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The Veiled Evolutionary Benefits of Conspicuous White African *Acacia* Thorns.

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ABSTRACT

Structural defenses, such as spines, are common in the savanna biome as a method in reducing herbivory on leaves and in particular, branches. There is however, little evidence as to why *Acacia* species have evolved conspicuously white thorns as opposed to camouflaged green or brown thorns. Goats were used in this study as a proxy for indigenous savanna browsers and were fed three *Acacia* species at three different sites (Kruger National Park, Mpumalanga and Western Cape, South Africa). The branches had either green-painted spines or white-painted spines. This gave the goats an initial choice between the two treatments and also allowed one to take a measurement of the two different feeding rates. Results showed that the white thorns do not act as an initial aposematic deterrent against browsing, as previously expected, but rather serve to facilitate herbivory on leaves to a certain degree, while still protecting the branches. There was also no difference in the browsers' feeding rate on the two different coloured thorns.

KEY WORDS: *Acacia*, aposematic colouration, feeding rate, herbivory, spinescence.

INTRODUCTION

Plants are frequently targeted by small and large herbivores and thus have evolved a variety of defenses. There are two ways in which plants cope with the pressures of herbivory; avoidance (e.g. poisons and resins; Fahn 1979) and tolerance (mechanical defense). Mechanical or structural defense includes spines (made of leaves), prickles (made of cortical tissues), hooks and thorns (made of branches; Lev-Yadun 2009). The need for plants to defend themselves has become widespread especially in African savanna ecosystems where ungulates can consume between 28-60% of net primary production and where there has been such a long co-evolutionary history between mega-herbivores (herbivores >1000kg) and plant defenses (Chew 1974, Grubb 1992, Rohner & Ward 1997 and Du Toit *et al.* 2005).

There are over 70 African *Acacia* species (Family: Fabaceae) with conspicuous white thorns defending their branches (Midgley 2004). However, the real reason for these obvious thorns is relatively unknown. Surely it would be more beneficial for the plants to posses more camouflaged thorns such as green or brown, compared to the existing white? Green thorns would be advantageous because this would increase the plants surface area of photosynthetically active material. The resultant added energy from the green thorns could then be utilized by the plant to increase its rate of growth, escaping the peak browsing level sooner. It would therefore be more energy efficient for plants to have green thorns. Brown thorns are less costly to produce ~~compared to~~ white, where the pigments need to be synthesized (Midgley 2004, and Lev-Yadun 2009). The *Acacia's* therefore, must surely have another evolutionary function for defending their branches with white thorns.

Aposematic colouration in animals is a well-known biological phenomenon used as a warning symbol to alert predators to a negative characteristic, usually poison, associated with the bright colours (Gittleman & Harvey 1980, Harvey & Paxton 1981, and Ruxton *et al.* 2004). While many studies have been carried out on the functioning of aposematic colouration in animals, relatively few have looked at the role of aposematic colouration in spiny plants as a means of browser deterrence until 2001 where the topic is now found in the literature (Lev-Yadun 2001, 2003, 2006, 2009; Midgley *et al.* 2001; Midgley 2004; Lev-Yadun & Ne'eman 2006; Rubino & McCarthy 2004; Ruxton *et al.* 2004; Speed & Ruxton 2005; Lev-Yadun & Gould 2008; Lev-Yadun & Halpern 2008 and Ronel *et al.*, 2009). Studies in Israel show species in the genera *Agave*, *Aloe* and *Euphorbia* to possess aposematic thorn colouration whereby browsers associated the coloured thorns with the poisons the plants possess and tended to avoid the plant (Lev-Yadun 2001).

Although *Acacia* species are not poisonous, in fact they are highly nutritious, the same hypothesis could apply with the *Acacia's* warning the browser off before it even attempts to forage on the tree. Most studies on spinescence have focused on herbivore feeding rate

in response to thorn density (Gowda 1996, Cooper *et al.* 2003) and spine length (Milewski *et al.* 1991, Gowda 1996, Young & Okello 1998). This study will focus rather on how the white colour of *Acacia* thorns acts as a type of aposematic deterrence described by Lev-Yadun (2009 unpublished).

The issues as to whether animals are responsive to different colours is highly debated in the literature (Cogan 1995 and Stoerig 1998) but recent research by Kelber *et al.* (2003) has shown that mammals can read optical signals, such as the conspicuous white thorns in the African *Acacia* genus. Although large mammalian herbivores probably do not see colour in the same manner as humans, they have dichromatic vision allowing them to distinguish contrasts between the thorn and the green "background" colour of the plant leaves (Kelber *et al.* 2003). *Acacia* species are of particular interest as a white signal is highly advantageous over any other colour as the contrast is highly emphasized even in colour-blind animals (Givnish 1990). It is also visible in low light intensities (Midgley *et al.* 2001). There exist many hypotheses that argue the purpose of defensive colouration in plants (Lev-Yadun & Inbar 2002 and Schaefer & Wilkinson 2004) but it can be assumed, ~~in corresponding view~~ consistent with Diamond (2005), that a single explanation of a certain trait may fail when complex environmental issues are of concern.

