

Regrowth patterns, defences and allocation of stored energy reserves in *Acacia* seedlings following herbivory and fire

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ABSTRACT

Disturbances, such as herbivory and fire are commonplace in savanna ecosystems. The effects of herbivory and fire on growth and defences of adult trees is a much studied field in plant ecology. However, there are comparatively few studies that have investigated the effects of herbivory and fire on seedling growth, defences and establishment. African *Acacia* trees are common and widespread, and are suggested to be keystone species in savanna ecosystems. They have been shown to significantly positively increase soil characteristics, such as soil moisture and infiltration, while also increasing spatial heterogeneity of savannas. These trees are distinguishable by their array of physical and chemical defences. Physical defences are either in the form of spines (physiologically costly to produce, thus considered inducible) or prickles (physiologically cheap to produce, thus considered constitutive), while condensed tannins are the most common form of chemical defences. Adult *Acacia* trees have been shown on several occasions to be highly resilient to disturbance events, primarily due to their large size. However, the effects of herbivory and fire on *Acacia* seedlings have been little studied despite their apparent importance for our understanding of African savanna ecosystem functioning.

In two separate experiments, this thesis aimed to investigate the individual and combined effects of simulated herbivory and fire on the regrowth, defences, and total non-structural carbohydrates (TNC) of the seedlings of several *Acacia* species, while also testing the Resource Availability Hypothesis (RAH) (14 *Acacia* species) and the Expanded Growth-Differentiation Balance Hypothesis (GDBH) (three *Acacia* species). The RAH (Coley, Bryant & Chapin 1985) and the GDBH (Loomis 1932) both assume that arid-adapted (resource-poor) species are slow growing, with low tissue turnover rates. Contrastingly, humid-adapted (resource-rich) species have fast growth rates and thus a high tissue turnover rate. Therefore, arid-adapted species are predicted to invest more carbohydrate reserves in defence after a disturbance event, in order to defend new photosynthetic material. Conversely, humid-adapted (resource-rich) species are predicted to invest carbohydrate reserves into increased growth after a disturbance event, in order to compensate for tissue loss.

The first greenhouse experiment found that, in accordance with the RAH, humid-adapted species displayed elevated growth rates compared to arid-adapted species. Overall, defences significantly increased after herbivory, but significantly decreased after fire. Herbivory was also

shown to significantly reduce TNC stores in roots more than fire. We found that arid-adapted species did indeed invest more carbohydrate stores (TNC) into defence, and displaying an increase in spine and prickle abundance, spine and prickle length, and condensed tannin concentration. Humid-adapted species displayed an increase in growth rather than in defence, with the majority of species only increasing one defensive trait. Humid-adapted species also displayed significant trade-offs (negative correlations) between new stem growth and defence traits, while arid-adapted species overall did not display any significant trade-offs between stem growth and increased defences. The majority of arid-adapted species also displayed an allometric effect for spine abundance and length, with an increase in one trait led to an increase in the other. Prickles were found to be inducible, despite the assumption that due to low physiological cost, they are not inducible. Herbivory and fire were shown to not be substitutable in their effects on *Acacia* seedlings in a controlled experimental setting.

The second greenhouse experiment tested the Expanded Growth-Differentiation Balance Hypothesis (GDBH) in the seedlings of *A. erioloba*, *A. karroo*, and *A. nilotica* using five levels of nutrient availability. Overall, spine abundance and spine length displayed a unimodal trend in all three species, with spine abundance and spine length being greatest at a nutrient availability of 800 mg/ ℓ. Spine abundance, spine length and condensed tannins increased significantly after herbivory, but were shown to significantly decrease after fire. We found that with an increase in nutrient availability, the growth of stems and roots, along with the production of TNC in roots and stems of all species significantly increased. Our data provide mixed support for the assumptions and predictions of the expanded GDBH. The regrowth of stems and physical defences were consistent with the GDBH. Chemical defences (i.e. condensed tannins) were however, inconsistent with the predictions of the GDBH.

We have shown that *Acacia* seedlings are highly resilient to disturbance events, while the growth responses of *Acacia* seedlings are underpinned by TNC stores in roots. Simulated herbivory and fire are often substituted for one another in controlled experiments. However, we have shown that the effects of herbivory and fire have significantly different effects on regrowth patterns of *Acacia* seedlings.

PREFACE

The data described in this thesis were collected in the Republic of South Africa from September 2010 to December 2011. Experimental work was carried out while registered at the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor David. M. Ward.

This thesis, submitted for the degree of Masters in Science, in the Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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Jeffrey Hean

January 2012

I certify that the above statement is correct

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DECLARATION 1 - PLAGIARISM

I, Jeffrey William Hean, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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 - a. Their words have been re-written but the general information attributed to them has been referenced
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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

Publication 1

Hean, J.W & Ward, D.M. Fire and herbivory are not substitutable: Evidence from regrowth patterns and mobilization of total-non-structural carbohydrates of seedlings of 14 *Acacia* species. Submitted to *Journal of Vegetation Science*

Author contributions:

JW Hean conceived paper with DM Ward. JW Hean collected and analysed data and wrote the paper. DM Ward contributed valuable comments to the manuscript.

Publication 2

Hean, J.W & Ward, D.M The effects of nutrient availability on growth patterns and mobilization of total non-structural carbohydrates in seedlings of three *Acacia* species. Submitted to *Functional Ecology*

Author contributions:

JW Hean conceived paper with DM Ward. JW Hean collected and analysed data and wrote the paper. DM Ward contributed valuable comments to the manuscript.

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Chapter 1

Resprouting, inducible defences and non-structural carbohydrate reserves of *Acacia* seedlings after fire and herbivory: A review

Introduction

The genus *Acacia* is common and widespread in African savannas and can be found in a variety of habitats, and can be considered to be keystone species in their environments (Munzbergova & Ward 2002; Shaw, Keesing & Osterfeld 2002). They play a key role in savanna ecosystem functioning by manipulating the surrounding soil structure, such as fixing nitrogen, promoting hydraulic lift, increasing soil moisture and water infiltration, as well as being a primary food source to numerous ungulate and insect species (Dube Mlambo & Sebata 2010). They are a valuable group of tree species that have been shown to increase the heterogeneity of the surrounding savanna (Belsky, Amundson & Duxbury 1989). Thus our understanding of *Acacia*-savanna interactions becomes key to building our knowledge on overall savanna ecosystem functioning. Despite the significant effects of fire and herbivory, as well as the importance of *Acacia* species throughout Africa, few studies highlight the effects of herbivory and fire on *Acacia* seedlings (Bond & Midgley 2001; Midgley, Lawes & Chamaille-Jammes 2010).

FIRE AND HERBIVORY AND THEIR EFFECTS ON REGROWTH AND DEFENCE

Fire and herbivory can be classified as large-scale disturbance events that shape and drive many *Acacia* savanna communities (Barnes & Midgley 2001), while also playing an integral role in the regeneration of numerous woody species (Pickett & White 1985). Thus, the short- and long-term effects of fire and herbivory on the establishment and recruitment of woody species is a vital component in our understanding of African savanna dynamics. As long-lived species, trees require particular adaptations to complete their demographic cycle in frequently- and intensely-disturbed habitats (Bond & Midgley 2001) Resisting disturbances such as fire and herbivory by large mammals is relatively easy for adult trees due to their large size (Palo, Gowda & Högberg 1993). However, it is more problematic for young individuals such as seedlings and saplings (Meyer et al. 2005). Consequently, our understanding of how these disturbance events affect seedling survival and recruitment is limited to fragments of literature (e.g. Gowda 1997; Fornara & Du Toit 2008; Tsumele, Mlambo & Sebata 2006; Dube et al.2010).

This review aims to determine the extent of the knowledge attained on the effects of fire and herbivory on the physiological properties of *Acacia* seedlings, as well as determining how disturbance events may govern seedling survival, growth and recruitment in African savannas. The current literature on *Acacia* species has focused on the encroachment of particular *Acacia* species on grasslands and savannas (e.g. Roques, O'Connor & Watkinson 2001; Kraaij & Ward 2006; Higgins et al. 2007; Balfour & Midgley 2008; Hagenah et al. 2009), various resprouting responses (e.g. Gignoux, Clobert & Menaut 1997; Bellingham & Sparrow 2000; Higgins, Bond & Trollope 2000; Bond & Midgley 2003; Chong, Edwards & Waycott 2007; Aleper, Lye & Moe 2008; Gignoux et al. 2009) and the production of chemical and physical defences of adult trees after herbivory and fire (e.g. Gignoux et al. 1997; Gowda 1997; Rohner & Ward 1997; Gadd, Young & Palmer 2001; Gowda & Raffaele 2003; Arimura, Kost & Boland 2004; Schindler, Fulbright & Forbes 2004; Zinn, Ward & Kirkman 2007; Aleper et al. 2008; Mboumba & Ward 2008; Ward 2010; Ward, Shrestha & Golan-Goldhirsh 2011)). There is, unfortunately, an apparent lack of detailed information on how *Acacia* seedlings respond to fire and herbivory (e.g. Schutz, Bond & Cramer 2009; Wigley, Cramer & Bond 2009; Cramer et al. 2010).

