainfall annually (Venter *et. al.* 2003), and are thus considered to be xeric savanna systems (Bond 2003). Fire return periods in the Pafuri region are generally greater than every ten years, while the area around Punda Maria burns approximately every 7 – 8 years (van Wilgen *et. al.* 2000). The Hluhluwe Game reserve is considerably wetter, with a mean annual rainfall in the vicinity of Hilltop camp of 950mm and 860mm for the Ledube area (D. Balfour personal communication). These regions are referred to as mesic savannas (Bond 1997). Rainfall decreases to the south of Hluhluwe in the Umfolozi Game Reserve, with an annual mean of approximately 700mm at the Umbondwe site, which would be considered to fall between the mesic and xeric savanna limits (Bond 1997).
Fire frequency in Hluhluwe-Umfolozi is correlated with rainfall, with the wetter regions burning more frequently than the drier areas (Balfour & Howison 2002). Fire return periods for the Hilltop, Ledube and Umbondwe study sites are approximately less than 2.5 years, 2.5 – 3 years and 5 – 8 years respectively (D. Balfour personal communication).

Sampling methods

Ten individuals of each of the eight study species were sampled. These species were selected to represent a range of structural defences of the genus *Acacia*. Four of the study species (*A. erubescens*, *A. nigrescens*, *A. caffra* and *A. burkei*) have only paired hooked thorns. Three species (*A. robusta*, *A. davyi* and *A. grandicornuta*) have paired spines only, while *A. tortilis* has both paired hooked thorns and paired spines.

Measurements

Three branches were cut from each individual at a branch diameter of 12mm. Thorn densities were calculated from counting the number of thorns on the last 50cm of the longest branch. Thorn measurements were made on the left hand thorn of the first five pairs of thorns moving outwards along the end 50cm of the longest branch. For *A. tortilis*, which has hooks and spines, five thorns of each type were measured. The following measurements were made on each thorn: length (thorn base to tip), gape width (perpendicular length from branch to thorn tip), angle of orientation (measured with 0° pointing back along branch to tree interior) and size of base (thorn attachment

Figure 2: Sock test being performed on *A. erubescens* at the Pafuri study site.

The third feeding simulation, the clamp test, made use of a large pair of wooden scissor apparatus with 8.5cm x 7.5cm plates as jaws. These were covered with plasticine. The clamps were then used to simulate an animal biting five branches of the study individual. The test was scored as the ratio of the number of thorn impressions to branch impressions that were made in the plasticine (Figure 4). A high score can be attained from this simulation by two means. Firstly, a low number of branch impressions can be caused by the thorns preventing the jaws actually closing onto the branch, and secondly, a high thorn density increases the number of thorn impressions. This test was performed three times on each study individual.

Figure 3: The apparatus used for the muzzle test (A). Scoring the muzzle test involved assessing the ratio of severe to superficial scratches left in the plasticine (B).

Figure 4: Plasticine 'jaw' plate of clamp. Note the branch and thorn impressions that have been made in the plasticine. The clamp test was scored as the ratio of thorn impressions: branch impressions

Nyala feeding trials

Feeding trials with nyala were carried out in the Centenary Game Capture Centre in Umfolozi Game Reserve. Branches of *A. burkei* and *A. caffra* were offered to ten nyala (eight females and two males) that had been held in the boma for three weeks. These *Acacia* species were chosen for initial trials as they represent an apparently well defended (*A. burkei*) and a less well defended species (*A. caffra*). Initially three and in later trials two branches of one species were tied at a height of 1.2m on the boma wall. A branch was tied up at the same height on the outside of the nyala boma to control for water loss. All branches were weighed using an electronic pan balance directly before being presented to the nyala. The branches were weighed again immediately after each feeding trial was concluded. The nyala were observed feeding on the branches from a distance of two to five metres. The number of bites taken in a feeding bout as well as the duration of the feeding bout was recorded. Feeding duration was measured from the moment an individual took its first bite until it dropped its head to move away from the branch it was feeding on. The feeding rate (g/min) of the nyala was calculated by multiplying bite size (g/bite) by bite rate (bites/min).The period of observation of each set of branches tied up in the boma varied between one to two and a half hours. This observation period varied in response to the nyala activity, as well as the time of day and weather conditions. All branches that were presented to the nyala were photographed before being tied up in the boma. Those that were fed on were then photographed again after the experiment so as to allow for a qualitative assessment of the nature of the damage done to each branch by nyala browsing.

Analysis

All analyses were carried out using Microsoft® Excel 2002 (Microsoft Corporation) and JMP™ 5. 0. 1. 2003 (SAS Institute Inc.).

Measurements

The mean values of the measured thorn parameters (angle, base, length and gape), thorn density and leaf length were calculated for each species. Where significant differences were detected by an analysis of variance in the dataset, the means for each species were tested against each other for significant differences at the 95% confidence level using the Tukey-Kramer HSD test.

Feeding simulations

The mean score of each species for each of the three feeding simulations was calculated and, where appropriate, was compared against the others using the Tukey-Kramer HSD test (95% confidence interval). The clamp test scores were also divided by thorn density in an attempt to distinguish between the two possible means of obtaining a high thorn: branch impression ratio as described earlier (i.e. high number of thorn impressions versus low number of branch impressions).

Indices

In order to quantify comparative defences of the *Acacia*s, the thorn dimension, thorn density and branching pattern data were combined into four indices. This is an attempt to put a value on different aspects of an individual's structural design only in terms of its ability to defend itself against herbivory. The stripping index is largely aimed at describing the effectiveness of a hooked thorn defence, and the biting index the effectiveness of a spiny defence. A branching index is also calculated to give some indication of the 'caginess' of an individual. These three indices are then combined into the defence index, which is an attempt to describe the overall degree of protection of the individual against herbivory, irrespective of its strategy (e.g. hooks vs. spines). In all of the indices thorn density is calculated as thorns/cm, all thorn parameter measurements are in centimetres and angles are measured in degrees.

a) Stripping index

$$\text{Stripping index} = \text{Thorn density} + \text{Base}*\text{Length}/\text{Gape} + (180 - \text{angle})/180$$

This index has three components, each aimed at describing an important feature necessary for protection against a stripping motion from the inside of the tree outwards. Thorn density is considered important as it seems logical that more thorns would confer a greater degree of protection. The middle term of the index is designed to quantify the strength of attachment of the thorn and also include a size parameter in the equation (Figure 5). The size of the base is the first important part of the attachment strength as this is the point of contact with the branch, but also is a potential indicator of the size of the thorn. Thorn length is also factored into this term as longer (bigger) thorns should inflict more pain than smaller thorns. Long thorns with large gapes offer a higher degree of leverage to bend or break them off when pulled against than would the same length thorn with a smaller gape. The length/gape ratio is thus also considered as being an important component of the strength of attachment term. The last term of the index is simply designed to score the orientation of the thorn, with a thorn pointing directly back along the branch being considered more effective against a stripping motion than a thorn whose point is angled up from the branch or even pointing towards the outside of the tree (Figure 6).

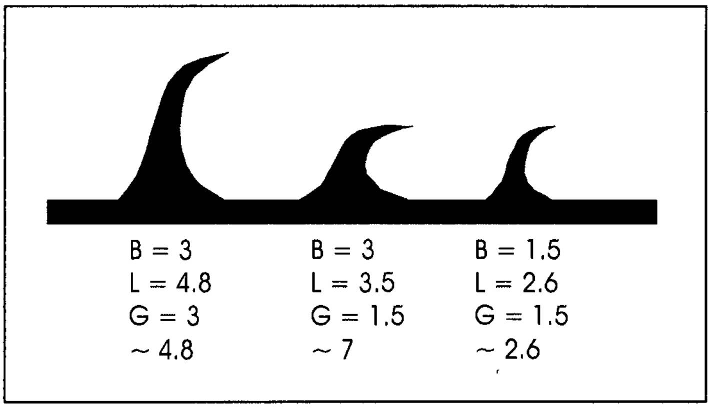

Figure 5: Effect of variation in hooked thorn base (B), length (L) and gape (G) when calculating the second term of the stripping index (~; B*L/G). The middle thorn is considered as being the best designed in terms of strength of attachment. The numbers in the illustration represent the relative size of each parameter for the three thorns presented.

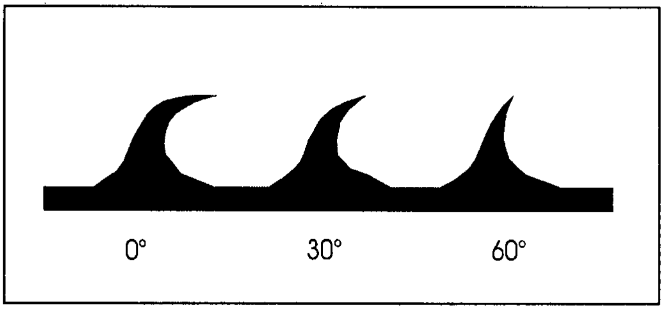

Figure 6: Variation in hooked thorn angle (approximate angle shown below thorns). The left hand thorn is considered to be best designed to defend against a pulling motion to the left along the branch, while the right hand thorn is considered as being the least well designed.

b) Biting index

Biting index = Thorn density + Length*Base + (90 – absolute (90 – angle))/90

The first term of the biting index assumes that the higher the thorn density the greater the degree of protection that is conferred. The second term attempts to describe the benefits of having longer thorns and more robust thorns when defending against branch biting (Figure 7). The third term of the equation maximises the score of thorns orientated at right angles to the branch (Figure 8). It is also does not discriminate between a thorn leaning 10° forward or 10° backwards on the branch, as both will score the same value for this term. The seemingly complicated nature of the third term of the index is partly a construct of the fact that thorn angle was measured from 0° being directed back along the branch towards the main stem of the tree.