define terms: hooks & spines are a subset of thorns. Hooks are epidermal and have no vascular tiss

Although thorns and hooks are designed to warn off herbivores as well as reduce feeding rate (Gowda 1996), observations from this study show it is relatively simple for an ungulate to pick around the thorns and access the palatable leaves. There is a higher cost for the plants to produce structural defenses compared to chemical defenses because more energy is invested in manufacturing the defensive structures rather than in growth and reproduction (Rohner & Ward 1997). Therefore, there must be an important evolutionary reason for these species to be allocating so many resources to producing spines. The wide range of responses by plants to herbivory suggests herbivory is costly to the plant (Rohner & Ward 1997). However, few studies have shown that herbivory, to a certain extent can, in fact be beneficial to the plant (Vickery 1972, Chew 1974, Dyer 1975, Dyer

whose?

too many references

the classic study is Paige & Whitham

& Bokhari 1976, McNaughton 1976, Owen &Wiegert 1976, Hendrix 1979, Owen 1980, Hilbert *et al.* 1981 and Inouye 1982). Owen &Wiegert (1976) suggest that plants tolerate a certain degree of herbivory in order to maximize plant fitness. Plant fitness can be defined as the relative reproductive success of the plant. Herbivory has been found to increases foliage density and via overcompensation, the plant produces a greater output of seeds. Although this hypothesis is surrounded with some uncertainty (Owen & Wiegert 1976 and Belsky 1986), Miller & Coe (1993) have shown this to be specifically evident in African *Acacia* species. They show *Acacia* seeds are appealing to ungulates (rounded shape, high nutrient content and scented; Gwynne 1969) and once consumed, the gut of the animal accelerates seed germination by scarifying the seed coat (Lamprey 1967 and Halevy 1974). Herbivory on *Acacia* species also reduces bruchid beetle (Bruchidae, Coleoptera) larvae infestation, which predate on the seeds. 99% of African *Acacia* seeds are invaded by the larvae (Southgate 1981). To compensate for the advantages of granivory, a fair degree of foliovory on the plant can therefore be tolerated by the plant.

Is it possible that *Acacia* thorns have a dual function of protecting their branches as well as fulfilling the criteria for Lev-Yadun's (2009 unpublished) aposematic colouration hypothesis? By displaying their thorns obviously, *Acacia's* could be using the honest defense mechanism, thereby warning animals of the pain they could inflict if they were browsed. Instead of camouflaging their defense mechanism, the thorns are advertised (Lev-Yadun 2009 unpublished).

By painting thorns on the branches of *Acacia tortilis, Acacia karroo* and *Acacia sieberiana* green and others white, the effect that the different colours have on herbivore choice and feeding rate could be determined. The experimental design is similar to Soltau *et al.* (2009), where they painted white variegation on non-varieted leaves to see the corresponding effect it had on herbivory. Firstly it was established whether the goats initially avoided the conspicuous white thorns of the varying *Acacia* species. It was predicted that the browsers would avoid the obviously defended white-thorned branches,

choosing the green-thorned branches the majority of the time. Secondly, when the white *Acacia* thorns were browsed on would the feeding rate differ between the two treatments? With the white-painted thorns forming a contrast to the green or brown leaf background, it is predicted that the browsers would be able to detect the thorns easier and pick around them, removing the leaves off the branch at a faster rate. This study contains a large diversity in goats, locations and *Acacia* species utilized therefore increasing the scale of this study. This makes it more reliable to assume the same result will occur in future studies on the same topic (Du Toit *et al.* 2005)

MATERIALS AND METHOD

The study took place at three different locations, using different numbers of goats and with three different *Acacia* species but with the same sampling method. The three *Acacia* species used in this study include *Acacia tortilis* (Forssk.), *Acacia karroo* (Hayne.) and *Acacia sieberiana* (DC. Burtt Davy). ~~All species belong to the family~~ Fabaceae.

The first study site was based at Scientific Services at Skukuza Research Camp in Kruger National Park (K.N.P.), Mpumalanga, South Africa (24°99' S, 31°59' E). Skukuza, a region of semi-arid savanna, is located in the southern section of the park. *Acacia* samples were taken from an area, which is constantly accessible to herbivores. The branches were fed to browsers (goats) at a rural farm 30 ~~kilometers~~ west of K.N.P.'s Paul Kruger gate, in the town of Justicia. The species used for sampling at this site was *Acacia tortilis*. ~~It is commonly known as the~~ umbrella thorn and occurs in low altitudes dry areas (Palgrave 2002). *A. tortilis* has a pair of short hooks and longer white thorns at the base of each leaflet on the branch. Pods are indehiscent and, along with their palatable leaves, are highly nutritious to livestock and game.