Responses of *Acacia* species to fire and herbivory

Woody species have varied responses to the removal of aboveground photosynthetic material and biomass (Arimura et al. 2004). Resprouting is a common response to the partial or complete removal of aboveground biomass (Bond & Midgley 2001, 2003, Nzunda, Griffiths & Lawes 2008), with fire being the most frequent cause of total biomass removal (Hoffman & Solbrig 2003). Savanna woody species experience topkill far more frequently than complete mortality after fire (Balfour & Midgley 2008) while frequent fires can eventually lead to overall mortality of individuals and/or cohorts (Hoffman & Solbrig 2003). Herbivory has been found to have similar effects on woody species (Belsky 1994; Augustine & McNaughton 2004). Belsky (1994), demonstrating that herbivory can significantly suppress new shoot growth in *Acacia* seedlings, although topkill through herbivory is rare. However, other researchers contradicted this notion and suggest that herbivory has no negative effects on regrowth of new shoots (see e.g. Du Toit et al. 1990; Rooke et al. 2004). Fornara & du Toit (2007) demonstrated that a high level of

herbivory induces a positive feedback loop in the growth of new shoots in *A. nigrescens*, analogous to grazing lawns.

PHYSICAL AND CHEMICAL DEFENCES: SPINES, PRICKLES AND POLYPHENOLICS

Plant defences come in numerous forms, ranging from numerous modified plant tissues that comprise physical defences, to toxic chemical compounds that are the product of plant metabolism, and have been the focus of frequent studies in plant ecology (e.g. Rohner & Ward 1997; Young & Ward 2002; Strauss & Agrawal 1999; Ward 2010; Hean & Ward 2011). Plant defences can be classified into two distinct groups, namely constitutive defences and induced defences. Constitutive defences have a fixed level of production while inducible defences vary and increase in concentration after herbivory (Karban & Baldwin 1997). Constitutive defences are assumed to be physiologically cheap to produce because they are produced at a fixed level (Karban & Baldwin 1997). Inducible defences however are only produced when necessary (i.e. after a disturbance event), thus are assumed to be physiologically expensive (Karban & Baldwin 1997).

Plant defences, both physical and chemical, have been shown to have a variety of responses to disturbance events. These responses may, however, be species-specific or environmentally dependent. For example, thorn re-growth has been shown to both increase and decrease with fire (e.g. Du Toit et al. 1990; Gowda & Raffaele 2003; Meyer et al. 2005; Aleper et al. 2008; Gignoux et al. 2009) and herbivory (e.g. Bazely, Meyers & da Silva 1991; Gowda 1997; Rohner & Ward 1997; Arimura et al. 2004; MacGregor & O'Connor 2004; Cash & Fulbright 2005; Ito & Sakai 2009), while the production of chemical defences (e.g. polyphenols, alkaloids, terpenes, oxalic acid) (Hanley et al. 2007) displays similar response to those induced by fire (Rohner & Ward 1997). The response of physical defences such as spines and prickles to fire and herbivory may not be consistent because of differences in their production costs. The predominant difference between spines and prickles (collectively known as thorns) is in the way each is produced. Thorns are woody, sharp-pointed modified branches, while spines can be defined as a sharp-pointed petiole, midrib, vein or stipule, making them essentially modified leaves (Ross & Gordon-Gray 1966; Bazely et al. 1991; Grubb 1992; Gutschick 1999). Thus, both are connected to the plants vascular tissue system. In comparison, prickles are sharp-pointed

outgrowths that are merely a modification of the epidermal or cortex cells (Bazely et al. 1991). Henceforth, I will use the term “spine” to indicate that there is a vascular connection and “prickle” to indicate an epidermal outgrowth.

Plants accumulate a wide variety of secondary chemical compounds that have various uses that range from hormonal mimicry to toxicity, and include alkaloids, terpenes and phenolics (Hagerman 2011). The defensive role that plant secondary metabolites play can be categorized as either qualitative or quantitative. Qualitative compounds are generally found in low concentrations (< 2% dry weight), are not dosage-dependent, and work best against non-specialised, generalist herbivores (Theis & Ler dau 2003). They are rapidly synthesized, low-cost water-soluble molecules that interrupt the biochemical and metabolic pathways of herbivores (Hagerman 2002; Theis & Ler dau 2003). Conversely, quantitative secondary metabolites are large, energetically costly, protein-precipitating molecules that often inhibit the digestion of protein in herbivores (Hagerman 2002). Because they are found in high concentrations in plants (5-40% dry weight), quantitative compounds are deemed to be dosage dependent, whereby the higher the intake of the compound, the less carbohydrates can be assimilated from plant tissue digestion (Theis & Ler dau 2003). Tannins constitute one of the most common groups of quantitative chemical defences. They can be defined as water-soluble phenolic compounds that have molecular weights that range from 500 to 3000, and have the unique ability to precipitate proteins (Hagerman 2011). Tannins constitute a diverse group of chemical compounds that play an intrinsic role in plant defence, and have been widely studied (e.g. Ward & Young 2002; Zinn et al. 2007, Hean & Ward 2011).

Role of carbohydrate reserves in *Acacia* regrowth and defence

Non-structural carbohydrates are generally comprised of reducing sugars (glucose and fructose), non-reducing sugar (sucrose), fructans and starch molecules (White 1973), and are accumulated when the demand for photosynthates by various plant processes, including growth, reproduction and differentiation, are met (McKey 1974, Fagerstrom, Larsson & Tenow 1987). Non-structural carbohydrates are important molecular energy components to plants, as they act as energy reserves when photosynthesis is limited, or to initiate growth following tissue removal through

herbivory or fire (White 1973, Van Der Heyden & Stock 1996, Schutz et al. 2009; Wigley et al 2009). Numerous studies have highlighted the fact that the responses of woody species are underpinned by non-structural carbohydrate stores (Trlica & Cook 1971; Ericsson, Larsson & Tenow 1980; Bowen & Pate 1992; Danckwerts 1993; Hendry 1993; Van Der Heyden & Stock 1995; Bell, Pate & Dickson 1996; Van Der Heyden & Stock 1996; Marquis, Newell & Villegas 1997; Bell & Ojeda 1999; El Omari et al. 2003; Kabeya & Sakai 2003; Dube et al. 2010; Kobe, Iyers & Walters 2010). The mobilization of these carbohydrate reserves has been shown to be an influential component of plant tissue regrowth (e.g. Van Der Heyden & Stock 1995, 1996; Kabeya & Sakai 2003; Schutz et al. 2009; Wigley et al. 2009), while Van der Heyden & Stock (1995, 1996) have shown experimentally that the removal of aboveground biomass by herbivory or fire significantly reduces the level of non-structural carbohydrates in below- and aboveground tissues. The greatest reduction in carbohydrate reserves can be observed in the roots, where non-structural carbohydrates are often stored (Hoffmann 1999; Chen, Hutley & Eamus 2003). Kobe et al. (2010) among others, through the use of the optimal partitioning theory, have shown that the mobilization of stored non-structural carbohydrates in woody plants can be modelled, allowing us an insight into the various pathways in which plants may respond to environmental disturbances (Bloom, Chapin & Mooney 1985).

Numerous greenhouse and field experiments have shown that there are several common trends in carbohydrate mobilization after disturbance that allows us to better understand some of the response mechanisms of savanna trees (see e.g. Danckwerts 1993; Bell et al 1996; El Omari et al. 2003). Savanna trees from semi-arid and arid (resource-poor) environments will invest more stored carbohydrate reserves into physical and chemical defences, to allow adequate protection of new photosynthetic biomass regrowth, which is slow and limited due to low soil moisture (Bryant Chapin & Klein 1983; Coley Bryant & Chapin 1985; Herms & Mattson 1992). Contrastingly, humid species (from resource-rich environments) will often adopt the converse, and invest more stored carbohydrate reserves into compensatory growth of photosynthetic biomass (see Bellingham & Sparrow 2000), as they are not generally limited in terms of soil moisture and nutrients. In either case, the fact that stored non-structural carbohydrates are imperative to plant regrowth after a disturbance event such as fire or herbivory is clear (Schutz et al. 2009; Wigley et al. 2009). Some have argued that stored carbohydrates may be of no

significant use after disturbance (Davidson & Milthorpe 1966; Richards & Caldwell 1985; Richards 1986). Rather, an increase in the production of photosynthates will compensate for the biomass loss and will provide sufficient nutrients to allow new biomass regrowth (Richards 1986). However, several experiments have shown that even a marked increase in photosynthates from the remaining photosynthetic material would not be sufficient to compensate for lost photosynthetic biomass and regrowth of new photosynthetic material (Dickson, Tomlinson & Isebrands 2000; Kabeya & Sakai 2003). Schutz et al (2009) however, determined that initial resprouting and root maintenance post-disturbance were supported from below-ground carbohydrate storage.

Inducible and Constitutive Defences

Constitutive defences are thought to be physiologically cheap compared to induced defences, as they are physical or chemical defences that are constantly present through evolutionary interactions with herbivores (Karban & Baldwin 1997). An inducible defence on the other hand, is the active increase of defensive traits by the plant in order to prevent/reduce tissue loss (Karban & Myers 1997; Hanley et al. 2007). Induced defences are considered to be physiologically costly to the plant, as they demand a greater allocation of stored resources, and consequently divert resources from other essential plant processes, such as growth and reproduction (Feeney 1976; Hanley et al. 2007). Inducible defences are a key component of *Acacia* physiology that is affected by herbivory or burning (Milewski & Young 1991; Gowda & Raffaele 2003; Milewski & Madden 2006; Hanley et al. 2007). Ward & Young (2002) and Ward (2010), amongst others, has shown that herbivory can trigger induced physical defences, such as thorns and prickles, while also demonstrating that there is not necessarily a trade-off between physical and chemical defences (also see Gowda 1997; Hanley & Lamont 2002; Ward & Young 2002; Walters & Heil 2007; Zinn et al. 2007; Mboumba & Ward 2008; Ward 2010).