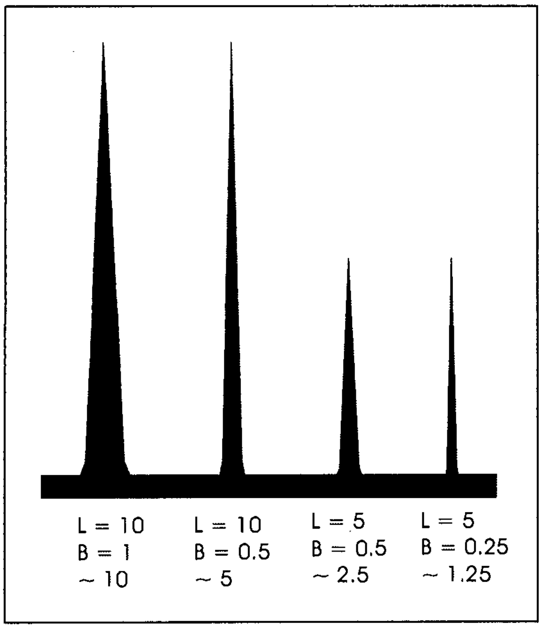

Figure 7: Effect of variation in spine length (L) and base (B) when calculating the second term of the biting index (~; L*B). The left hand thorn is considered as being the best designed in terms of defending against branch biting. The numbers in the illustration represent the relative size of each parameter for the three thorns presented.

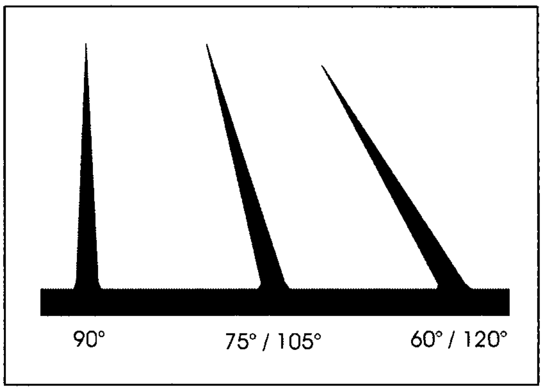

Figure 8: Variation in spine angle (approximate forward/backward orientation angle shown below thorn). The left hand thorn is considered to be best designed to defend against a biting which would come from the top and bottom of the frame, while the right hand thorn is considered as being the least well designed to defend against biting.

c) Branching index

Branching index = Total branch length / Longest branch length

This index is the same as the 'branching ratio' equation used by Archibald & Bond (2003). A higher score indicates a higher degree of branch ramification (Figure 9). A second method proposed for determining the shape of branching of a tree (L. Kruger personal communication) is to calculate the ratio of the actual length of the longest branch to the direct distance between the tip and base of the longest branch. Measurements to calculate this ratio were not obtained for *A. erubescens* and *A. tortilis*.

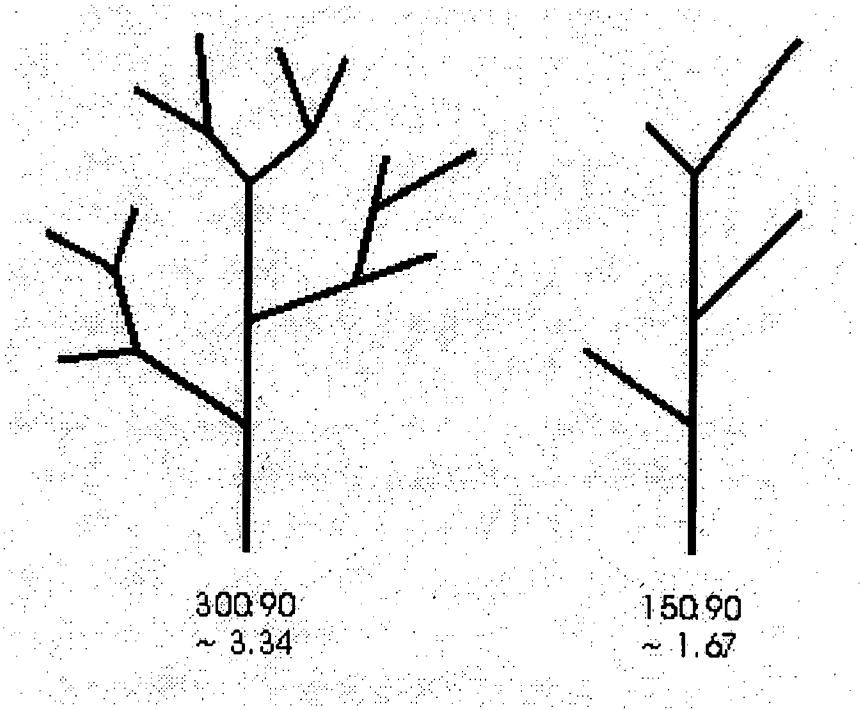

Figure 9: The branching index is the ratio of the total length of branches to the length of the longest branch. The lengths of the longest branch in both illustrations are equal, but the branch on the left scores more highly in the branching index due to its greater total length of branches (~ denotes branching index score).

d) Defence index

Defence index = Stripping index + Biting index + (Branching index/10)

The overall defence index simply sums the three indices describing components of *Acacia* structural defences. The branching index weighting is scaled down so that it has a similar weighting to the other two component indices. The defence index score for *A. tortilis* was calculated by summing the stripping index and biting index scores derived for the hooks and spines of this species when calculated separately, rather than using averaged thorn dimensions across the two types.

e) Feeding trials versus indices

The ratio between the feeding rates of nyala on *A. burkei* and *A. caffra* was compared to the ratio between the defence indices calculated for these two species. Similarly, the ratios between the feeding rates measured by Cooper & Owen-Smith (1986) for kudu, impala and goats on *A. burkei*, *A. caffra* and *A. tortilis* were compared to the defence scores calculated in this study. As an addition to make up for the lack of comparable spiny species, feeding rates on *A. karoo* and *A. nilotica* were equated with the defence indices of *A. grandicornuta* and *A. robusta* respectively. This decision was based on the relative similarity of the rankings of these species in the feeding rate gradient of Cooper & Owen-Smith (1986) and the defence index gradient of this study from *A. tortilis* to *A. davyi*. This comparison of feeding rates relative to defence indices gives some indication of how well the indices reflect the ability of a plant to defend against biomass loss. It does not, however, distinguish between the different types of material that a plant loses to herbivory (i.e. leaves versus branches). The feeding simulation scores of each species were plotted against their index scores, and the lines of best fit analysed. The effect of changing various parameters in the make up of the indices was explored and some variations are presented.

The mean species score for each of the four indices was calculated and variance in the dataset analysed for significant differences. Where differences existed, the means were compared with the Tukey-Kramer HSD test and a 95% confidence limit. A stripping index and biting index score was also calculated separately for the hooks and spines of *A. tortilis*. These were included in the analysis of index variation between species.

Results

The structural defences of *Acacia*'s show a large degree of variation both between and within species. This variability will naturally affect the confidence in any index which attempts to put a single number to how well defended a species is. It is hoped that the efforts to cross check indices against real and simulated browsing will confer a reasonable degree of support for the methods proposed.

Measurements

The mean thorn dimensions for the individuals of each species measured in this study are shown in Table 1. The analysis of variance showed significant variation in the thorn angle and base size datasets (angle: $F_{9,\,1566} = 359.77$; base: $F_{9,\,1566} = 291.27$; $p < 0.0000$). A significant distinction in the design of spines and hooks is apparent in terms of mean thorn angle (spines = 100.2°, hooks = 38.7°; $t_{1048} = -45.793$; $p < 0.0001$). Spine angles range from 86.3° (*A. grandicornuta*) to 107.4° (*A. robusta*), while hook angles vary from 28.7° (*A. burkei*) to 64.3° (*A. caffra*). The mean base size of hooks (4.9mm) is significantly larger than that of spines (2.6mm; $t_{1048} = 21.42$; $p < 0.0001$), with only *A. robusta* (3.7mm) of the spiny species having a thorn base comparable to that of the hooks only species. The hook base of *A. tortilis* (1.9mm) is significantly smaller than its mean spine base (2.8mm), and also the bases of the hooks only species included in the study (Tukey-Kramer HSD $q^* = 3.168$; $p < 0.05$).

Gape and length dimensions (Table 1) are closely correlated for hooks ($r = 0.83$) and spines ($r = 0.99$). The analysis of variance showed significant differences to exist in the hook and spine datasets for both gape and length (hook gape: $F_{4,\,745} = 100.19$, hook length: $F_{4,\,745} = 126.00$, spine gape: $F_{4,\,821} = 54.71$, spine length: $F_{4,\,821} = 52.82$; $p < 0.0001$). Of the hooks only species, *A. burkei* (6.6mm) had the longest mean thorn length and *A. caffra* (3.6mm) the shortest. The mean spine length of *A. tortilis* (45.6mm) was greater than that of *A. robusta* (30.5mm), despite the fact that the longest spines measured in this study belonged to *A. robusta* individuals. The high

Table 1: Mean thorn dimensions (angles in degrees (°); base, gape, length in mm) and standard deviation (s. d.) of the eight *Acacia* study species. *A. tortilis* refers to the mean values for the hooks and spines of this species combined, while *A. tortilis* (hooks) refers to hook measurements only and *A. tortilis* (spines) refers to spine measurements only. Rows not having the same letter in the 0.05 columns are significantly different at the 0.05 level (Tukey Kramer HSD). 0.05* indicates that significance between rows was calculated separately for hooks and spines. Mean values for hooks and spines do not include *A. tortilis* data. Significant differences between hook and spine means at 0.001 level (*t* Test) is indicated by ***.