The second and third study sites were based in Cape Town, Western Cape, South Africa. *Acacia sieberiana* was used at the second study site in Pinelands (33°56' S, 18°30'E). It was collected from a garden in Camps Bay (33°57' S, 18°22'E), where it grows

unbrowsed. The paperbark thorn is a flat topped, deciduous tree preferring grassland and open bushveld. The pods are also indehiscent and popular among game and livestock (Schmidt *et al.* 2004). Goats used at this site were located in an "eco-village" in Pinelands. The Oude Molen village was created with the aim of establishing a working model of a sustainable eco-village in Cape Town and goats, horses and sheep are located on the premises. The third species, *Acacia karroo*, was purchased as seedlings from Kirstenbosch nursery, Newlands (33°99' S, 18°43' E). The sweet thorn is an evergreen species and is the most common and widespread in South Africa, occurring in all 9 provinces (Schmidt *et al.* 2004). This species, similar to *A. sieberiana*, has only straight thorns, with no hooks at the base of each leaflet on the branch. The goats used at this site were located in Philippi, a rural area on the outskirts of Cape Town (34°01' S, 18°33' E). These all-male goats were kept together in a large pen, ~~on sale to the local people~~.

The ~~test subjects~~ were domestic goats (~~*Capra aegagrus hircus*~~). Goats are horned, foregut fermenting ruminants. They are browsers, preferring woody shrubs to grasses and have been used in this study as a proxy for how indigenous browsers might impact plants with spinescent defenses. The herbivores were all kept in fenced pens and fed at varying times of the day.

Experimental design and Protocol

Sampled *Acacia* trees were in close proximity to one another (>10 m apart) and therefore it was assumed that they have similar growing conditions (e.g. soil quality, rainfall received, equal disturbance etc). Branches were collected at a similar height on the tree (0.5-2 m above ground). Three possible treatments were implemented on the discrete branches: the thorns on the branches were either left alone, unpainted, as a control, painted with white paint or painted with green paint. One third of the branches were painted to have white thorns to control for the possible effects paint may have on the goat's choice. The paint that was used was a water-based paint, and the green coloured paint was mixed to match the colour of the *Acacia* leaves. The two branches of

contrasting colour, chosen for each trial matched in length, branching pattern, leaf density and thorniness to maximize uniformity. Each pair matched was allocated at random to a treatment. The thorns were painted on the branches up to 30 cm from the terminal of each branch (~~using a standard meter stick~~), to ensure uniformity.

Branches were fed to the goats at the different locations in the same manner. Very little vegetation was within reach of the enclosure, so there were few other food sources to influence the goats' feeding attention. For each trial, a choice of two different thorn branch conditions was compared. Feeding rate was determined in each case using a variety of measurements including bites per second, duration spent at each branch within an allocated minute, mass removed, ~~per second~~, mass removed per bite (study site 1) and amount of leaflet removed ~~per minute~~ (study site 2 and 3). At the first study site the two branches were weighed prior to the feeding and after feeding with a 30g Micro-Line Scale (Pesola). The method was revised for the following two sites as the weight loss was so small even if all the leaves were removed by the goat. The numbers of leaflets on the branches were thus counted before and after feeding at sites two and three.

The two branches of differing treatment were placed into the fenced arena for feeding at equal distance from the goat. At the first study site, beginning at the initial bite, the goat was given one minute to feed on the branches and the time spent on each branch was recorded. At the second and third study site, the goat was allocated 30 seconds to one minute to feed on each treatment, depending on the size of the branch and the density of the leaves. An observation of the first branch colour chosen by the goat was recorded after each trial. Both factors (feeding rate and initial choice) should show the difference in preference between the two branches. The time of feeding included both time eating the branch and time spent in close proximity to the branch. Each new trial switched whether the branch type was held to the left of the goat or to the right of the goat. Six goats were used in the first study, five in the second and 15 in the third.

May I assume that the goats did not all feed together?

Data analysis

Initial choice – painted branches vs no paint

To determine if there was significant difference in the goat's choice between painted and unpainted branches a chi-squared test was conducted between these two treatments. The expected results would be a completely equal choice with 50% of the goats choosing unpainted branches and 50% choosing painted-white thorned branches. As there was only one degree of freedom, a Yates' Correction was implemented. Paired t-tests were also implemented for time spent at each branch and number of bites on each branch (both variables were normally distributed) and a Wilcoxon test was used for the relative weight loss (weight loss over initial weight), as it is a derived value. ~~All these statistical tests were confirming that goats did not prefer unpainted branches over painted branches in any way.~~

Initial choice – green vs white

Once it was verified that goats did not prefer unpainted branches and did not avoid painted thorns in anyway, another chi-squared test was used to determine if there was a difference in the first choice between green and white thorned branches. As above, an equal choice of each treatment was expected and as there was only one degree of freedom, a Yates' Correction was implemented.

Feeding rate

(i). Time spent at green branches was compared to the time spent at the white branches recorded form the first study site with a paired t-test (data was normally distributed).

(ii). Wilcoxon test for two dependent samples was used to determine if there was variability in relative weight loss. The non-parametric test was used, due to relative weight's derived nature. Relative weight loss was chosen over absolute weight loss, as the relative value minimizes statistical differences due to natural differences in branch weight. The box-and-whisker plots were also utilized to visualize if any trends were

present for time spent at each colour branch and relative weight loss from each colour branch.