Plant defence hypotheses

While the mobilization of non-structural carbohydrates has been shown to underpin the regrowth of below- and above-ground biomass subsequent to a disturbance event, the overall pattern of regrowth is dictated by genetics, soil nutrient availability, climate and water (e.g. Coley et al. 1985; Myers 1987; Bazely et al. 1991; Glynn et al. 2003). There are, however, several hypotheses that have been formulated in an attempt to better understand the regrowth patterns of plants after biomass loss. Four of these hypotheses will be outlined and discussed, to test through green house experiments on several African *Acacia* species. The hypotheses that I will review will be the expanded Growth-Differentiation Balance hypothesis (GDBH) (Loomis 1932, 1953), the Optimal Defence hypothesis (ODH) (Feeney 1976), the Carbon-Nutrient Balance hypothesis (CNBH) (Bryant et al. 1983), and the Resource Availability hypothesis (RAH) (Coley et al. 1985).

The ODH is known to be the theoretical basis for many plant defence hypotheses, but tends to be rather vague in its details. Contrastingly, the Resource Availability hypothesis (RAH) and the GDBH have very similar premises, although the GDBH is more advanced than the RAH in that the GDBH explains the physiological trade-offs between growth and differentiation at the cellular and tissue levels (Stamp 2003). I note, however, that the RAH is an evolutionary model, while the GDBH is a phenotypic model (Table 1). Of these hypotheses, the GDBH is deemed to be the most theoretically most mature, as outlined by Stamp (2003). I note that there are few examples in which a plant defence hypothesis has been completely rejected, therefore making all hypotheses mentioned to be viable candidates for testing.

Table 1: Defence hypotheses can be grouped as phenotypic and evolutionary defence hypotheses. Some of these hypotheses assume that there is a trade-off between growth and defence and others do not.

Phenotypic Model	Trade-off	Evolutionary Model	Trade-off
Carbon-Nutrient Hypothesis	No	Apparency Theory	Yes
Expanded Growth-Differentiation Balance Hypothesis	Yes	Optimal Defence Hypothesis	Yes
		Resource Availability Hypothesis	Yes

THE OPTIMAL PLANT DEFENCE HYPOTHESIS

The optimal defence hypothesis (ODH) (Feeny 1975, 1976) consists of several sub-hypotheses that address how the defensive requirements of plants will vary in relation to herbivory (McKey 1974; McKey 1979; Rhoades 1979; Stamp 2003). The ODH predicts that plants will defend their leaves and other plant parts from herbivores in relation to their value (Stamp 2003), whereby easily-replaced, less critical tissues and organs will be less well defended than hard-to-replace, indispensable ones (Mattson et al. 1988). Furthermore, patterns of defensive investment will reflect the frequency and severity of herbivory experienced by populations over evolutionary time (Feeny 1976; Rhoades 1979; Chew & Courtney 1991). Plant defences are assumed to be costly to the plant, because they divert resources from growth and reproduction, while herbivory is considered to be the principal mechanism that drives the quantitative patterns of plant defence (McKey 1974; Feeny 1976; Fagerstrom et al. 1987). As plant resources are often limited, any investment into defensive traits must maximise plant fitness in order to optimize the investment into defence rather than growth. However, trade-offs between allocation to defence and growth or reproduction are possible and evident in some instances (Vrieling & van Wijk 1994; Bergelson & Purrington 1996; Stamp 2003), while there are numerous underlying reasons for possible trade-offs and why defence may be detrimental to the plant (Herms & Mattson 1992). The ecological costs of defence (resistance) include deterrence of mutualists (e.g., pollinators, mycorrhizal fungi), reduction in competitive ability, traits that deter one enemy but attract another, and traits that confer resistance against one enemy but constrain resistance to another (Herms & Mattson 1992).

When herbivores are present, well-defended individuals should have higher fitness than individuals with lower levels of defence, and *vice versa* (Herms and Mattson 1992). This idea thus leads us to the apparency subhypothesis (genotypic expression of defence) of the ODH, which assumes that a plant will increase its defences as its interaction with herbivores increases, i.e. as the risk of herbivory and subsequent potential biomass loss increases (i.e. as the plant part becomes more “apparent” to the herbivore), a plant should increase its defences in order to maximise its future fitness (Stamp 2003). The downfall of the apparency hypothesis is its definition. Feeny (1976) used oak trees as an example of an apparent plant, because oak trees are

large and conspicuous trees that are easily found by herbivore predators. Thus they should have a high level of investment in secondary metabolites for defences. Conversely, crucifers (Feeny 1976) are deemed to be unapparent plants less likely to be found by herbivores. Thus the investment in defences should be lower. However, the problem still remains of how best to define an apparent plant. The assumption is that, by Feeny's (1976) definition, trees (including other woody species) should be deemed apparent. However, numerous studies have refuted the assumption of the apparency hypothesis, because there was no correlation between "apparency" of a given plant population and investment in defence (Herms & Mattsson 1992; Stamp 2003).

The apparency hypothesis was thus, despite much theoretical potential, disregarded as an adequate plant defence hypothesis, and was subsequently incorporated in the ODH years later (Herms & Mattsson 1992). Evaluating the tradeoff between growth, reproduction and defence is complicated by the idea of a "third party" tradeoff with tolerance (Herms & Mattsson 1992; Stamp 2003). Tolerance (traits that reduce the impact of damage on plant fitness), as opposed to resistance (traits that reduce the amount of damage) (Stowe et al. 2000) to tissue loss is a growth property, reflecting intrinsic growth rate, carbohydrate reserve storage capacity, allocation pattern, flexible photosynthetic rate, flexible nutrient uptake, and developmental plasticity (Rosenthal & Kotanen 1994). Just as secondary metabolites are a product of various selective pressures besides herbivory, so too is tolerance (Stamp 2003, but see Orians & Ward for counter-argument). Plants exhibit variation for tolerance to herbivory (Fineblum & Rausher 1995; Mauricio 1998; Strauss & Agrawal 1999), and tolerance can counter the potential negative impact of herbivory on reproduction (Maschinski & Whitham 1989; Stamp 2003). Plants appear to have one of three strategies that will optimize their fitness: 1) well-developed defence (resistance) and poor tolerance, 2) well developed tolerance and poor resistance, or 3) an intermediate balance of both (Van Der Meijden, Wijn & Verkaar 1988; Stamp 2003). For example, damage may have no effect on alkaloid or polyphenolic (chemical defences) concentrations of a plant species that typically experiences little herbivory but maintains a high constitutive level of defence, whereas damage results in lower alkaloid concentration in a second species that suffers high herbivory but exhibits substantial regrowth, and damage increased alkaloids in a third plant species that experienced little herbivory but also slow regrowth (van Dam, van der Meijden & Verpoorte 1993; Ruiz, Ward & Saltz 2002). Evidence indicates that

defence and tolerance negatively co-vary (e.g. Bilbrough & Richards 1992; Fineblum & Rausher 1995; Strauss & Agrawal 1999), but that defence and tolerance can also co-occur (e.g. Rosenthal & Kotanen 1994; Mauricio 1998). However, plant tolerance in the absence of herbivores may impose a cost to fitness (Herms & Mattson 1992). In conclusion, defences do have a cost in terms of construction and maintenance, but there is likely to have been selection to reduce that cost (Stamp 2003).

The ODH has the following assumptions; a) secondary metabolites have genetic variation upon which selection can act, b) the production of secondary metabolites is principally selected for by herbivore pressure, and lastly c) plant defences inevitably reduce herbivore attack (Strauss et al. 2002; Stamp 2003; Ito & Sakai 2009). Herbivory, according to the ODH, exerts the greatest selective pressure on plant defences. Hence, the magnitude of defence that a plant displays can be directly selected for by herbivory, while the type of defence (physical or chemical) attempts to reduce tissue loss through herbivory. There is a large amount of literature that highlights the selective pressures of herbivory on plant defences. This indicates that many, or even all, plants should develop strategies to minimize loss. However, many plants are able to withstand large episodes of herbivory with little cost to their overall fitness (e.g. McNaughton 1983; Paige & Whitham 1987). Nevertheless, a vast spectrum of various secondary metabolites has evolved in plants that are primarily utilized in antiherbivore defence e.g. McKey 1974; Fagerstrom et al. 1987; Gowda 1997; Rohner & Ward 1997; Hyvarinen et al. 2000; Traw 2002; Wallace & Eigenbrode 2002; Ward & Young 2002; Wackers & Bonifay 2004; Adler et al. 2006; Donaldson & Lindroth 2007; Walters & Heil 2007; Kaplan et al. 2008; Ito & Sakai 2009). Used in conjunction with physical defences, such as thorns, spines or prickles, plants have evolved effective antiherbivore defences that limit the amount of biomass that can be removed by herbivore predators.

THE CARBON-NUTRIENT BALANCE HYPOTHESIS

The Carbon: Nutrient Balance hypothesis (CNBH) attempts to model how the supply of environmental carbon and nutrients manipulates the *phenotypic* expression of defence by plants (Bryant et al. 1983; Tuomi et al. 1988, Tuomi, Fagerstrom & Niemela 1991).