Thorn type		Angle		Base		Gape		Length	
and species	n	Mean (s. d.)	0.05	Mean (s. d.)	0.05	Mean (s. d.)	0.05*	Mean (s. d.)	0.05*
Hooks									
A. burkei	150	28.7 (20.2)	E	6.7 (1.9)	A	5.0 (1.3)	A	6.6 (1.7)	A
A. caffra	150	64.3 (22.3)	C	3.5 (1.5)	D	3.1 (1.1)	C	3.6 (1.4)	C
A. erubescens	150	32.0 (20.9)	DE	4.2 (1.2)	C	2.9 (0.8)	C	3.9 (1.1)	C
A. nigrescens	150	29.7 (15.7)	DE	5.3 (1.6)	B	4.0 (1.2)	B	5.3 (1.7)	B
A. tortilis (hooks)	150	36.6 (21.1)	D	1.9 (0.6)	G	2.9 (1.1)	C	3.5 (1.3)	C
Mean	600	38.7 (24.8)	***	4.9 (2.0)	***	3.8 (1.4)	***	4.9 (1.9)	***
Spines									
A. davyi	150	106.9 (14.4)	A	1.6 (0.5)	G	13.2 (5.4)	D	14.5 (5.9)	D
A. grandicornuta	150	86.3 (10.1)	B	2.6 (1.2)	EF	16.4 (14.6)	CD	17.0 (15.2)	CD
A. robusta	150	107.4 (13.5)	A	3.7 (0.9)	D	29.4 (27.6)	B	30.5 (27.9)	B
A. tortilis	263	60.3 (32.1)	C	2.3 (0.8)	F	20.9 (22.8)	C	21.6 (22.9)	C
A. tortilis (spines)	113	91.8 (8.3)	B	2.8 (0.8)	E	44.9 (14.1)	A	45.6 (14.4)	A
Mean	450	100.2 (16.1)	***	2.6 (1.2)	***	19.7 (19.6)	***	20.7 (19.9)	***

mean for *A. tortilis* is based on the fact that short spines were largely hooked, and thus classified and measured separately. Spine length in *A. robusta* was very variable (standard deviation = 27.6mm). The mean spine gape (19.7mm) and length (20.7mm) measurements were significantly larger than those of hooks (3.8mm & 4.9mm; gape: $t_{1048} = -19.353$, length: $t_{1048} = -19.854$; $p < 0.0001$).

Mean thorn densities (Table 2) varied significantly between species in the analysis of variance ($F_{9,290} = 49.23$; $p < 0.001$). *A. tortilis* had the highest thorn density (56.4 per 50cm), a feature largely attributable to the large number of hooks per unit length of branch (52.1 per 50cm). The spine density of *A. tortilis* was very low (4.5 per 50cm), but field observation indicates that this is a very variable feature between individuals (see also Gowda 1996). The mean thorn density of hooked species (39.8 per 50cm) was slightly lower than that of spinescent species (43.1 per 50cm), but this difference was not significant at the 95% confidence level ($t_{208} = -1.850$; $p = 0.0657$).

Significant variance was found in the mean leaf length dataset ($F_{7, 2078} = 1148.78$; $p < 0.0000$). The mean leaf length data presented in Table 3 show *A. caffra* (133.9mm) of the hooked species and *A. davyi* (22.7mm) of the spinescent species to have significantly longer leaves than the other species included in the study (Tukey Kramer HSD $q^* = 2.952$; $p < 0.05$). The leaves of *A. tortilis* (26.3mm) were significantly smaller than those of any other species (Tukey Kramer HSD $q^* = 2.952$; $p < 0.05$), with the next smallest leaves belonging to *A. erubescens* (46.0mm) whose leaves are on average almost twice as long. The mean leaf length of hooked species (80.9mm) is significantly larger than that of the spiny species (69.3mm) when *A. tortilis* is included as a spinescent species($t_{2084} = 6.579$; $p < 0.0001$). This comparison is not significant when *A. tortilis* is excluded from the spinescent species group ($t_{1836} = -1.134$; $p = 0.2570$).

Table 2: Mean thorn density (number of thorns per 50cm of branch) and standard deviation (s. d.) of the eight *Acacia* study species. Rows not having the same letter in the 0.05 column are significantly different at the 0.05 level (Tukey Kramer HSD). Mean values for hooks and spines do not include *A. tortilis* data.

Thorn type and species	n	Thorn density Mean (s. d.)	 0.05
Hooks			
A. burkei	30	47.6 (10.0)	ABC
A. caffra	30	38.1 (17.1)	D
A. erubescens	30	39.0 (9.4)	CD
A. nigrescens	30	34.5 (8.1)	D
A. tortilis (hooks)	30	52.1 (11.5)	A
Mean	120	39.8 (12.5)	
Spines			
A. davyi	30	48.3 (11.3)	AB
A. grandicornuta	30	42.7 (15.5)	BCD
A. robusta	30	38.2 (8.4)	D
A. tortilis	30	56.4 (10.9)	A
A. tortilis (spines)	30	4.5 (4.5)	E
Mean	90	43.1 (12.6)	

Table 3: Mean leaf lengths (mm) and standard deviation (s. d.) for the eight *Acacia* study species. Rows not having the same letter in the 0.05 column are significantly different at the 0.05 level (Tukey Kramer HSD). Significant differences between hook and spine means at the 0.0001 level (t Test) is indicated by ***.

Thorn type and species	n	Leaf length Mean (s. d.)	 0.05
Hooks			
A. burkei	300	66.7 (16.5)	C
A. caffra	300	133.9 (27.4)	A
A. erubescens	250	46.0 (7.7)	E
A. nigrescens	206	66.7 (16.0)	C
Mean	1056	80.9 (39.2)	***
Spines			
A. davyi	300	122.7 (27.2)	B
A. grandicornuta	300	51.5 (11.2)	D
A. robusta	182	69.3 (18.8)	C
A. tortilis	248	26.3 (6.4)	F
Mean (incl. *A. tortilis*)	1030	69.3 (41.2)	***
Mean (excl. *A. tortilis*)	782	83.0 (38.0)	

The mass of *A. grandicornuta* thorns was found to be significantly correlated with the product of their length and base ($R^2 = 0.9314$; $F_{1,49} = 2450.027$; $p < 0.0001$). Using the equation of the line of best fit for this relationship, the mass of the spines of *A. davyi*, *A. grandicornuta*, *A. robusta* and the hooks and spines of *A. tortilis* were estimated. The thorn mass of *A. burkei* was significantly correlated to length and base parameters when the thorn was modelled as a cone ($R^2 = 0.8323$; $F_{1,11} = 471.695$; $p < 0.0001$). The resulting equation was used to estimate the thorn mass of the hooks of *A. burkei*, *A. caffra*, *A. erubescens* and *A. nigrescens*.

The thorn mass per 50cm of branch was calculated per individual of each species using the measured thorn densities (Figure 10). There was significant variation between the estimated thorn mass of the different species ($F_{7,72} = 19.5442$; $p < 0.0001$). *A. robusta* had a significantly higher estimate of mean thorn mass (3.19 g/50cm; Tukey Kramer HSD $q^* = 3.122$; $p < 0.05$) than all other species. *A. grandicornuta* had the second highest mass estimate (1.77 g/50cm), followed by *A. burkei* (0.99 g/50cm). The lowest estimate was for *A. caffra* (0.14 g/50cm). There was a significant difference between the estimated mean thorn mass per 50cm of branch of hook only and spine only species ($t_{68} = -6.235$; $p < 0.0001$; Figure 11), with spines averaging 1.94 g/50cm and hooks 0.43 g/50cm.

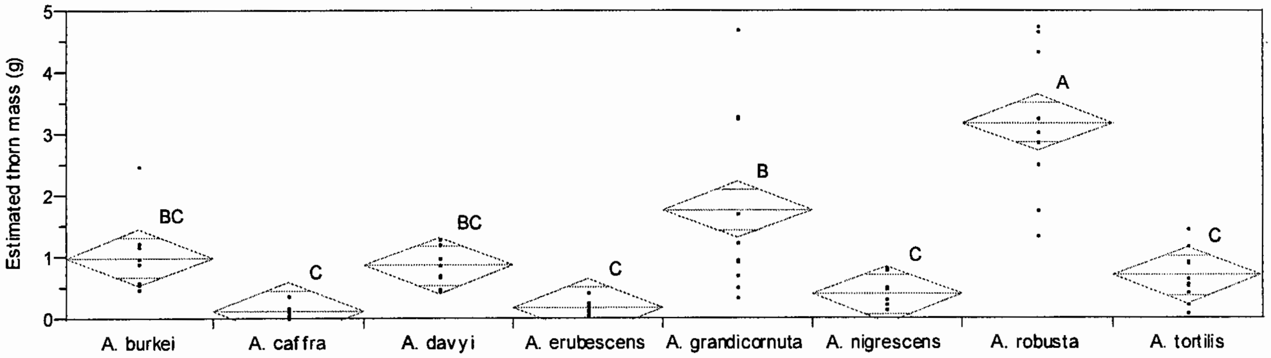

Figure 10: Estimated thorn mass (g) for each species. Hooked thorn masses were estimated using the regression line from *A. burkei* thorns when modelled as a cones and plotted against mass ($R^2 = 0.8323$; $F_{1,11} = 471.695$; $p < 0.0001$). Spine masses were estimated using the regression line from *A. grandicornuta* spines when length * base was plotted against mass ($R^2 = 0.9314$; $F_{1,49} = 2450.027$; $p < 0.0001$). The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using the Tukey Kramer HSD test ($q^* = 3.122$).

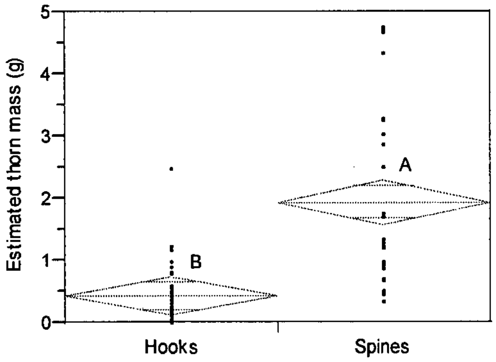

Figure 11: Estimates of mean thorn mass (g) for hooks and spines. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using a t test ($t_{68} = -6.235$; $p < 0.0001$).