(iii). Further Wilcoxon tests were used to compare the numbers of bites per second, relative weight removed per second, relative weight removed per bite for each branch treatment and the number of leaflets removed per minute for each treatment. All Wilcoxon tests were used to compare differences that could arise in each trial, not differences that may arise independent of trial.

Avoid single-sentence paragraphs

All statistical tests were conducted using Statistica version 5.2 (Stat Soft, Inc. 2007).

RESULTS

Goats demonstrated no preference or aversion toward paint in the control test when comparing white painted thorns and normal, unpainted thorns ($X^2_{(1)} = 0.9$, $P > 0.05$). All measures of feeding rates in the first study, i.e. number of bites taken from each branch ($t_{[8,1]} = 1.859$, $P = 0.304$), total time spent at each branch ($t_{[9,1]} = 1.833$, $P = 0.201$), and relative weight removed from each branch ($W_s = 13.00$, $P = 0.484$) were unaffected by the white paint control ~~as well~~. As goats exhibited no preference, the data from the branches painted white and painted green, and normal, unpainted and painted green tests were combined for further statistical analyses.

Goats in the first study site showed no inclination toward either colour spine, based on initial selection, choosing branches with each colour equally; 17 white branches and 17 green branches ($X^2_{(1)} = 0.0294$, $P > 0.05$). The second and third study sites in Cape Town showed the same results with goats choosing 11 white branches from the 25 replicates and 14 green branches. The goats did not avoiding the painted white thorns ($X^2_{(1)} = 0.36$, $P > 0.05$; Fig 1.).

Further statistical tests displayed no difference between feeding rates on either of the two coloured branches. The number of bites/sec ($W_s = 252.00$, $P = 0.437$), relative mass

removed/sec (W_s = 261.00, P = 0.532) and relative mass removed/bite (W_s = 247.00, P = 0.984) in the first study were not significantly different. However, trends for relative biomass removed (W_s = 218.00, P = 0.264) and total time spent at each branch ($t_{[33,1]}$ = 1.53, P = 0.136) emerged (Figs 2 and 3). Goats tended to spend more time at branches with green-painted thorns, however they removed less biomass compared to branches with white thorns. Further data was collected in study sites two and three, ~~in an attempt to make this trend clearer~~. However no significant difference resulted in the ~~amount~~ of leaflets removed from the green branch compared to the white painted branch (W_s = 108.5, P = 0.37). No significant difference in feeding rate between the two treatments emerged.

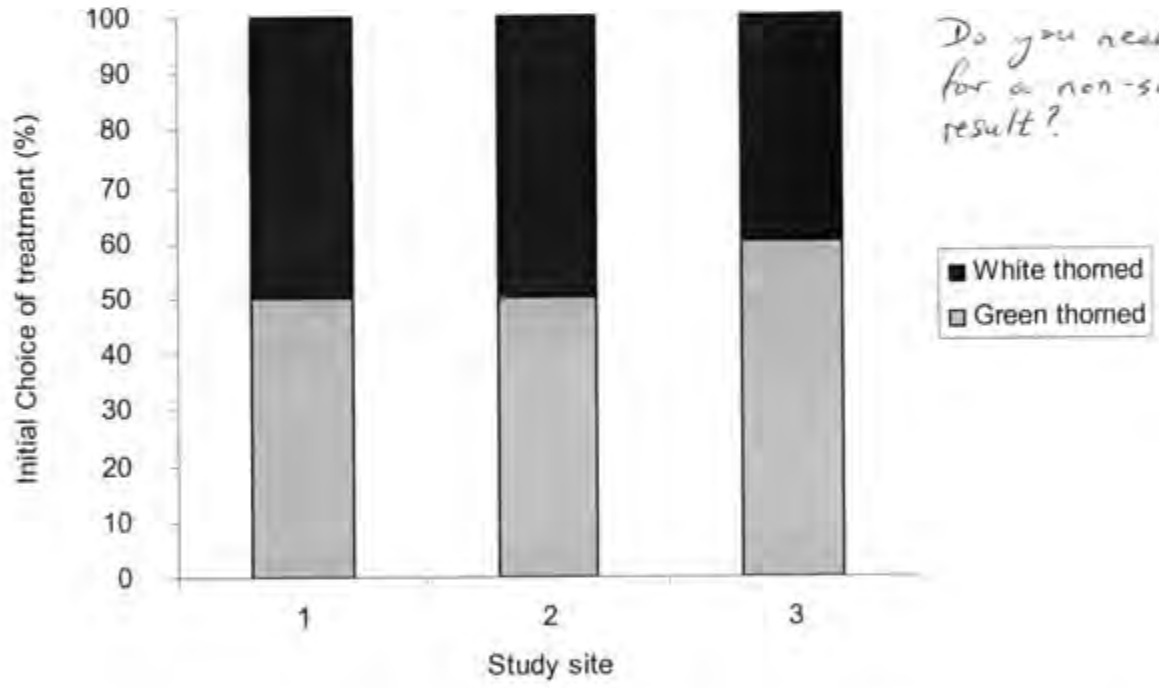


Figure 1: Results from all three study sites showing the near equal choice by browsers between painted green and white thorned *Acacia* branches.