Low Carbon

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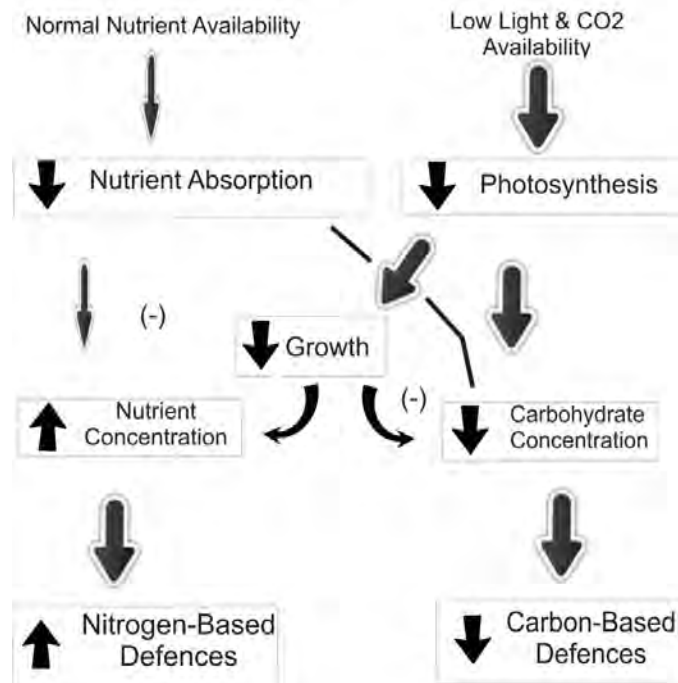


Fig. 1a: Effect of low carbon levels on plant growth and defence allocation. Thickness of arrow indicates strength of relationship, while arrows in boxes signify increase (↑) or decrease (↓) (Bryant et al. 1983).

Low Nutrients

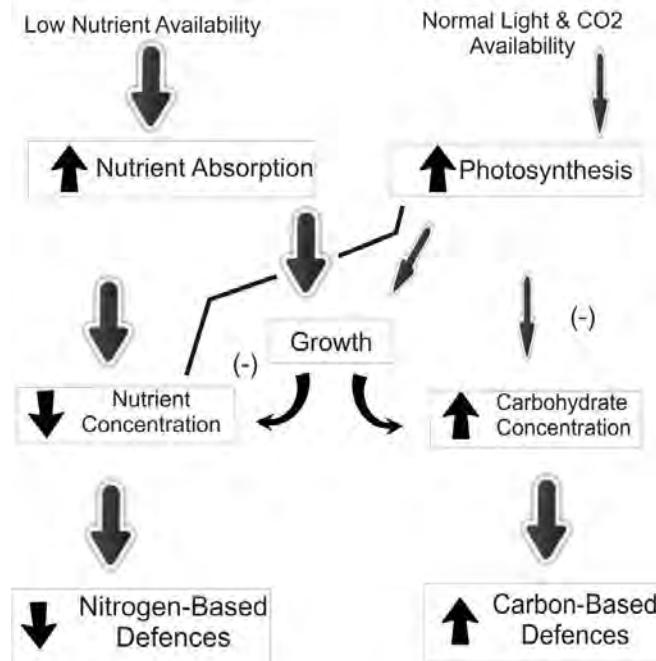


Fig. 1b: Effect of low nutrient levels on plant growth and defence allocation (Bryant et al. 1983). Thickness of arrow indicates strength of relationship, while arrows in boxes signify increase (↑) or decrease (↓)

The CNBH was originally developed to help explain the influence of soil nutrients and shade on the chemistry of plant defences through the effects of the ratio of carbon: nutrients in plant tissues (Bryant et al. 1983). The original description of the CNBH notes that there is a distinctive threshold, whereby allocation to defence only comes *after* growth/regrowth (therefore no cost to defence is predicted) underwent much scrutiny, and subsequent criticism (see e.g. Karban & Baldwin, 1997; Hamilton et al., 2001, Stamp, 2003). The hypothesis was altered into the definition that is commonly known and accepted today, that, “if the carbon: nutrient ratio acquired by a plant controls allocation of resources to plant functions, then the phenotypic expression of that plant’s genetic potential for defences will be affected” (Bryant et al. 1983, Stamp 2003). The expression of plant phenotype varies from complete genetic determination, to great plasticity in response to environmental conditions. For example, a plant species that grows in a nutrient-poor environment that has a high constitutive level of defence may be well defended for several reasons. Firstly, such genotypes may be selected for in resource-poor environments through herbivore pressure, and secondly, a surplus of carbon in plant tissues may be shunted into the production of allelochemicals, and lastly, it may be a combination of these two (Tuomi et al. 1988, 1991). According to the CNBH, plant defence accrues from a combination of fixed and flexible allocation (Bryant et al. 1983; Herms & Mattson 1992, Stamp 2003). The “baseline plus,” occurs when plants have a fixed amount of carbon that they will allocate to defence, proportional to growth (Stamp 2003). Environmental variation may affect the carbon: nutrient balance, whereby subsequent carbon surplus beyond use for growth, may be shunted into the plant’s defences as flexible allocation. Thus, plant defence may be a combination of baseline and *flexible* allocation.

The “variable plus,” (*sensu* Bryant et al. 1983) occurs when plants have a *fixed* allocation to defence, but this allocation is directly proportional to both growth and carbon surplus (Herms & Mattson 1992). When environmental conditions affect the carbon: nutrient balance, carbon surplus beyond use for growth may be shunted into defence (i.e. flexible allocation), but in this instance, defence is an amalgamation of shifting-but-fixed and flexible allocation. In either instance, changes in the carbon: nutrient balance will trigger varied responses from plants, and thus alter the phenotypic expression of the genetic defence potential of the plants. The assumptions of the CNBH include, 1) carbon gain and plant growth depend on mineral nutrient

reserves of plant tissues, 2) plant growth is allocated to carbon whenever mineral nutrient supply is adequate, 3) surplus carbon is allocated to defence or storage after carbon requirements for growth are met, 4) nutrient limitation has a greater limiting effect on growth than does photosynthesis, and 5) herbivory is the primary selecting force for constitutive secondary metabolite defences, and these defences reduce herbivory as a whole (Bryant et al. 1983; Tuomi et al. 1988; Luxmoore 1991; Tuomi et al. 1991; Herms and Mattson 1992; Stamp 2003).

Along with these assumptions, however, the CNBH does not assume that the general type (nitrogenous versus non-nitrogenous) or the amount of defence exhibited by a plant is driven by herbivory (Bryant et al. 1983; Tuomi et al. 1988; Stamp 2003). Lastly, the CNBH acknowledges that plant genetics ultimately control defences, and that the range of defensive expressions exhibited by plants is genetically determined through environmental pressures (Bryant et al. 1983; Luxmoore 1991; Tuomi et al. 1988, 1991; Stamp 2003). A meta-analysis of the CNBH (147 papers reviewed by Koricheva et al. 1998) showed that the responses of secondary metabolites, such as condensed tannins and terpenoids, did not fit the predictions of the CNBH. However, this may be due to flaws in the experimental design of the experiments (Stamp 2003). Nonetheless, the CNBH only predicts the allocation of plant resources (C-based and N-based resources) pre-disturbance, and does not predict the responses of plant defences (Stamp 2003), making the CNBH a poor plant defence hypothesis. In order to fully test the CNBH, the genetic defence capabilities of the species need to be established (Stamp 2003), making adequate testing of the hypothesis labour intensive and implausible in many cases. The CNBH can also only be applied to woody species that experience prolonged episodes of light and shade (namely temperate species), thus making it unsuitable for tropical and savanna based species.

THE RESOURCE AVAILABILITY HYPOTHESIS AND EXPANDED GROWTH-DIFFERENTIATION BALANCE HYPOTHESIS

The Resource Availability hypothesis (RAH) (also known as the Growth Rate hypothesis) (Herms & Mattson 1992) and the expanded Growth-differentiation Balance (GDB) hypothesis (Stamp 2003), provide a theoretical framework for predicting how plants will balance allocation between growth-related processes (i.e. stems, roots, leaves, and any cell elongation processes)

and differentiation which includes, among others, defensive traits over a range of environmental conditions (Loomis 1932, 1953). The GDBH, while explaining the evolutionary aspects of the ODH and the RAH or growth-rate hypothesis, also includes a model of the evolution of plant allocation trajectories that are molded by selective pressure by herbivores (Stamp 2003).

Water, light and nutrients are essential resources upon which all plants are dependent for growth. Research has shown that there is competition for available photosynthates between growth processes and secondary metabolism (Veihmeyer & Hendrickson 1961; Mooney & Chu 1974). Consequently, the carbon demands of all functions cannot be met simultaneously and thus, a trade-off must be expected (Lorio 1986). However, Rohner & Ward (1997) showed in their study of *Acacia raddiana* and *A. tortilis* that there is no negative correlation between physical and chemical defences after tissue loss, while Young & Ward (2002) found no trade-off between growth and condensed tannin concentration. The RAH is centred on plant growth rate, and predicts that as the maximal growth rate of a plant decreases (i.e. nutrient availability is reduced), the level of defences increases in order to protect new photosynthetic material from future herbivore attack (Herms & Mattson 1992; Stamp 2003). The RAH assumes that 1) the maximal growth rate is determined by resource availability, such that arid-adapted (resource-poor) species should have low maximal growth rates, while humid-adapted (resource-rich) species should have high maximal growth rates; 2) herbivory is the primary driving force for the production of secondary metabolites (i.e. defences); 3) slow turnover of plant tissues is advantageous in resource-limited environments; and 4) high turnover of plant tissues is advantageous in resource-rich environments. Although the RAH was widely accepted and thoroughly tested, the model is ultimately flawed (Figure 2). The x -axis of the model predicts that at the upper end of the defence investment, all leaf mass will be invested in defence, which is highly improbable (Stamp 2003). Despite having easily testable assumptions, the RAH has had mixed support, with several studies showing no correlation between growth rate and defence investment, nor between herbivory and defences (tannin concentration) (see Herms & Mattson 1992, Stamp 2003 for review). Endara & Coley (2011) (meta-analysis of 50 papers on the RAH) found that, overall, plants with high growth-rates are less well defended than plants with low growth-rates. Plant species with slow-growth rates were found to maximise both physical and

chemical defences, while fast-growing species generally invested only in secondary metabolites (polyphenolics and condensed tannins).