Feeding simulations

The variation in mean sock test scores (Figure 12) was significant ($F_{7,72} = 38.64$; $p < 0.0001$). *A. nigrescens* (6.59kg) and *A. tortilis* (6.36kg) provided the greatest resistance to a stripping motion of the test, and *A. caffra* (1.15kg) and *A. davyi* (0.43kg) the lowest. When the sock test scores for the different thorn types were analysed (Figure 13), hooks were shown to have a significantly higher sock test mean (4.52kg) than spines (1.76kg; $t_{68} = 5.519$; $p < 0.0001$).

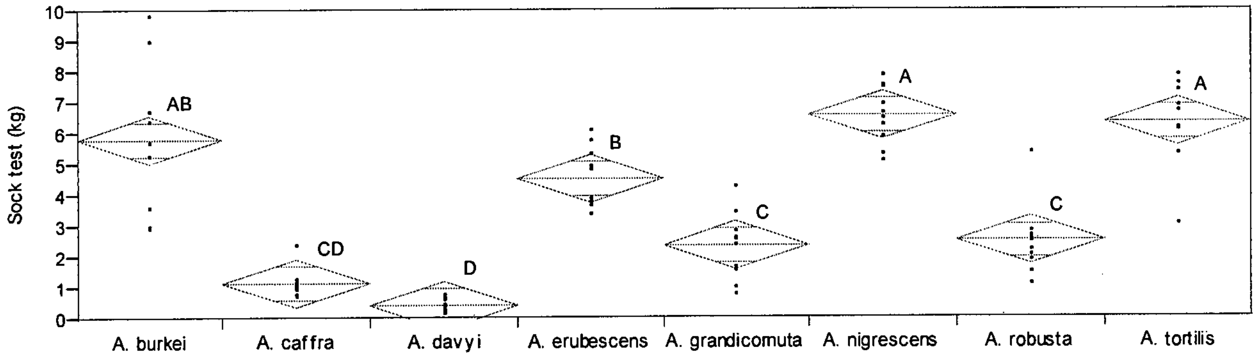

Figure 12: Mean sock test scores (kg) calculated for each species. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using the Tukey Kramer HSD test (q* = 3.122).

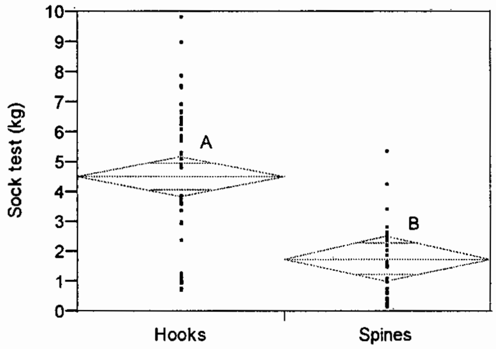

Figure 13: Mean sock test score (kg) for hooks and spines. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using a t test ($t_{68} = 5.519$; $p < 0.0001$).

There was a significant difference between the mean scores of each species for the muzzle test ($F_{7, 72} = 39.74$; $p < 0.0001$; Figure 14). *A. grandicornuta* had the highest proportion of severe scratches (0.63), followed by *A. nigrescens* (0.57). *A. caffra* (0.25) and *A. davyi* (0.12) had the lowest scores. There was no significant difference between the mean scores of hooks versus spines on the muzzle test ($t_{68} = 0.33$; $p = 0.75$; Figure 15).

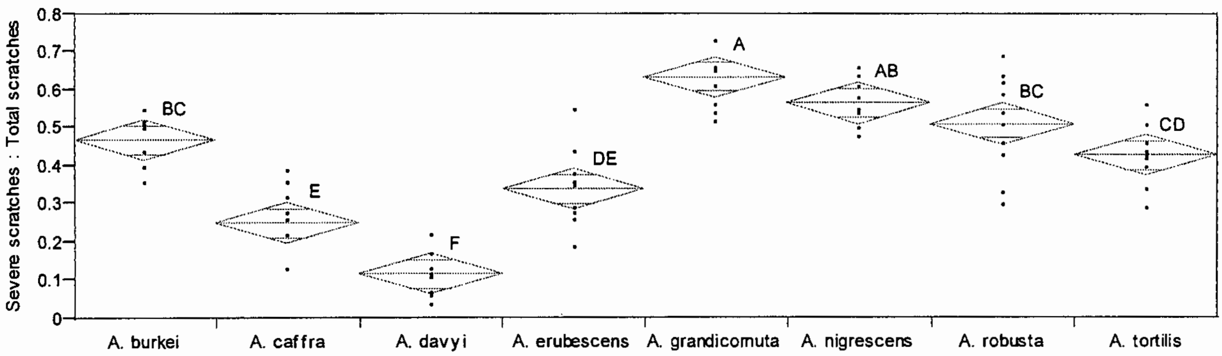

Figure 14: Mean muzzle test scores (severe scratches: total scratches) calculated for each species. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using the Tukey Kramer HSD test (q* = 3.122).

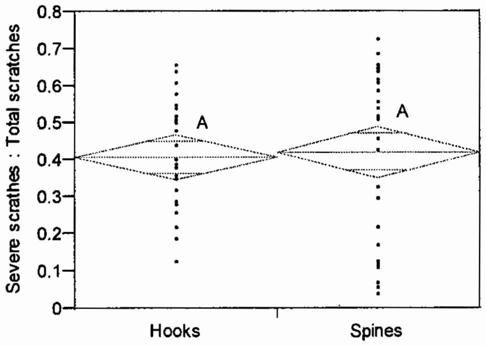

Figure 15: Mean muzzle test score (severe scratches: total scratches) for hooks and spines. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using a t test ($t_{68} = 0.33$; $p = 0.75$).

The clamp test also showed significant variation between the mean scores for each species ($F_{7,72} = 8.79$; $p < 0.0001$; Figure 16). *A. robusta* (10.2) had the highest ratio of thorn: branch impressions, followed by *A. grandicornuta* (7.67). The lowest scores were those of *A. caffra* (2.62) and *A. erubescens* (2.57). Spinescent species scored significantly higher in the clamp test than did hooked species ($t_{68} = -4.772$; $p < 0.0001$; Figure 17). Dividing the clamp test score by thorn density had no effect on the top four ranked species in the test, and only switched the ranking of *A. caffra* and *A. davyi* in the lower rankings.

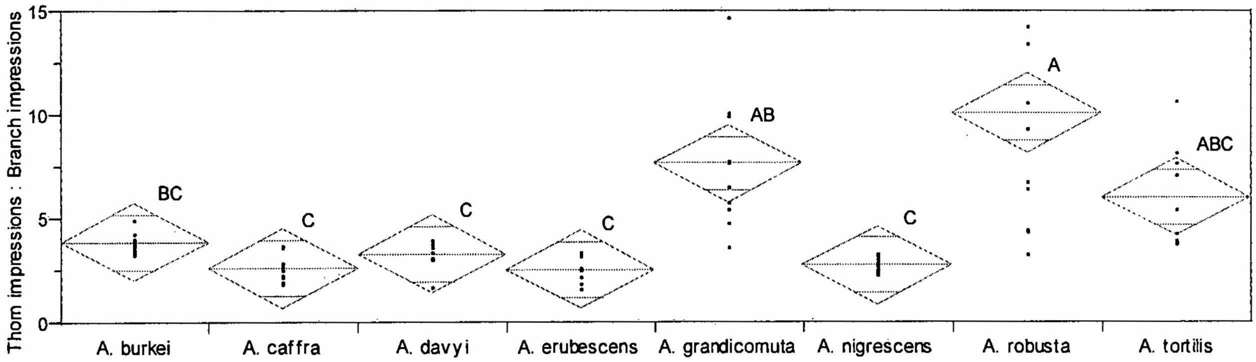

Figure 16: Mean clamp test scores (thorn impressions: branch impressions) calculated for each species. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using the Tukey Kramer HSD test ($q^* = 3.122$).

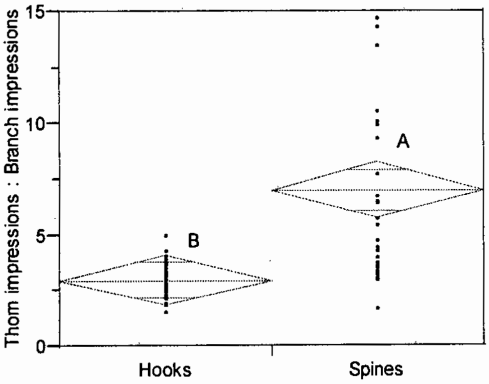

Figure 17: Mean clamp test score (thorn impressions: branch impressions) for hooks and spines. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using a t test ($t_{68} = -4.772$; $p < 0.0001$).

Feeding trials

Despite having been kept in the bomas for three weeks, the nyala were still very wary of any human activity around them. In total, ten branches of *A. burkei* were presented to the nyala through six observation sessions lasting in total 7 hours 30 minutes. Of these branches, only three were fed on for more than five bites. Seven branches of *A. caffra* were presented to the nyala in four sessions lasting 5 hours 15 minutes in total. Two branches were fed on for more than five bites. The results are thus very limited and no statistical comparisons can be made. Bite size, bite rate and feeding rates were calculated for those branches which were fed on for more than five bites (32 bites from *A. burkei* were included, and 27 from *A. caffra*), and the results are presented in figure 18. The bite rate on *A. burkei* was higher than that on *A. caffra* (*A. burkei*: 22.6 bites/minute versus *A. caffra*: 19.1 bites/min), but larger bite sizes were taken from *A. caffra* (*A. caffra*: 0.73 g/bite versus *A. burkei*: 0.41 g/bite). The result is that the feeding rate on *A. caffra* was higher than that on *A. burkei* (*A. caffra*: 13.93 g/min versus *A. burkei*: 9.24 g/min).