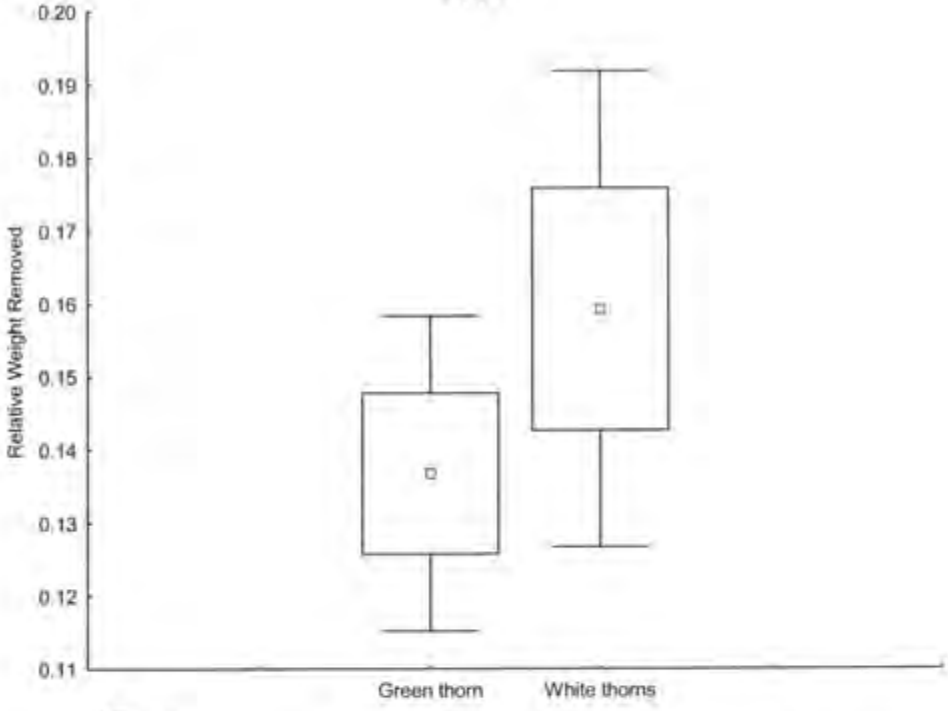


Figure 2: Displays the comparison between the relative weight removed (initial weight-final weight/initial weight) from *Acacia tortilis* branches with green-painted and white-painted thorns by goats in a one minute period in site 1. Significance?

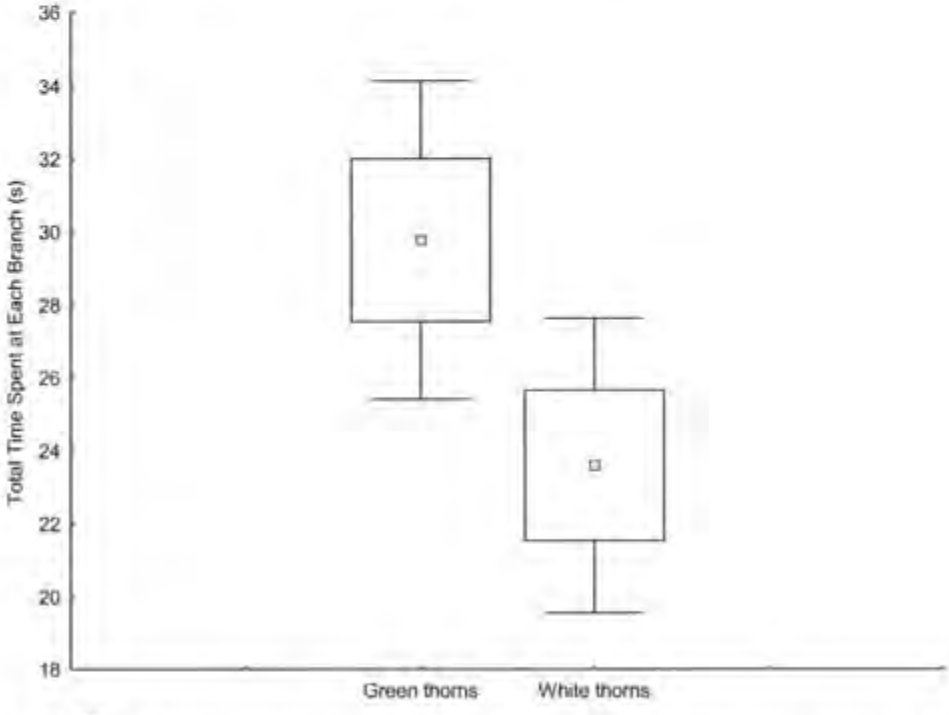
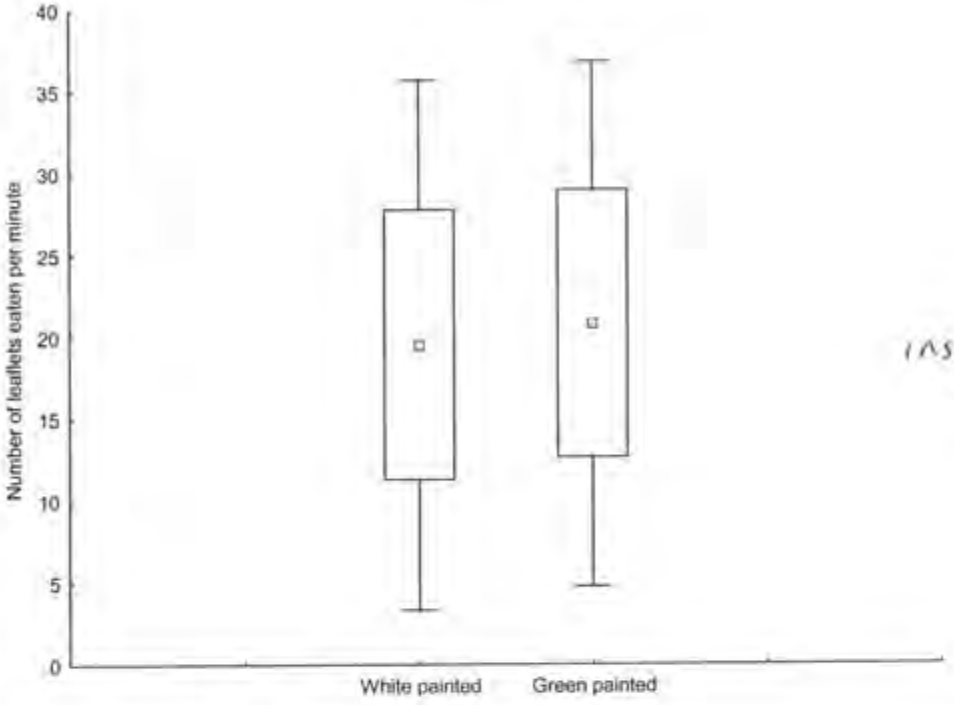