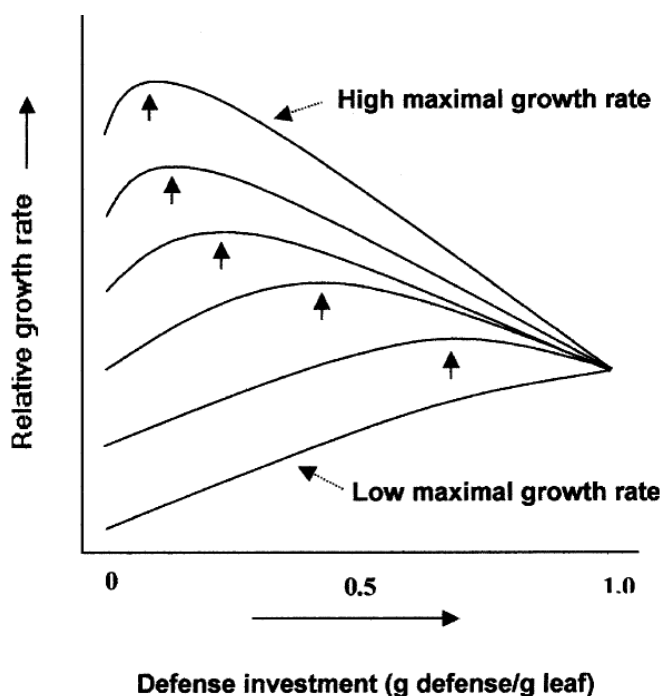


Fig. 2: Effect of defence investment on realized growth according to the Resource Availability Hypothesis. Each curve represents a plant with a different maximum inherent growth rate. Levels of defence that maximize realized growth are indicated by arrows (Coley et al. 1985).

Overall, the RAH has shown that, in evolutionary terms, resource availability is a driving force that overrides the cost of defence, and also indicates that intrinsic growth rate and defence are significantly shaped by abiotic factors of such magnitude that they ultimately determine basic defensive profiles (Stamp 2003). In order to overcome the limitations of the RAH, the expanded Growth-Differentiation Balance hypothesis (GDBH) was developed (first by Loomis (1932), but later expanded by Herms & Mattson (1992). The GDBH dictates that “any environmental factor that slows growth more than it slows photosynthesis can increase the resource pool available for allocation to differentiation-related products” (Stamp 2003). For example, a shortage of nutrients and water has been shown to slow growth significantly, while photosynthesis has been shown to be less sensitive to such limitations (Herms & Mattson 1992, Stamp 2003). Under these circumstances, carbohydrates are accumulated when growth demands are met, and may be

converted subsequently into defensive traits, such as secondary metabolites, with low cost to plant fitness (Herms & Mattson 1992, Stamp 2003).

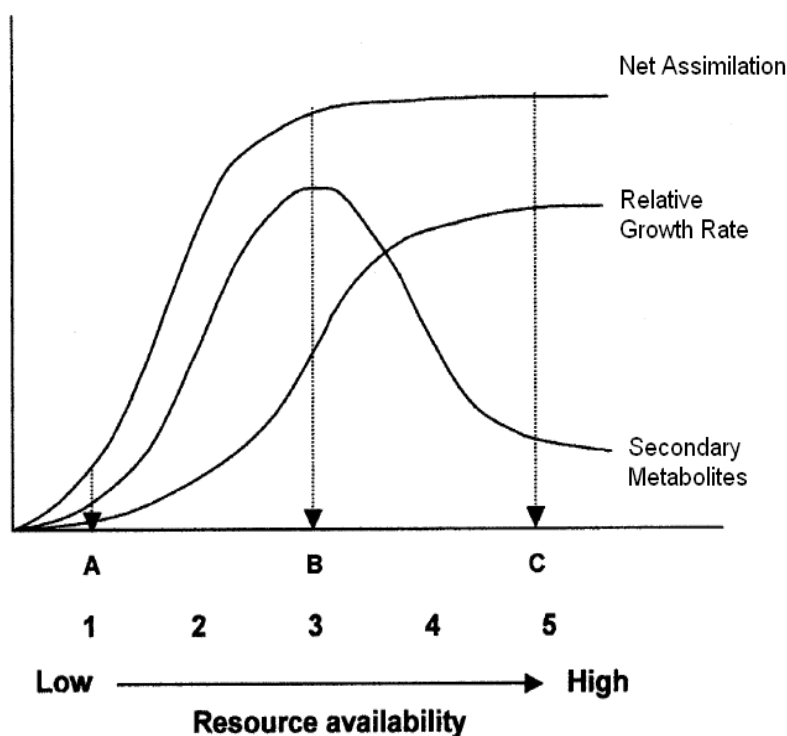


Fig. 3: Relationship of net assimilation rate, relative growth rate, and differentiation (specifically secondary metabolism) across a resource gradient according to the Growth-Differentiation Balance hypothesis. Resource availability affects growth more than it does photosynthesis. At point A, both growth and photosynthesis are constrained by low resource availability. At point B, growth is more constrained than photosynthesis and thus there is more allocation to differentiation. At point C, growth is less constrained and thus there is more allocation to growth. Stamp (2003) notes that a minimum of five resource levels spread along the gradient are necessary in order to determine this pattern (Adapted from Herms & Mattson (1992)).

There is a continuum of varying habitat types that range from resource-poor environments (e.g. arid environments) that support little or no plant growth, to environments that are very resource-rich (e.g. humid environments) that can potentially support rapid plant growth (Coley et al. 1985; Grubb 1992; Gutschick 1999). Despite the fact that Loomis (1932) first created the basis for the GDBH hypothesis, it was Herms and Mattson (1992) that were the first to utilize the GDBH hypothesis to explain how the *physiological* tradeoff between growth and

differentiation processes interacts with the selective forces of competition and herbivory to shape plant life-history strategies. Herms & Mattson (1992) proposed that competition in resource-rich environments will select for a growth-dominated strategy, whereas the stresses of resource-poor environments will select for a strategy that is focused on differentiation (Herms and Mattson 1992).

Due to the fact that nutrients are not readily replaced or available in resource-poor environments, the growth rates of plants that reside there are inherently slow, and subsequently tend to have long-lived leaves and twigs (Coley et al. 1985). The benefit of having a slow turnover of plant tissue in resource-poor environments is that it limits the unnecessary loss of essential nutrients. Each time a plant part is shed, it carries with it approximately 50% of its maximum nitrogen and phosphorous pool (Coley et al. 1985; Gutschick 1999). Contrastingly, plant species that reside in resource-rich environments have the potential for rapid growth rates, and thus have a high tissue turnover rate. Hence, the loss of plant parts is not necessarily detrimental to growth rate (Coley et al. 1985). Rapid turnover of plant parts, and the associated and inevitable loss of carbon and nutrients is not a strong influence on plants growing in resource-rich environments due to the higher availability of light and nutrients (Grime 1977; Chapin 1980; Coley et al. 1985; Myers 1987; Gignoux et al. 1997; Stamp 2003; Ito & Sakai 2009).

Plant species living in high-resource environments exhibit a characteristic set of traits, such as high capacity to absorb nutrients, high respiratory and light-saturated photosynthetic rates, and can take advantage of pulses in resource availability through biochemical and morphological plasticity (Mooney, Gulmon & Johnson 1983; Coley et al. 1985; Grubb 1992; Gutschick 1999). The combination of the RAH and the expanded GDBH, and following the reviews of Herms and Mattsson (1992) and Stamp (2003), we predict the following;

- 1) resource availability, excluding light availability, has a more positive effect on growth than on photosynthesis,
- 2) resource availability determines the maximal relative growth rate. Thus, the preferred environment in which the plant species grows will be reflected by the inherent growth rate,

- 3) competition for photosynthates between growth and differentiation processes leads to an inevitable tradeoff,
- 4) defensive secondary compounds are selected for my herbivory,
- 5) plant defences reduce herbivore attack, and
- 6) plant defences are considered to be costly, as they divert resources away from growth.

Considering these assumptions, the expanded GDBH also makes the following predictions:

a) plants in resource-poor environments should be limited in both growth and photosynthetic capability, as available resources are mobilized to preferential growth processes rather than to differentiation (i.e. defence) processes (Waring and Pitman 1985). The constraint of growth processes should yield low growth rate and moderate defence levels (Herms and Mattson 1992; see Figure 3, *point A*),

b) Plants experiencing intermediate resource availability should have an intermediate accumulation of above- and below-ground biomass, but a high level of defence (Loomis 1932, 1953), as the intermediate level of nutrients will constrain growth but not photosynthesis (Chapin 1980; Korner 1991; Luxmoore 1991). Defences, such as secondary metabolites, will accumulate in plants experiencing intermediate resource availability owing to the availability of a pool of accumulated photosynthate, and because defences should be relatively inexpensive (Figure 3, *point B*),

c) Individual plants growing under high resource availability should allocate a greater proportion of photosynthate to growth rather than defence (differentiation traits) as the high level of nutrients does not limit photosynthesis or growth (Loomis 1932; Herms and Mattson 1992) (Figure 3, *point C*).