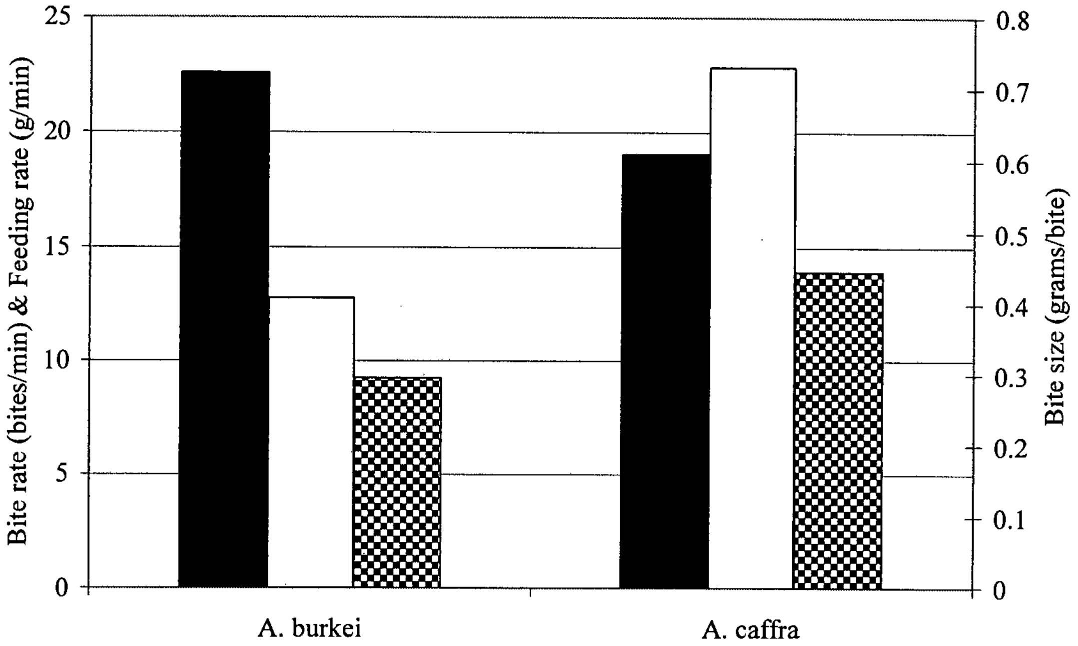

Figure 18: Bar chart showing bite rate (black), bite size (white) and feeding rate (chequered) of nyala on *A. burkei* (32 bites) and *A. caffra* (27 bites) in the feeding trials.

The feeding style of the nyala differed between *A. burkei* and *A. caffra*. The nyala were only noted to get their lips hooked on the thorns of *A. burkei*. Examination of the branches after nyala browsing showed *A. burkei* leaves to often be bitten in half, so that the animals' lips would presumably be kept out of reach of the thorns (Figure 19). When feeding on *A. caffra* however, leaves were cropped off right down to the branch (Figure 20).

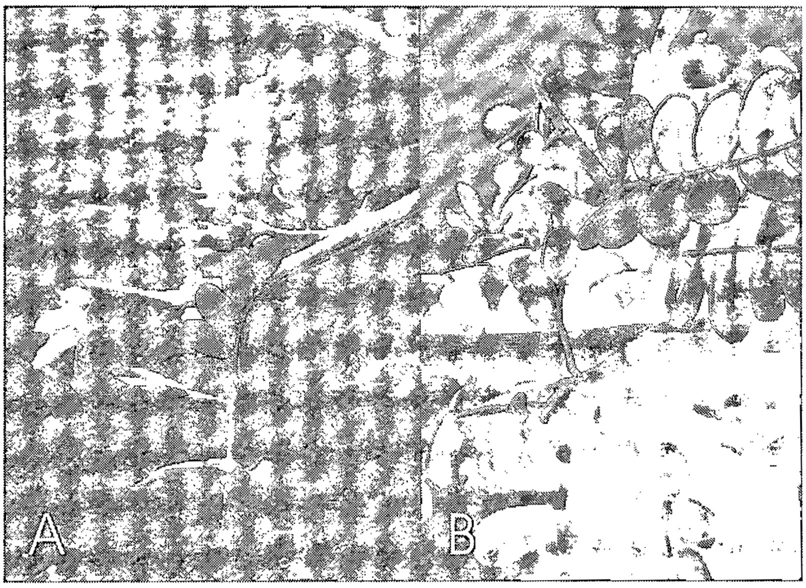

Figure 19: Close up of A. burkei branches (A & B) after being fed on by nyala in the feeding trials. Note how the leaves have been bitten in half, reducing the risk for the animals of catching their lips on the thorns.

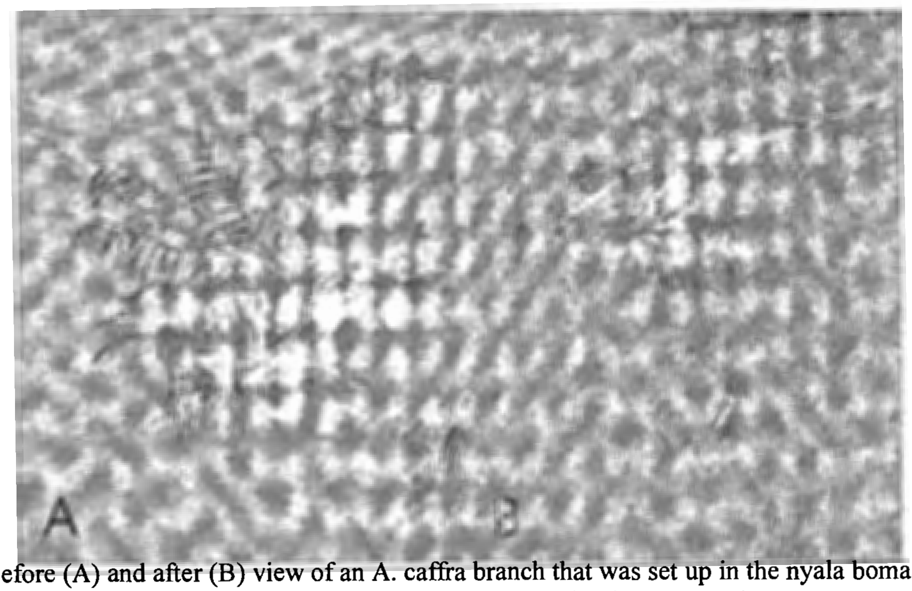

Figure 20: Before (A) and after (B) view of an A. caffra branch that was set up in the nyala bomas during the feeding trials. Note how the leaves have been bitten off right down at their base, in contrast to the leaves of A. burkei in figure 19.

Indices

There was significant variation in the stripping index dataset ($F_{8, 81} = 50.26$; $p < 0.0001$; Figure 21). *A. burkei* (2.70) scored significantly higher than *A. nigrescens* (2.23) which had the second highest score, followed by *A. erubescens* (2.18). *A. davyi* (1.56) and the spines of *A. tortilis* (0.78) had the two lowest mean scores for this index. A separate analysis of hooks versus spines showed the mean hook score (2.20) to be significantly higher than the mean spine score (1.39; $t_{88} = 9.617$; $p < 0.0001$; Figure 22). The mean and standard deviation of all scores for the stripping index was 1.96 ± 0.44.

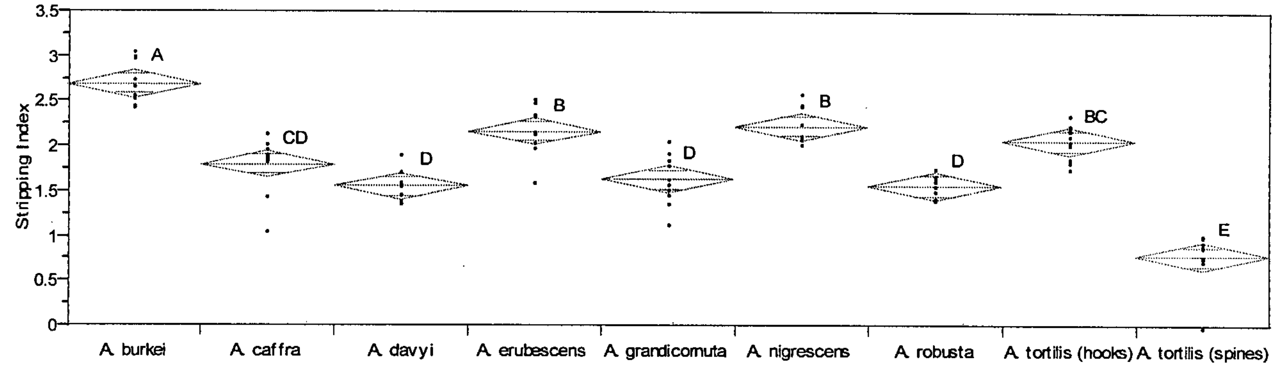

Figure 21: Mean stripping index scores calculated for each species. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using the Tukey Kramer HSD test ($q^* = 3.187$).

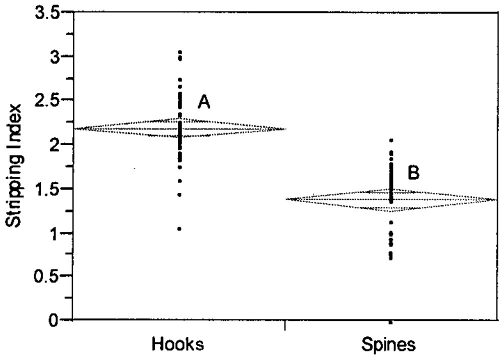

Figure 22: Mean stripping index score for hooks and spines. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using a t test ($t_{88} = 9.617$; $p < 0.0001$).

The biting index (Figure 23) scored *A. robusta* (2.85) as the best defended against biting, followed by *A. grandicornuta* (2.38). There was significant interspecific variation between the mean biting index scores ($F_{8, 81} = 15.16$; $p < 0.0001$). *A. nigrescens* (1.32) and *A. erubescens* (1.31) scored the lowest in the biting index. The mean score of spiny species (2.32) was significantly higher than that of hooked species (1.50; $t_{88} = -8.179$; $p < 0.0001$; Figure 24). The mean and standard deviation of all biting index scores was 1.94 ± 0.61.