Figure 3: illustrates comparison of total time spent physically (feeding or examining) at branches with *Acacia tortilis* green-painted and white-painted thorns by goats in site 1.



insign. heart = unimportant

Figure 4: Demonstrates the insignificant difference in the number of leaflets removed per minute from green-painted and white-painted *Acacia* thorns from sites 2 and 3 combined.

Is it worth displaying this?

Table 1: Summary of all measurements of feeding rates taken from all three study sites and the corresponding P values illustrating insignificant differences between white-painted and green-painted thorns of *Acacia* species.

Feeding Rate Measurement	**Significance**
Absolute time spent at each branch	P = 0.137
Absolute weight removed	P = 0.786
Relative weight removed	P = 0.264
Absolute weight removed/second	P = 0.309
Relative weight removed/second	P = 0.532
Bites/second	P = 0.437
Absolute weight removed/bite	P = 0.911
Relative weight removed/bite	P = 0.984
Leaflets removed/min	P = 0.370

* $P < 0.05$

DISCUSSION

Despite the vast amount of studies on the evolution of aposematic colouration in animals, the advantageous function it has regarding spiny plants is only just beginning to receive the attention it needs. Simcha Lev-Yadun proposed that coloured thorns have evolved as visual warning signals to herbivores, often by mimicking the physical appearance of poisonous plants, making animals learn to avoid the plant (Lev-Yadun 2001, 2003). This is generally known as Batesian mimicry. In this study, however, when offering 59 white-thorned and 59 green-thorned *Acacia* branches to goats, they chose the white-thorned branches 28 times and the green-thorned branches 31 times. This near 50% split demonstrates that thorn colouration has no effect on browser initial branch choice (Fig. 1). White thorns of *Acacia* therefore neither act as an aposematic deterrent, facilitating avoidance behaviour, nor as an attractant, in contrast to the predictions of Lev-Yadun (2009) and Milewski *et al.* (1991). Since the formation of this white defense structure requires extra energy expenditure (Midgley 2004), other possible evolutionary drivers were explored as to why this genus evolved to bare white thorns.

Painted white and painted green thorns had no difference in the extent of herbivore damage when comparing the differences in feeding rate and biomass removed (Fig. 2-4). Studies have shown that, in general, thorns reduce the feeding rate of herbivores by decreasing their average bite size, thus they remove less plant biomass per unit time (Cooper & Owen-Smith 1986 and Ronel *et al.* 2009). Animals tend to spend less time foraging on defended plants as it could forage more efficiently on a plant without defenses. Green thorns are more cryptic compared to the conspicuously white *Acacia* thorns because they match the green leaves. It was thus expected that they should further diminish browser feeding efficiency as the herbivore takes more time to safely consume leaves or branches around the camouflaged thorns in fear of injury. Within each experimental trial, white thorns should have essentially given each goat a feeding rate advantage over the green-thorned option, as they could more easily identify and evade the individual obstructions. Since white thorns allow herbivores to feed more easily on

leaves, goats should have consumed more leaves off a white-thorned branch than off a green-thorned branch in a set time period. During the experiment, it was observed that most of the goats initially attempted biting branches with their molars, only to receive a cheek or eye full of thorns. After their first effort, individuals quickly switched to a front-teeth approach, selectively picking out single leaves amid the thorns. There was however, no difference found in the feeding rates values that should have corresponded with a higher feeding rate in white-thorned branches. This result could perhaps be due to the relatively short length of the branches used in the experiment. Longer, more densely leaved, or better-defended branches would possibly reveal underlying differences.