Application of plant defence theories

Each of the plant defence theories that I have reviewed has a unique application in plant ecology. However, for my purposes, I will only be testing two of the three theories, and will subsequently use my findings to either support or refute the assumptions of the theories. The carbon-nutrient balance hypothesis is an excellent conceptual model in the manner in which it explains how a

plant may shift its defensive strategy from nitrogen-based to carbon-based defences. However, for the purpose of this thesis and the subsequent experimental design, this hypothesis requires that the levels of nutrients and carbon need to be controlled for (and greater genetic control- Stamp 2003). Unfortunately, the experimental design that I followed did not allow for this hypothesis to be adequately tested (savanna *Acacia* trees always grow in high-light environments and hence have adequate carbon available). Thus, the CNBH was not used to explain trends in plant responses to fire and herbivory. Therefore, the Resource Availability Hypothesis (RAH) was tested in chapter 2. The GDBH (which is related to the RAH) can be thoroughly tested in a fertilizer treatment experiment. Thus, it was tested in chapter 3 of this thesis. As indicated in figure 3, to adequately and thoroughly test the GDBH, five levels of nutrients need to be included to allow a concise and clear trend to appear. In the second greenhouse experiment, I tested this hypothesis, and try to establish the trend in the variation of plant defences as resource availability increases. Figure 3 indicates a trend for secondary metabolites only, but I used these predictions and also applied them to physical defences (spines and prickles) because it should be appropriate to this type of differentiation too. Because the predictions of the RAH and the GDBH are closely linked to one another, there is repetition of definitions of plant defence hypotheses present in chapters 2 and 3. Each chapter was submitted individually to scientific journals for peer review, hence the repetition of terms and definitions.

The Resource Availability hypothesis will be tested in the first greenhouse experiment. Preliminary findings by Hean & Ward (2011) have provided evidence that does not support RAH, as only one species (*A. robusta*) out of seven species tested supported the predictions of the RAH. Following the experimental layout and procedures outlined by Hean & Ward (2011), the RAH were tested on a much larger scale using seedlings of 14 African *Acacia* species. I predicted that *Acacia* seedlings would provide evidence supporting the RAH, with 1) species from arid (resource-poor) environments allocating more stored resources towards the production of physical and chemical defence after simulated herbivory and fire compared to humid (resource-rich) species, because the loss of biomass for arid species will incur a greater cost to fitness than humid species; 2) fire and simulated herbivory treatments would not have a significant effect on prickles abundance and regrowth length, because they are comparatively less costly to produce than spines which have a vascular system (Bazely, Myers & Da Silva 1991); 3)

stored non-structural carbohydrates would be significantly greater in arid species than humid species, because carbohydrate demands for growth in arid species will be met relatively quickly compared to humid species allowing arid species to accumulate more photosynthate into TNC.

I make the following predictions with regard to the second experiment which manipulated nutrient availability:

- 1) Regrowth would increase linearly, as predicted by the Expanded GDBH,
- 2) Defensive secondary metabolites (i.e. tannins) would display a positive unimodal trend with increasing nutrient availability,
- 3) Physical defences (i.e. spines and prickles) would display a similar trend to secondary metabolites with increasing resource availability (see Figure 3),
- 4) Stored non-structural carbohydrates would significantly increase with increasing nutrient availability, and
- 5) Arid species would differ from humid species in spine abundance, spine length, and secondary metabolites.

Chapter 2 has been submitted to the *Journal of Vegetation Science*, and chapter 3 has been submitted to *Functional Ecology*.

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Chapter 2

Comparison of regrowth patterns, defences and mobilization of total non-structural carbohydrates of seedlings of 14 *Acacia* species from arid and humid environments

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Abstract

Question: What are the separate and interactive effects of simulated herbivory and fire on vegetation regrowth and defence traits and what are the consequences for total non-structural carbohydrate (TNC) storage on seedlings of 14 *Acacia* species? Do the assumptions of the Resource Availability Hypothesis (RAH) apply to *Acacia* seedlings?

Location: Arboretum, Pietermaritzburg, South Africa.

Methods: We selected species from humid and arid environments to test the assumptions of the RAH. Additionally, we chose species from each environment that had either spines (with a connection to the vascular tissue) or prickles (epidermal origin). Using a two-factor, completely randomized experiment, we tested the RAH on 14 indigenous African *Acacia* (*A. ataxacantha*, *A. caffra*, *Acacia erioloba*, *A. erubescens*, *A. fleckii*, *A. gerrardii*, *A. hebeclada*, *A. karroo*, *A. mellifera*, *A. nilotica*, *A. schweinfurthii*, *A. sieberiana*, *A. tortilis*, and *A. xanthophloea*).

Herbivory was simulated by cutting seedlings at the first node (point at which the cotyledons were attached) with garden shears, and fire was simulated with a hand-held blowtorch, burning the entire seedling for 8s. We measured seedling regrowth, spine and prickle abundance, spine and prickle length, condensed tannin concentration and total non-structural carbohydrates.

Results: In accordance with the RAH, arid-adapted species placed greater investment into defence rather than growth. The significant reduction in TNC in roots subsequent to herbivory and fire treatments confirmed that *Acacia* seedlings are reliant on their roots as carbohydrate reserves. Humid-adapted species with spines exhibited more trade-offs between growth and defences than arid species with spines, while humid species with prickles exhibited more trade-offs between new stem growth and defences than humid species with prickles. None of the species exhibited a trade-off between defensive traits, although several species showed significant positive correlations between defence traits.

Conclusions: *Acacia* seedlings are highly resilient to severe tissue loss through herbivory and fire, and have the ability to resprout and increase their defensive capabilities despite the apparent increased demands of limited carbohydrate reserves. Fire and herbivory were shown to not be substitutable in their effects in a controlled experimental environment.

Keywords: fire; optimal plant defence; prickles; Resource Availability Hypothesis; seedlings; simulated herbivory; spines; tannins.

Introduction

Terrestrial ecosystem composition and functioning is strongly influenced by disturbance events, and can play a central role in the regeneration of numerous woody plant species (Pickett & White 1985). Disturbance in savannas occurs in various forms, but is dominated by fire (natural or anthropogenic) and herbivory by insects, rodents and large mammal herbivores (Barnes 2001; Bond & Midgley 2011; Bond & Midgley 2003; Maclean et al. 2011). Fire is a major disturbance factor in many biomes ranging from savannas to tropical rainforests (Laurance 2003) although the season, intensity, and frequency of fire are key determinants of its impacts on vegetation (Hoffman 1999; Higgins et al. 2000; Gambiza et al. 2005; Higgins et al. 2007). Seedling recruitment and stand development are often constrained by high intensity herbivory (Weltzin, Archer & Heitschmidt 1998) and fire (Bond 2008). Due to their large size, adult trees are able to withstand disturbance events with relative ease, although it becomes more problematic for seedlings and saplings (Meyer et al. 2005). As long-lived species, trees require particular adaptations to complete their demographic cycle in frequently- and intensely-disturbed habitats (Bond & Midgley 2001).

Research has produced inconsistent results concerning the overall effects of disturbance events (Fornara & Du Toit 2008), while outlining the roles of seasonal timing and site location as having significant influences (e.g. Bond et al. 2001; Scogings & Mopipi 2008). The concept of the persistence niche (Midgley & Bond 2001) emphasizes that, in frequently disturbed habitats, the survival of seedlings relies on the production of new stems and shoots, to persist in spite of possible recurrent removal of some or all aboveground biomass (Gignoux et al. 2009). Shoots of several woody species in semi-arid southern African savannas have a tendency of dying back when stressed by disturbance factors such as herbivory, fire or drought (Frost & Robertson 1987). A plant may respond to disturbance events via tolerance (survive physical damage without loss of fitness (Tsumele et al. 2006), compensation (increased growth of new shoots after tissue damage/loss (Strauss & Agrawal 1999)), and/or induced defence (Karban & Baldwin 1997).

A common response to disturbances such as fire and herbivory is resprouting/coppicing (henceforth referred to as coppicing) (Bellingham & Sparrow 2000; Bond & Midgley 2011; Bond & Midgley 2003). Coppicing occurs when secondary trunks and stems are produced from suppressed buds on the stem or roots (Del Tredici 2001), and is an evolutionarily adaptive attribute that enables survival after considerable physical damage and loss of above-ground biomass (Hodgkinson 1998). Woody species that occur in disturbance-prone terrestrial environments, particularly African savannas, typically exhibit these types of responses to fire and herbivory (Cruz et al. 2003). However, coppicing potential may vary among species, and is primarily governed by life history, physiological, and disturbance event traits (Gomez Sal et al. 1999). The resprouting ability of woody species is, among other contributing factors, dependent on the use and mobilization of stored carbohydrate reserves (often contained in roots and lignotubers) to allow re-establishment of photosynthetic capacity (Buwai & Trlica 1977; Bowen & Pate 1992). The decrease in the amounts or concentrations of non-structural carbohydrates following defoliation implies a causal role for these compounds in initiating regrowth (Schutz et al. 2009; Wigley, Cramer & Bond 2009).