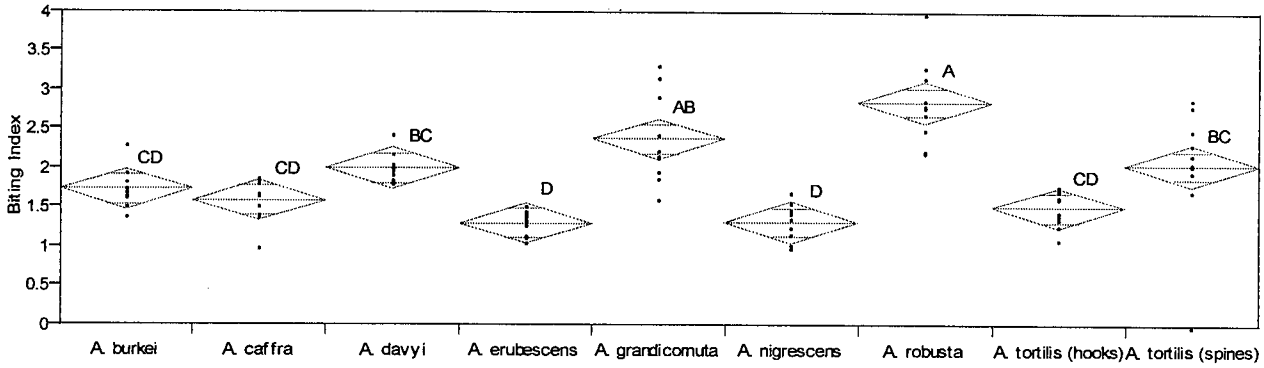

Figure 23: Mean biting index scores calculated for each species. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using the Tukey Kramer HSD test($q^* = 3.187$).

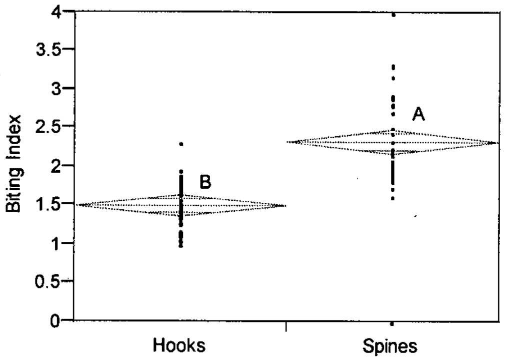

Figure 24: Mean biting index score for hooks and spines. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using a t test ($t_{88} = -8.179$; $p < 0.0001$).

The branching index means for *A. nigrescens* (6.91) and *A. erubescens* (6.91) were the two highest calculated (Figure 25). There was significant variation between species ($F_{7,2} = 10.81$; $p < 0.0001$). The two lowest branching index scores measured were for *A. burkei* (3.28) and *A. robusta* (2.32). The analysis of mean branching index score between hooked (5.40) and spiny (4.21) species was only significant at the 95% confidence level ($t_{68} = 2.277$; $p = 0.0259$), but was slightly higher for hooked species (Figure 26). The mean and standard deviation for the branching index score was 5.11 ± 2.31.

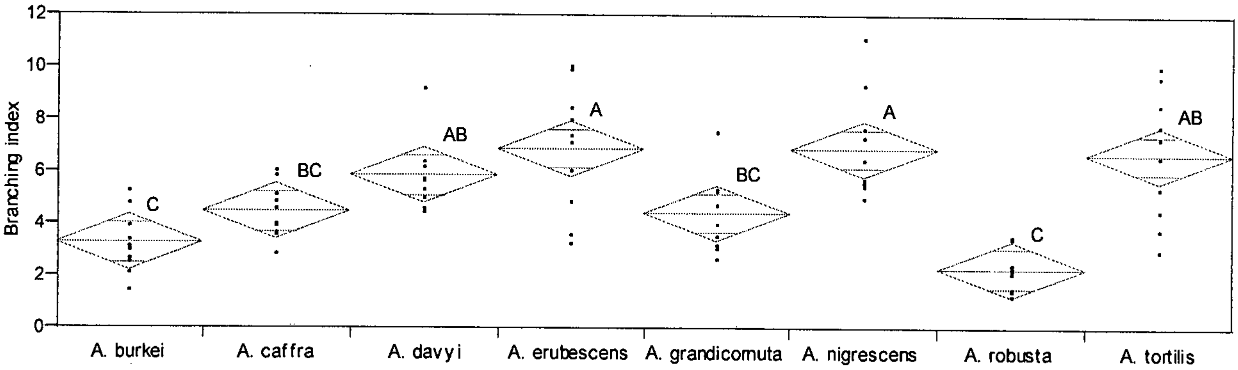

Figure 25: Mean branching index scores calculated for each species. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using the Tukey Kramer HSD test($q^* = 3.122$).

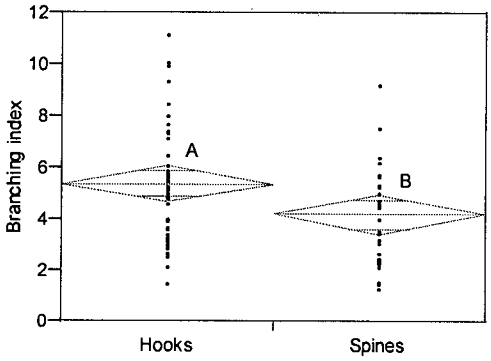

Figure 26: Mean branching index score for hooks and spines. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using a t test ($t_{68} = 2.277$; $p = 0.0259$).

The defence index when calculated only incorporating a scaling factor for the branching index showed significant difference between species means ($F_{7, 72} = 23.05$; $p < 0.0001$; Figure 27). The Tukey Kramer HSD test revealed the score of *A. tortilis* (7.09) to be significantly higher than all other species (Tukey Kramer HSD $q^* = 3.121$; $p < 0.05$), but that the means of the remaining species were not significantly different. *A. burkei* (4.76) and *A. robusta* (4.65) had the next highest defence index scores after *A. tortilis*. The two lowest scoring species were *A. davyi* (4.15) and *A. caffra* (3.85). No significant difference existed between the combined defence index scores of hooks only and spines only species ($t_{68} = -1.141$; $p = 0.2577$; Figure 28).

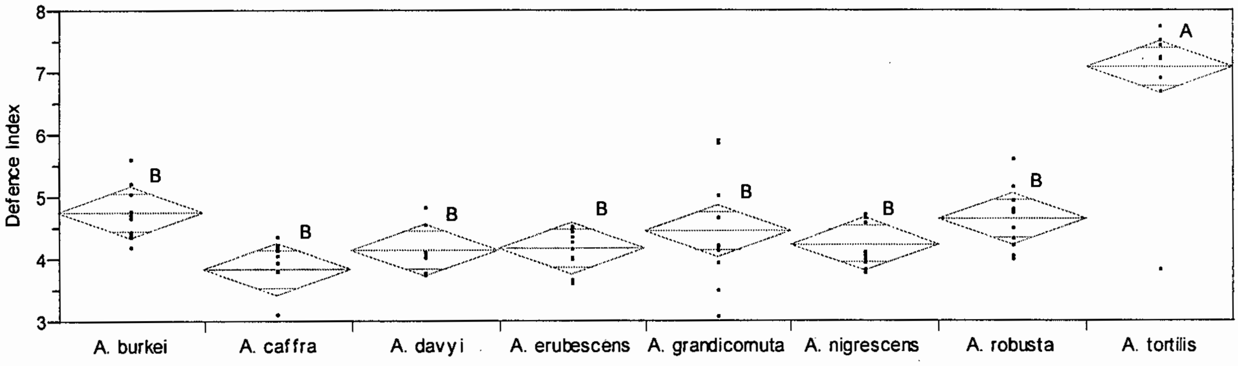

Figure 27: Mean defence index scores calculated for each species. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using the Tukey Kramer HSD test ($q^* = 3.122$).

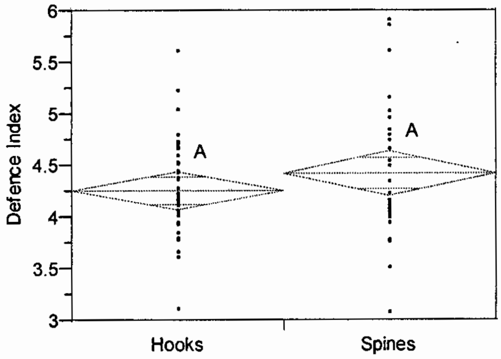

Figure 28: Mean defence index score for hooks and spines. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using a t test ($t_{68} = -1.141$; $p = 0.2577$).

The defence index and feeding rates are inversely related. Therefore, the ratio between the feeding rates on two species needs to be plotted against the inverse ratio of their defence indices. If the defence indices of two species are scaled accurately relative to the feeding rates on the two species, then dividing the inverse ratio of their defence indices by the ratio between their feeding rates would be one. This ratio between defence indices^{-1} and feeding rates was plotted against the feeding rate ratio between the species for all possible species pairings, and shown in figure 29. If the defence index was scaled correctly relative to feeding rates, all points should have a y value of one, and the regression line a gradient of zero.

Using the proposed defence index, the gradient of the line of best fit is -3.05 and the mean is 1.97 (Figure 29, solid line). The four solid circle points in figure 29 with y values greater than 1.8 are all of the species pairings that include *A. caffra*. The mean value of all defence indices^{-1}/ feeding rate ratio species pairings including *A. caffra* is 2.84, and pairings excluding *A. caffra* is 1.27. Modifying the biting index by increasing the contribution of the second term (length * base) by multiplying it by 10 (i.e. the second term becomes 10 * length * base) has the effect of changing the gradient of the line of best fit to -0.864 and the mean to 1.23 (Figure 29, dashed line). The mean of *A. caffra* defence indices^{-1}/ feeding rate ratio pairings using the modified index is 1.47, and 1.01 when these pairings are excluded. Using the modified defence index, the means and standard deviations of the contributing indices are: stripping index 1.96 ± 0.44, biting index 6.17 ± 4.56 and branching index 0.51 ± 0.23.