Upon first glance, these outcomes do not explain the actual evolutionary advantage of white thorns in *Acacia*s. If a plant is going to expend energy creating a mechanical defense, why invest more resources, by synthesizing pigment and sacrificing photosynthetic ability, when it is actually facilitating predation (Midgley 2004)? The explanation proposed in this study is that the *Acacia* evolved aposematic colouration in order for it to gain the indirect or veiled benefits of herbivory, but still possess thorns to avoid total destruction. The *Acacia* genus is generally highly palatable, compared to other savanna species (Milewski *et al.* 1991) and thus is regularly browsed upon (Miller 1994 and Or & Ward 2003). As Gowda (1996) concluded, the thorns of most *Acacia* species exist to protect the branches rather than the leaves, thus controlling levels of pruning (shoots and leaves) rather than the level of picking (leaves alone). This slows the feeding rate and regulates the bite size of herbivores. Milewski *et al.* (1991) showed clear evidence that *Acacia* thorns prohibit giraffe from browsing on whole branches. Removal of shoots or branches is much more detrimental to the plant and thus requires the bulk attention of defensive structures (Midgley *et al.* 2001). This branch-protection hypothesis was evident in the feeding behaviour observed in this study, as goats never consumed portions of our test branches, except for occasional thorn-less new growth tips. They did however, manage to get at leaves hidden amongst most sections of the branches by the manipulation method described by Bond *et al.* (2004), whereby the goat can pluck out

single leaves or leaflets around a thorn. The costs of pigment production for the conspicuous defense organs is most likely offset by the energetic advantage of branch or shoot biomass saved by the thorns (Gowda 1996).

The question remains why *Acacia's* have evolved white thorns to assist a browsing ungulate in finding its leaves, while still investing heavily in branch defenses. Since colouration of thorns is so widespread, the phenomenon is probably not a random evolutionary trait (Lev-Yadun 2001). *Acacia's* possess two types of pods; dehiscent (pods split and seeds dispersed by gravity or wind) and indehiscent (pods only release seeds when mechanically in heavy rain or due to browsing; Miller 1994). It has been proposed that indehiscent seeds, which all *Acacia* species in this study possessed, have evolved specifically for ungulate consumption forming a mutualistic relationship between the plant and the herbivore as pods are hard, therefore resisting chewing damage, and are strongly scented (Gowda 1997, Miller & Coe 1993, Miller 1994). While most studies are focused on the negative effect of herbivory on plant fitness (see references in Paige & Whitham 1987), many studies have shown another perspective, that plant-herbivore interaction can, to a certain degree be beneficial to the plant, especially within the genus *Acacia* (Chew 1974, Dyer 1975, Dyer & Bokhari 1976, McNaughton 1976, Owen & Wiegert 1976, and Inouye 1982).

Firstly, there is substantial evidence that a certain degree of herbivory in general has the ability to alter plants' nutrient flow, even though leaf biomass is reduced (McNaughton 1985, Jaramillo & Deltling 1988, Coughenour *et al.* 1990 and du Toit 1990). Chapin (1980) found stimulated root nutrient uptake (in particular phosphorus) after clipping tundra sedges. A small level of herbivory on *Acacia's* can mimic pruning and thus improved fitness by keeping the plant younger (du Toit 1990). Gowda (1997) found *A. tortilis* to have higher leaf nutritional value and higher compensatory growth in the presence of herbivores, suggesting herbivory is not in fact hindering their growth or survival. Trees in the *Acacia* genus have the ability to fix nitrogen because of a

mutualistic relationship with root nodules (Paige & Whitham 1987). Nitrogen, which is usually limited in the soil, is accessible more readily by the *Acacia* trees (Norby *et al.* 2000 and Craine *et al.* 2008). *Acacia's* may therefore have a unique ability to balance the amount of nutrients lost by foliovory, encouraged by the contrasting white thorns, as they are capable of gaining other nutrients from below ground.