Plant defences

Physical defences are generally in the form of thorns (also known as spines or prickles). Spines and prickles are effective physical defences that deter mammalian browsers by impeding bite size and foraging rate, as well as overall herbivory on individual plants (Cooper & Owen-Smith 1986; Milewski, Young & Madden 1991). A spine is a modified sharp-pointed petiole, midrib, vein or stipule, and is connected to the plant via a vascular tissue system (Ross 1979; Bazely et al. 1991; Gutschick 1999). Conversely, prickles are sharp-pointed outgrowths that are merely a modification of the epidermal or cortex cells (Bazely et al 1991).

Chemical defences are also important in defence against herbivores (Du Toit et al. 1990), because compounds such as tannins bind to protein molecules, thereby reducing protein digestion and nutrient availability after ingestion (Ward & Young 2002; Scogings et al. 2004). Fire and herbivory have been shown repeatedly to have a significant effect on the production of induced physical and chemical defences (Karban & Myers 1989; Rohner & Ward 1997; Wigley et al. 2009; Hean & Ward 2011). Induced defences are assumed to be costly to plants (Karban &

Baldwin 1997) and trade-offs with other life history traits or among different strategies of defence are assumed to exist. However, more recent studies have indicated that species may increase investments in both physical and chemical defence strategies subsequent to a disturbance event (Gowda 1997), while not compromising on growth (Ward & Young 2002; Ward 2010).

Plants depend on several essential elements for growth and survival, including water, nitrogen and phosphorus among others. The Resource Availability hypothesis (RAH) (Coley et al. 1985) (also known as the Growth Rate hypothesis (Stamp 2003)) predicts that the growth rate of plants is positively correlated with resource availability, and the investment into defences subsequent to tissue damage or loss is determined by available resources (Coley et al. 1985). Thus, as the growth rate of a plant decreases, the investment into defences will increase in order to defend new tissue growth. In nutrient-rich environments, Coley et al (1985) predicted that plants should invest in regrowth, while in nutrient-poor environments plants should invest in defence to minimize the probability of consumption. In a meta-analysis, Endara & Coley (2011) determined that overall, the majority of studies that have tested the RAH support its predictions. On closer inspection however, it is evident that the studies used in the Endara & Coley (2011) meta-analysis were predominantly based on forest ecosystems. However, the RAH has come under much scrutiny over the past few years, because several studies have refuted its outcomes and predictions (e.g. Ward & Young 2002; Hean & Ward 201, see Herms & Mattson 1992 and Stamp 2003 for review).

African *Acacia* trees

Throughout Africa and the Middle East, *Acacia* trees (family Fabaceae, subfamily Mimosaceae) are common and highly abundant (Ross 1979; Rohner & Ward 1997). They constitute a significant proportion of the diet of numerous large mammalian herbivores (Du Toit et al. 1990; Gowda 1997; Rohner and Ward 1997; Bond & Midgley 2003; Maclean et al. 2011). *Acacia* species abundance has been linked to such important community and ecosystem variables as species diversity (Dean et al. 1999), soil-water infiltration (Dougill et al. 1998), size and availability of nutrient pools (Hudak et al. 2003) and productivity (Belsky 1994). *Acacia* species

are protected from herbivory by elongated and lignified spines or prickles (Ross 1979; Gowda 1997) and condensed tannins (Gowda 1997; Rohner & Ward 1997).

Based on the Resource Availability Hypothesis, we make the following predictions, 1) arid-adapted species will invest more carbohydrate reserves than humid species into physical and chemical defence to protect new photosynthetic material, 2) herbivory will induce increased investment in physical and chemical defences, while fire will have a significantly negative effect on defensive traits due to nutrient loss (Hean & Ward 2011), 3) species with spines will display a greater response to herbivory and fire than prickles, because the production of prickles is less costly to the plant (Bazley et al. 1991), 4) herbivory will have a significantly greater negative effect than fire on total non-structural carbohydrate reserves in stems and roots, because herbivory will deplete reserves allocated to primary metabolism (Van Der Heyden & Stock 1996).

Materials and Methods

Seed collection

A. ataxacantha, *A. caffra*, *A. erioloba*, *A. erubescens*, *A. fleckii*, *A. gerrardii*, *A. hebeclada*, *A. karroo*, *A. mellifera*, *Acacia nilotica*, *A. schweinfurthii*, *A. sieberiana*, *A. tortilis*, and *A. xanthophloea* were used in testing the effects of fire and herbivory on resprouting ability of each species. We selected these species based on the clear division into arid and humid species and secondarily on the differentiation of the presence of either prickles or spines (Table 1). The experiment was conducted at the Tainton arboretum tunnels, situated on the University of KwaZulu-Natal, Pietermaritzburg campus, KwaZulu-Natal, South Africa. Seed collection took place throughout South Africa where populations were densest. Seeds of each species were collected from several adult trees, and seeds were manually scarified with nail clippers prior to germination.

Experimental design

A two factor, fully randomized design was used, with fire and simulated herbivory being the two factors. Levels of factors were control (no burning and no cutting), burning only, cutting only, and burning and cutting. Species were laid out into blocks and planted in 20 l pots, using a 2:1

(sand to wood mulch ratio) potting medium. One seedling of each was planted in each pot, with treatments being replicated 15 times and randomized within species blocks. Pots were laid out on an automated mist bed, with watering taking place every 3 h for 1 min. Seeds were allowed to germinate and grow for 15 weeks. Burning was achieved through the use of a butane-fuelled blow torch, using a flame of approximately 20 cm (Balfour & Midgley 2006) held at a distance of 15 cm from seedlings being treated (approximately 1000° C), and the entire seedling was burnt from first node (point at which cotyledons were attached) to apex for 8s. Simulated herbivory was achieved with the use of hand-held garden scissors and cutting at the first node. We recognize that this simulation may not accurately replace the effects of mammalian herbivory due to unreplicable effects of saliva (Teague, 1988; Bergman, 2002; Rooke, 2003) but we believed that it would be more useful to ensure that similar levels of herbivory were applied (Hanley and Fegan, 2007). Bergman (2002) notes that there is no consistent effect of saliva on plant growth across plant species. Seedlings were cut at the first node (i.e. where the first lateral branch is produced) (Dube et al. 2010). Seedlings that were exposed to both fire and simulated herbivory were burned first, and then allowed to grow for 10 days before being cut at the first node. All seedlings were then allowed to grow for a further 21 d.

Above- and belowground biomass

The effect of the various treatments on biomass re-growth for each species was determined by measuring and comparing dry weight (DW) of roots and above-ground material (Trlica & Cook 1971). Individual seedlings were carefully removed from their allocated pots and all soil washed off the roots. Photosynthetic material and roots were separated by cutting at the first node, and dried in a 60 °C oven for 48 h. Photosynthetic material and roots were directly compared for each individual seedling by weighing the dry material.

Spinescence

The effects of simulated burning and herbivory on overall spine and prickle abundance and length were measured. Total spine and prickle abundance were recorded for each seedling per species. Spine and prickle length were recorded using vernier calipers to measure the total length of each individual spine or prickle present.

Condensed tannin analysis

Photosynthetic material was used in the determination of condensed tannin concentration. The photosynthetic material was dried in a 60 °C oven for 48 h, and then ground in a Wiley milling machine to pass through a 40 mesh sieve (Van Der Heyden & Stock 1996). In a 50 ml screw cap centrifuge tube, we added 2.5 ml of 70 % acetone to 0.25 g of dried plant material, which was then sonicated for 10min. The sample was then centrifuged for 10 min at 2250 g. The supernatant was pipetted off and placed in a refrigerator for later use. Condensed tannin analysis was performed using the acid butanol assay as described by Hagerman (2011). Standards are expressed in quebracho equivalents, as recommended by Hagerman (2011).

Non-structural carbohydrates

Total non-structural carbohydrates were determined from a 0.25g sample from above-ground biomass. As with the determination of tannin concentration, all plant material was dried in an oven for 48 h at 60 °C, then ground with the aid of a Wiley mill to allow the material to pass through a 40 mesh sieve (Van Der Heyden & Stock 1996). Total non-structural carbohydrates were extracted for each species in a two-step process according to Tolsma et al. (2007). A standard curve was created using pure amylose (Knutson & Grove 1994). The sample was standardized by calculating $\% \text{ amylose}_{\text{corrected}} = (\% \text{ amylose}_{\text{uncorrected}} - 6.2) / 93.8$, where $\% \text{ uncorrected amylose} = \% \text{ calculated from the standard curve}$. The samples were repeated in triplicate to avoid contamination and biased readings from cellulose lint (Tolsma et al. 2007).

Statistical analysis

The means of stem and root length, stem and root weight, stem and root total non-structural carbohydrates (TNC), spine and prickle length and abundance, as well as tannin concentration responses to simulated burning and herbivory were compared. Significant differences in the aforementioned variables were determined by comparing the calculated means in a two-factor ANOVA (SPSS version 18 for Windows), using simulated herbivory and burning as independent factors (i.e. Cut-No cut; Fire-No fire). Through the use of a general linear model, arid and humid species were compared across all physiological attributes (GenStat version 12 for Windows).

Species and habitat type (arid vs. humid) were used as blocking factors in the following linear model: $y = \text{herbivory} + \text{fire} + \text{herbivory} \times \text{fire} + \text{species} + \text{habitat}$. Each physiological attribute previously investigated was tested and compared for arid and humid species. Pearson's correlations (with Bonferroni correction for multiple comparisons) between stem length and thorn abundance, thorn length and condensed tannins were conducted to determine whether there were any trade-offs between growth and defence traits, and between the various defence traits.