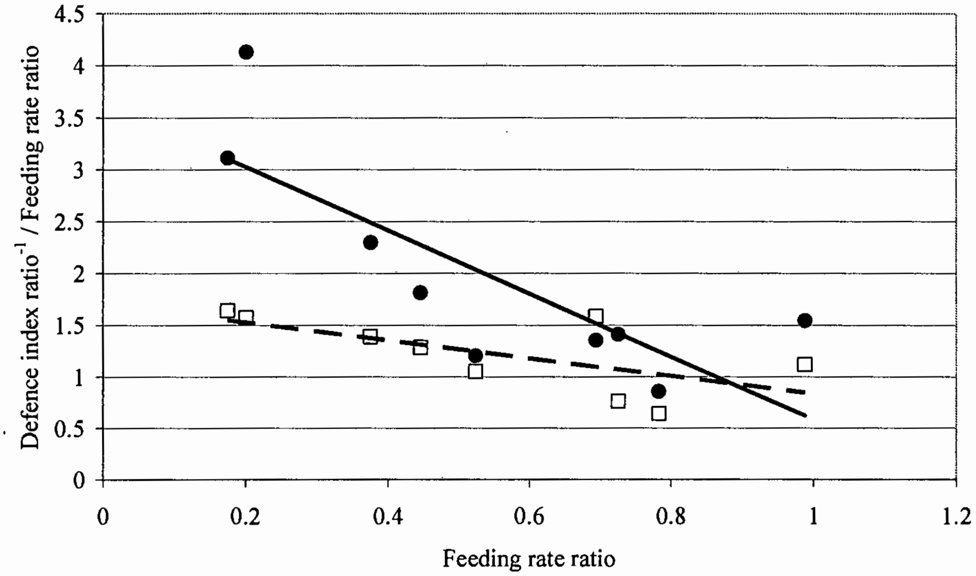

Figure 29: Plot of the ratio between feeding rates on nine species pairings and the ratio of their defence indices^{-1} divided by the feeding rate ratio. The solid circles (●) represent the unmodified defence index, and the solid line the line of best fit through these points ($y = -3.0509x + 3.6337$; $R^2 = 0.6373$, $p = 0.010$). The open squares (□) represent the modified defence index score which has a greater weighting of the contribution of thorn size to its component biting index. The dashed line is the line of best fit for the modified defence index ($y = -0.864x + 1.697$; $R^2 = 0.4269$, $p = 0.056$).

The modification to the defence index suggested by scaling it against feeding rates significantly altered the ranking order of species' defences (Figure 30). *A. robusta* becomes the most well defended species (16.24), scoring significantly higher than all other species including the next ranked *A. tortilis* (9.94; Tukey Kramer HSD q* = 3.121; $p < 0.05$). *A. erubescens* is ranked second lowest (5.73) and *A. caffra* the lowest (5.12). The mean score of species with spines (10.83) is significantly higher than those with hooks (6.69) using the modified defence index (Figure 31; $t_{68} = -4.148$; $p < 0.0001$).

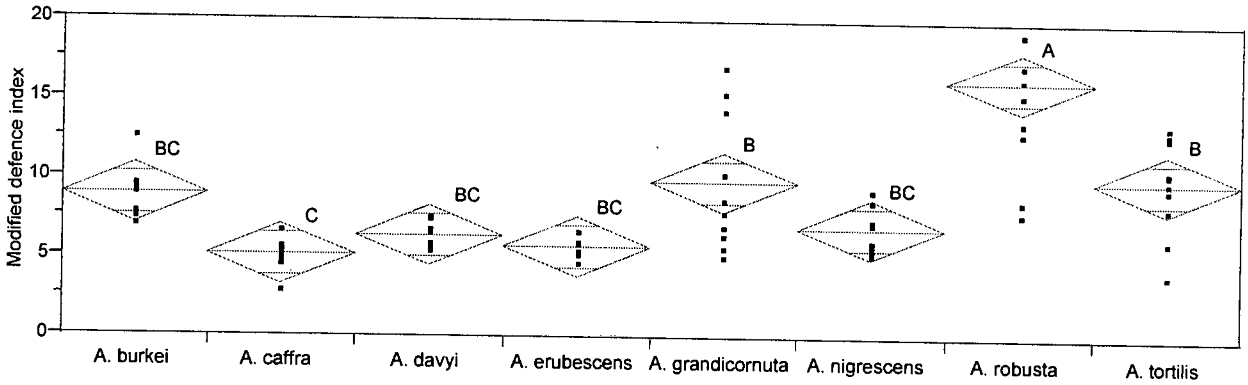

Figure 30: Mean modified defence index scores calculated for each species. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are not significantly different from one another at the 95% confidence level when compared using the Tukey Kramer HSD test (q* = 3.122).

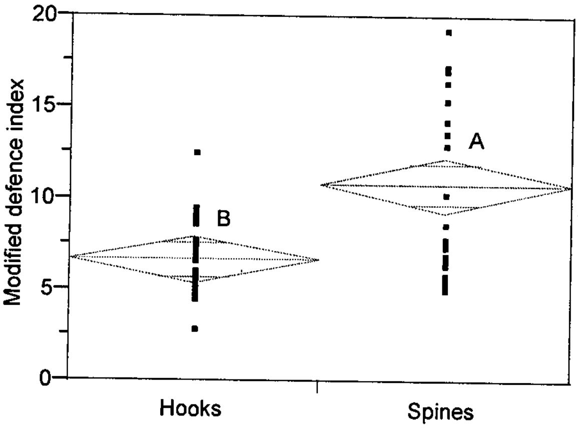

Figure 31: Mean modified defence index score for hooks and spines. The upper and lower points of the species diamonds indicate the 95% confidence interval for each species, and the centre line the mean for the species. The means of species sharing the same letter are significantly different from one another at the 95% confidence level when compared using a t test ($t_{68} = -4.148$; $p < 0.0001$).

The gradient, R^2 value and p value of the lines of best fit when plotting the feeding simulation scores against index scores is shown in table 4. The correlation between feeding simulation scores and index scores was generally low, with only the stripping index versus sock test R^2 value (0.58) being greater than 0.3. A number of pairings did however have significant relationships. The stripping index was significantly and positively related to the sock test scores ($F_{1,78} = 107.83$, $p < 0.001$), but not to the clamp or muzzle test simulations. The biting index had a significant and positive relationship with the clamp test scores ($F_{1,78} = 26.34$, $p < 0.0001$). The branching index showed a fairly close relationship with all three feeding simulations, although this was only significant for the clamp test ($F_{1,78} = 6.16$, $p = 0.0152$). The branching index was negatively related to the clamp and muzzle test, but positively related to the sock test. The unmodified defence index was most significantly related to the sock test ($F_{1,78} = 19.81$, $p < 0.0001$), but also showed a close relationship with the clamp test ($F_{1,78} = 6.00$, $p = 0.0165$). The modified defence index was positively and significantly related with all three feeding simulations, and is also the only index that had a significant relationship with the muzzle test score ($F_{1,78} = 9.68$, $p = 0.0026$).

Table 4: Relationships between the three feeding simulations and the various indices. The values in the gradient and R^2 (coefficient of determination) rows refer to the line of best fit for the respective plots of index versus feeding simulation. P-level values in **bold** type are significant at the 95% confidence level.

		Sock Test	Clamp Test	Muzzle Test
Stripping index	Gradient	0.16	-0.02	0.48
	R^2	0.58	0.01	0.03
	p-level	**0.0001**	0.3349	0.1560
Biting index	Gradient	0.02	0.11	0.88
	R^2	0.00	0.25	0.03
	p-level	0.5345	**0.0001**	0.0996
Branching index	Gradient	0.02	-0.02	-0.26
	R^2	0.04	0.07	0.04
	p-level	0.0847	**0.0152**	0.0707
Defence index	Gradient	0.20	0.08	1.10
	R^2	0.20	0.07	0.03
	p-level	**0.0001**	**0.0165**	0.1262
Modified defence index	Gradient	0.54	0.73	10.45
	R^2	0.06	0.25	0.11
	p-level	**0.0284**	**0.0001**	**0.0026**

Discussion

The most obvious distinction in *Acacia* defences is that between hooked thorns and straight spine thorns. Their specific designs are geared to function in distinctly different ways, although both with the same end goal: to limit the feeding damage by large mammal herbivores. Assessing their designs from descriptive measurements reveals hooks to have their tips generally pointed back along the branch into the tree, and spines to generally be orientated at right angles to the branch. The tips of the thorns are literally the points that inflict the pain on herbivores, pain being the currency of deterrence. The assessment thus that hooks are geared to inflict pain when stripping along the branch and spines when approaching the branch from right angles seems a logical one. This difference in primary functioning demands different design parameters, and it is interesting to note that hooked thorns, although much smaller than spines, have larger bases. Strength of attachment thus seems to be an important component of hooked thorn design. On the other hand, size appears to be an important component in spine design. Spine size is also however very variable along branches, indicating that not all spines that begin to develop are generally required (considering the cost at which they come to the plant) to reach their maximum size. Hooks are much more uniform in their size, with each being relatively more fully developed.

The density of *Acacia* thorns is a parameter which, interestingly, shows no significant variation between the means of the hooked species and the spiny species included in the study. Thus, despite vastly different designs, the number of thorns presented per length of branch does not differ significantly. It could be contended that phylogenetic constraints elevate spine densities to higher than what are required for an adequate defence, based on the fact that many spines don't develop fully.

Feeding simulations

The sock test was closely correlated with the stripping index and would appear to be a good rough guide as to how well defended hooked species are. It has little relevance to spiny defences, and thus when comparing across types of defences it would need to be used in conjunction with another method. The clamp test was significantly related to the biting index, although it had less predictive power for the biting index than did the sock test for the stripping index. Despite the seemingly coarse nature of the clamp test, it did manage to distinguish between the different degrees of defence of spiny *Acacia*s as predicted by the biting index. The effect of thorn density causing an exaggeration in clamp test scores was found to be minimal. These two simulations thus seem to offer a viable rough substitute for the arduous task of measuring thorns for calculating the stripping index (sock test) and biting index (clamp test).