Secondly, herbivores play an integral role in seed dispersal (Miller & Coe 1993). *Acacia* seeds prefer establishment in open areas away from the shaded canopy of the parent plant and therefore seed survival is increased when a dispersal agent, such as a browser, is involved. Studies have found that the body size of the browser is correlated to the probability of seed survival during feeding, the distance the seed is dispersed as well as the probability of the seed being deposited in an open area (Miller & Coe 1993, Rohan & Ward 1999 and Or & Ward 2003). The fruits of the *Acacia* are positioned at the intersection of the pair of white thorns, thus the thorns could act as a visual marker for herbivores locating the nutritious seeds. If the seed is consumed by an ungulate the protective testa is broken down much faster in the gut because of the gastric acids compared to natural mechanical weathering (Lamprey 1967). Most seeds that pass through the gut remained unharmed (Lamprey *et al.* 1974) and once it is released by the animal through defecation it is a fair distance from the plant, enhancing survival. It is also advantageous for *Acacia* seeds to be consumed as Miller (1995) has shown that germination of the uneaten seeds is much lower (3.2%) compared to seeds which have been eaten. The dung provides a nourishing microclimate for the seed to germinate (Owen 1980). The proposed hypothesis that the function of white thorns is to obviously display the location of the seeds needs to be further researched.

Further insight supporting the "white thorns act as a fruit attractant" hypothesis mentioned above and possibly the greatest advantage of herbivory on *Acacia's* are the impact ungulates have on Bruchid beetle infestation. Bruchid beetles (Bruchidae, Coleoptera) also predate on the fruits, infesting and ultimately destroying the seeds, and

often damaging the tree. An example given by Miller (1996) shows that *Bruchidius uberatus* can destroy 60% of the seed crop of *A. nilotica*. *A. tortilis* in particular is widespread across eastern, southern and northern Africa and thus can possess the highest number of Bruchid infesting species (Miller 1996). Mammalian herbivores are mainly attracted to the fresh fruits and pods still remaining on the tree and can thus remove and disperse the Acacia seeds before the bruchids have a chance to invade (Milewski and Madden 2006, Miller 1996). The beetle larvae can be destroyed in the stomach acids or crushed by the teeth during mastication (Or & Ward 2003). Levels of bruchid infestation are much lower in the presence of herbivores (0-45%) than in the absence of them (25-99%, Or & Ward 2003). It would therefore be evolutionary advantageous for *Acacia* species to evolve conspicuous white thorns so herbivores learn where the fruits are positioned on the tree. Domestic goats have not co-evolved with *Acacia's* like other African browsers thus the knowledge about the function of thorn coloration remains unknown to them yielding in risky results from this method of the study. The reliability of using a goat as a proxy for an African browser should therefore be questioned as a familiar natural herbivore, such as an impala (*Aepyceros melampus*) could possibly recognize the colouration advantage over a domestic goat.

Future research on the reasons behind aposematic thorn colouration in *Acacia's* should focus on the existence of pathogens and bacteria, which could have existed on the thorns in the past. Thorns have the ability to pierce through the skin of the animal therefore directly introducing the microorganisms into the animals system, which can be more dangerous than the actual physical damage form the thorn (Lev-Yadun & Helpern 2008). By mimicking a possibly harmful bacteria, which are white in colour, *Acacia's* could have evolved to focus their pigment production to mimic this form of browser deterrent. The concern of the use of the domestic goat as a substitute for a natural African browser becomes questionable again. Perhaps the goats have no evolutionary knowledge of this bacterium and therefore the white thorns did not affect their initial choice of treatment. The toxic fungal sclerotia for example, are generally associated with conspicuous colours

such as black, yellow, violet and white (Lev-Yadun 2001). Other studies in the field have been done on flora in Europe and North America, which have white fungal hyphae visible to the herbivore and the evidence shows browsers avoiding these individuals (Lev-Yadun 2006). However, bacteria on the thorns of the African *Acacia* species in particular, has not yet been explored and much of the literature on microorganisms on plants is sited in medical journals with little reference to the ecological implementations.

One main concern in this study is that the goats only had a choice of branches containing thorns. The function of the white thorns could possibly still act as an aposematic deterrence when its neighboring plant possesses no thorns. It would then be expected that the browser would chose the less obviously defended plant over the conspicuously harmful *Acacia*. A simple explanation for the *Acacia's* to possess white thorns is that they are merely an outcome of convergent evolution where a few savanna plants evolved thorns and over time out competed many other species (Lev-Yadun & Gould 2008). In order to keep up with the increased pressures of herbivory, *Acacia's* mimicked these other plants attaining a similar feature, which was the characteristic white thorns. This is commonly known as the Neutral theory, whereby plant traits are a relic of past evolutionary outcomes (Soltau *et al.* 2009).

CONCLUSION

The most conclusive evidence of this study is that white colouration of thorns serves as neither a visual aposematic deterrent nor an attractant for mammalian herbivores. The feeding rate, no matter what the measurement method used, was not significantly different between browsers feeding on green or white thorns. This possibly suggests that white thorns evolved to attract a certain amount of granivory and folivory due to their highly conspicuous nature and subsequently harnessing the benefits of herbivory, while still protecting the branches, which are integral to the plant's overall survival. Seeds of white-thorned trees would have more nutrients due to higher fertilization levels, survive in greater proportions due to decreased bruchid beetle predation, and would be dispersed

further from the parent plant, giving it a greater chance of colonizing a new area and enhancing genetic variability, thereby positively selecting for the white thorn colouration trait. My results therefore support Rubino and McCarthy (2004), who suggest that aposematic colouration in plants is beneficial in the long run.

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