The muzzle test was most useful as a predictor of overall defence. This test is most valuable in terms of the dynamics of its operation, in that it captures information about hooks and spines. The muzzle is manoeuvred in and out of the branches, mimicking attempts to gain access to branches, with the motions giving both thorn designs an opportunity to inflict damage. Interpreting the impressions in the plasticine and scoring them as severe or superficial is however a rather subjective process, which could limit the widespread comparability of this test of defences. This test scored *A. grandicornuta* as the most well defended species, followed by *A. nigrescens*.

Feeding trials

The feeding trials are the most reliable means for scaling the indices to actual effects of defences on herbivores. It is thus unfortunate that the response of the nyala to the experimental setup of this study was not more rewarding, but never the less some important observations were made. The results obtained for the nyala are very similar to those of Cooper & Owen-Smith (1986) for impala feeding on *A. burkei* and *A. caffra* (although this study used wet mass and Cooper & Owen-Smith used dry mass). The attempts at scaling the indices using data from Cooper & Owen-Smith (1986) showed that an emphasis of the size term in the biting index equation resulted in a better fit of the defence index with measured feeding rates. The modification of the defence index results in the mean biting index score being three times higher than the mean stripping index contribution, with the effect that spinescent species score significantly higher than the hooked species. The feeding rate data does not distinguish between the loss of branch and leaf material, only quantifying the biomass lost per unit time. It is thus not unexpected that scaling the defence indices against feeding rates should suggest increasing the contribution of the biting index to the defence index, as branch biting constitutes a higher biomass loss than does leaf stripping. Branch biomass loss and leaf biomass loss do not necessarily equate directly for the plant, and the cost of herbivory may be measured better in terms of the expense of the resources invested in the material lost. The benefits derived from the better hooked defence of *A. burkei* versus *A. caffra* would appear to be an overall lower feeding rate, but also that the leaves of *A. burkei* are not completely removed when browsed, potentially allowing them to maintain some degree of utility to the plant.

Indices

The logic behind the development of the indices is presented in the methods section, and the resulting rankings of species are largely in line with what would be expected. The indices are based on assumptions which, together with the actual index design are naturally open to criticism. For example, the stripping index is designed to give the maximum score to strongly attached thorns which point directly back along the branch. No upper limit is specified for when a thorn is well enough attached, a point beyond which investment in a larger base may only form a cost, with no real added defence benefit. Optimising thorn angle at 0° could also be questioned, with the true optimum perhaps lying around 30° to coincide with potentially the most frequent angle of incidence of the leaf picking lips of browsers.

Criticism can also be levelled at the biting index which is essentially designed to assess a spiny defence. It assumes an optimal thorn angle of 90°, which while possibly optimal for preventing biting, does not account for other potential benefits of different spine orientation. Spines which lean forward may further limit herbivory by increasing the risk of having an eye stabbed, while spines which lean slightly back (e.g. *A. nilotica*, personal observation) may threaten the soft underside of an animal's neck as it withdraws its head from the canopy. The biting index scores bigger spines as better, and while this may adequately reflect the benefit gained by reducing biting up to a point, the way in which the defence works may change as thorns get bigger than herbivore gapes. As discussed by Midgley *et. al.* (2001), large white spines may act as a visual deterrent to herbivores. Very large spines may also act in a similar way to a caged branch architecture, so that while their tips may be easier to avoid, their long shafts act as a barrier to getting to leaves further down the branch.

The branching index (from Archibald & Bond 2003) is a simple and effective way of quantifying the degree of ramification. This measurement, however, does not necessarily incorporate the 3-dimensional branching structure of individuals as the same branching pattern whether presented in a flat plane or contorted into a box shape scores the same value. An alternative method proposed by L. Kruger is to calculate the ratio of the actual length of the longest branch to the direct distance between the tip and base of the longest branch. This method however ignores the number of branching points along a branch. It is likely that a combination of the methods of Archibald & Bond (2003) and that proposed by L. Kruger would be most useful, as both techniques incorporate important aspects of the branching structure.

The optimal branching structure may also vary for different species. An idea formulated during the fieldwork for this study is that increased branch flexibility may improve the defence of some hooked *Acacia* species (e.g. *A. caffra*). High branch flexibility would mean that when an animal got hooked in one place, the branch would bend with it as it tried to free itself, and in the process may get hooked by another branch. This idea is analogous to a spider's web, and would likely require lower degrees of branching resulting in longer lengths of branches.

Combining the stripping, biting and branching indices into an overall defence index is an attempt to provide a useful 'all-in-one' index. The merits of this can be questioned though, as hooks and spines, while both being designed to limit herbivory, neither defend the same parts of the plant, nor do they optimally defend against the same suite of herbivores. Scaling the contribution of the stripping and biting indices is thus made very challenging. While spines may protect the plant's biomass more effectively (see feeding trials section), the contribution of hooks may be more profitable in terms of the trade off of the cost of the defence and the protection derived for leaves (but seemingly not branches). It is thus necessary to have a clearly defined picture of what resources are most limiting in an environment and what plant parts are most at risk (dependent on the suite of herbivores), in order to effectively address the trade-offs surrounding defensive investments.

Life history implications

The aim of producing indices that adequately capture the variation in defences between different populations of *Acacia*s was inspired by the need to assess the role of herbivory in different environments when attempting to understand variation in *Acacia* life history strategies (e.g. Archibald & Bond 2003). This study suggests that there is variation in the degree of *Acacia* defences between species. It is interesting to note that the two least well defended species of each thorn type (i.e. *A. caffra* – hooks and *A. davyi* – spines) had very similar leaf sizes, both significantly larger than all other species in this study. This could indicate that these less well structurally defended species may opt for a higher growth rate to attempt to boost them to above the browse line, or may be better defended chemically. Skarpe *et. al.* (2000) report high levels of herbivory on *A. caffra* relative to other *Acacia*s in their study area (including *A. erubescens* and *A. tortilis*), and it is also possible that this species is simply able to tolerate this high browse pressure. Bond & Loffell (2001), however, report *A. caffra* and *A. davyi* as being severely impacted by giraffe browsing, largely being restricted to areas inaccessible to giraffe.

A. tortilis was ranked as the most well defended species, and has significantly smaller leaves than all other species assessed (see also Midgley 2001). Small leaves are suggested as being a further defensive mechanism (Milewski *et. al.* 1991, Gowda 1996) as they necessitate the animal manoeuvring in closer to the thorns when feeding. Spines are associated with leaves, and the very high thorn density of *A. tortilis* implies a high density of small leaves along the branch (although this was not measured). This high thorn density constitutes a cost to the plant, and it is thus seems logical that the spine to hook ratio of *A. tortilis* decreases dramatically above the reach of browsers (personal observation, Milewski *et. al.* 1991). The defensive attributes of *A. tortilis* suggest that it has evolved in an environment where herbivory has been a major selective pressure, and it is consequently well adapted to cope with this pressure.

*Acacia*s are the archetypical savanna tree, a biome where fire and large mammal herbivory generally play a very influential top down controlling role. Fire can not be defended against with the same suite of attributes that limit large mammal herbivory. The costs associated with adequately defending against both fire and herbivory are likely to be overwhelming for plants, and thus different suites of defensive traits and/or species are likely to characterise environments where the relative impact of fire and herbivory are different. Phenotypic plasticity would thus be expected to be an *important* trait of determining the limits of *Acacia* distributions.

Predictions

Hooks and spines differ in their ability to defend against different sized herbivores. Hooks are better suited to defending against small browsers, which are able to manoeuvre quite effectively between larger and more obvious spines. Spines offer a better defence against the branch biting feeding technique of larger herbivores. It is thus seems reasonable to suggest that the distributions of hooked and spiny *Acacia*'s should vary with patterns of herbivore body size distributions. Olff *et. al.* (2002) model the global distributions of herbivore body size (2kg<) in relation to the generally inversely related plant-available water and plant-available nutrients. Their results predict an increase in larger herbivores with an increase in water availability, as this results in a greater plant biomass, but which is generally of a lower quality. Smaller herbivores tend to increase as nutrient availability increases, but water availability decreases (Olff *et. al.* 2002). Hooked defences should increase with smaller herbivore predominance, which would essentially correlate with more arid but higher nutrient regions. Spines, however, should be more frequently observed in more moist regions where herbivores are predicted to be larger (Olff *et. al.* 2002). This highlights the need for the potential impacts of large mammal browsers to be properly considered when introducing them to a region.

At a more local scale, hooked thorn species are also predicted to be more prevalent in areas less accessible to larger herbivores, such as on rocky hill slopes. Spinescent species on the other hand would be predicted to be more frequently encountered in flatter regions such as valley bottoms and open savannas. The predicted distributions of hooks and spines place spinescent species in more fire prone landscapes (i.e. more mesic) while hooked species are predicted to occur in areas less prone to burning (i.e. more xeric and rocky areas; Bond 1997). It would thus be interesting to investigate the distribution of traits conferring fire tolerance in the genus *Acacia*. The argument made here would anticipate spinescent species showing a greater potential to pursue a Gulliver-type life history strategy (Bond & van Wilgen 1996), while hooked species are predicted to show a less plastic response to burning.

Conclusion

This study directly addresses the task of quantifying the variability in *Acacia* defences in order to provide a tool for relating this feature, which is a response to large mammal herbivory, across different savanna selective environments. The feeding simulations offer a means of roughly assessing variation in *Acacia* defences, but which may not give the required consistency to allow for confident comparison between different studies and workers. The indices however are precise and allow for broad scale comparability. The large variability in operation of *Acacia* defences requires a holistic approach in assessing them, and the robustness of the indices is in need of further verification in order to determine whether they adequately describe the different components of *Acacia* defences. Direct empirical assessment of the response of large mammal herbivory to natural variation and experimental manipulation of *Acacia*s defences is the only reliable means for substantiating the value of the proposed defence indices.

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