Results

Stem and root length

There were distinct response patterns of *Acacia* seedlings from arid and humid habitats to disturbance events, as well as a distinction between species with spines or prickles. Overall, all species with spines had significantly shorter stems subsequent to herbivory ($P < 0.001$, error d.f. = 44) and fire ($P < 0.001$, error d.f. = 44, Fig 1a,b), while the stem length of both humid and arid species with spines showed a significant interaction effect ($P < 0.001$). Species with prickles displayed similar trends to species with spines, whereby all species with prickles exhibited significantly shorter stem lengths subsequent to simulated herbivory ($P < 0.001$, error d.f. = 44) and fire ($p < 0.001$, error d.f. = 44), as well as a significant interaction effect ($P < 0.001$). *A. erubescens* ($F = 0.470$, $P = 0.494$, error d.f. = 44) and *A. ataxacantha* ($F = 0.234$, $P = 0.631$, error d.f. = 44) were the only two species where burning did not have a significant effect on stem length (Fig 1a). Herbivory and fire significantly reduced the root length of all species ($P < 0.001$, error d.f. = 44), while all species displayed a significant herbivory \times fire interaction ($P < 0.001$).

Above- and below-ground biomass

Herbivory and fire had a significant negative effect on stem biomass across all species, although burning did not have a significant effect on the stem biomass of *A. ataxacantha* ($F = 1.603$, $P = 0.212$, error d.f. = 44) and *A. schweinfurthii* ($F = 1.603$, $P = 0.212$, error d.f. = 44). Simulated herbivory and fire significantly reduced the root biomass of all species ($P < 0.001$, error d.f. = 44, Fig 2). All species showed a significant herbivory \times fire interaction effect for stem and root biomass ($P < 0.001$).

Physical defences

A. hebeclada ($F=20.349$, $P< 0.001$, error d.f.= 44), *A. erioloba* ($F=4.542$, $P= 0.039$, error d.f.= 44), *A. karroo* ($F=63.264$, $P< 0.001$, error d.f.= 44), and *A. tortilis* ($F=89.622$, $P= 0.001$, error d.f.= 44) all displayed a significant increase in spine abundance after herbivory (see Fig 3). *A. gerrardii* was the only humid species with spines that did not have a significant change in spine abundance after herbivory ($F=0.302$, $P= 0.585$, error d.f.= 44). *A. nilotica* was the only humid species to exhibit a significant increase in spine abundance to herbivory ($F=25.968$, $P< 0.001$, error d.f.= 44) and fire ($F=5.696$, $P= 0.021$, error d.f.= 44). *A. xanthophloea* and *A. sieberiana* displayed significant increases in spine abundance subsequent to fire only (Fig 3b). Fire significantly reduced spine abundance in all arid species with spines (Fig 3a). *A. karroo* ($F= 1.913$, $P= 0.174$, d.f.= 44) and *A. gerrardii* ($F= 0.256$, $P= 0.615$, d.f.= 44) were the only species where spine abundance that did not exhibit a significant interaction effect.

Several *Acacia* species showed inducible changes in prickles abundance, consistent with the findings of Bazely et al. (1991). *A. erubescens* exhibited a significant increase in prickle abundance subsequent to herbivory ($F=23.270$, $P< 0.001$, error d.f.= 44) and fire ($F= 7.40$, $P< 0.001$, error d.f.= 44), and was also the only arid species with prickles that exhibited a significant herbivory×fire interaction ($F=4.601$, $P= 0.037$, d.f.= 44). *A. fleckii* demonstrated a significant increase in prickle abundance subsequent to herbivory ($F=5.926$, $P= 0.019$, error d.f.= 44), while fire significantly reduced prickle abundance ($F= 8.912$, $P= 0.005$, error d.f.= 44). *A. mellifera* had a significant increase in prickle abundance subsequent to herbivory only ($F=31.230$, $P< 0.001$, error d.f.= 44). Neither herbivory nor fire had any significant effect on the prickle abundance in *A. ataxacantha*, *A. caffra* or *A. schweinfurthii*, nor was there a significant interaction effect ($P> 0.05$).

Fire was shown to have a significant negative effect on the spine and prickle length for all species (both arid and humid), with the exception of *A. ataxacantha* where fire had no significant effect on prickle length ($F= 2.386$, $P= 0.130$, error d.f.= 44). However, herbivory also increased spine length in *A. karroo* ($F=58.939$, $P< 0.001$, d.f.= 44; Fig 4a). The interaction of herbivory×fire had a significant effect on spine length in *A. erioloba* ($F=30.597$, $P< 0.001$, d.f.= 44) and *A. karroo* ($F= 4.576$, $P= 0.035$, d.f.= 44) only. Overall, humid and arid species with

prickles did not have a significant herbivory×fire interaction, with the exception of *A. ataxacantha* ($F= 55.586$, $P< 0.001$, d.f.= 44).

Chemical defences

Condensed tannin concentration was found to be highly variable in seedling response to herbivory and fire. Herbivory increased tannin concentration in *A. hebeclada* ($F= 5.221$, $P= 0.027$, error d.f.= 44, Fig 5a), *A. erioloba* ($F= 4.510$, $P= 0.039$, error d.f.= 44, Fig 5a), *A. karroo* ($F= 7.140$, $P= 0.011$, error d.f.= 44, Fig 5a) and *A. tortilis* ($F=5.695$, $P=0.021$, error d.f.= 44, Fig 5a). All arid species with spines displayed a significant herbivory×fire interaction. Herbivory and fire did not have a significant effect on condensed tannins of *A. gerrardii*, *A. nilotica*, and *A. xanthophloea*. *A. sieberiana* exhibited a significant increase in tannin concentration after herbivory ($F= 18.539$, $P< 0.001$, error d.f.= 44, Fig 5c), while condensed tannins were shown to be significantly lower after fire ($F=15. 831$, error d.f.= 44, $P< 0.001$). *A. sieberiana* was the only humid species with spines that displayed a significant herbivory×fire interaction ($F= 8.752$, $P= 0.005$, d.f.= 44). *Acacia ataxacantha*, *A. caffra* and *A. schweinfurthii* did not exhibit a significant change in their tannin concentration after herbivory or fire, nor did they show a significant interaction effect ($P> 0.05$).

Total nonstructural carbohydrates (TNC)

Overall, there were two distinct trends subsequent to herbivory and fire in terms of total non-structural carbohydrates. TNC were significantly lower in new stems following the herbivory treatment for all species (see Fig 6), while fire had no significant effect on the TNC of new stem growth. Both simulated herbivory and fire significantly reduced TNC in roots across all species ($P< 0.001$, error d.f.= 44, Fig 6). *A. gerrardii* ($F= 10.191$, $P= 0.002$, error d.f.= 44) and *A. sieberiana* ($F= 1.760$, $P= 0.019$, error d.f.= 44) were the only species that displayed a significant herbivory×fire interaction effect for stem TNC, while all species displayed a significant interaction effect for root TNC.

Arid vs. humid species

Arid and humid species with spines or prickles were found to be significantly different in several physical traits. Humid species with spines had longer stems ($P < 0.001$), greater spine abundance ($P = 0.017$), higher condensed tannin concentrations ($P < 0.001$) and greater TNC concentration in both stems and roots ($P < 0.001$ for stems and roots) than arid species. However, arid species with spines were found to have greater spine lengths than their humid counterparts ($P = 0.001$). Root length ($P = 0.777$) and root biomass ($P = 0.463$), as well as stem biomass ($P = 0.296$) were not significantly different between arid and humid species with spines. Overall, humid species with prickles exhibited longer stems ($P < 0.001$), greater stem ($P < 0.001$) and root ($P < 0.001$) biomass, higher tannin concentrations ($P = 0.001$), and greater stem and root total non-structural carbohydrates ($P < 0.001$ in both instances) than arid species with prickles. Arid species, however, displayed longer prickles ($P < 0.001$) and greater prickle abundance ($P < 0.001$) than humid species.

Trade-offs

Herbivory

Trade-offs for species with spines were only exhibited between new stem growth and physical defences. Overall, there was a trade-off between new stem growth and condensed tannin concentration in humid species with spines ($r = -0.364$, $P < 0.001$). Arid species with spines displayed a significant negative correlation (i.e. trade-off) between stem length and spine abundance ($r = -0.303$, $P = 0.003$), and new stem growth and spine length ($r = -0.654$, $P < 0.001$). Humid species with spines also exhibited a negative significant correlation between stem length and spine abundance ($r = -0.640$, $P < 0.001$) (Table 2). *A. gerrardii* however, did not display a significant correlation between stem growth and any defence traits (Table 2). Arid species with prickles displayed significant negative correlations between new stem growth prickle length ($r = -0.538$, $P < 0.001$), and condensed tannins ($r = -0.579$, $P < 0.001$).

Conversely, humid species with prickles displayed a significant positive correlation between stem length and prickle abundance ($r = 0.358$, $P = 0.002$) and prickle length ($r = 0.425$, $P < 0.001$), while they displayed no significant relationship between new stem growth and

condensed tannin concentration (Table 2). *A. ataxacantha* and *A. schweinfurthii* displayed significant negative correlations between new stem growth and prickles length (Table 2). Arid species with spines exhibited a significant trade-off between spine abundance and condensed tannins ($r = -0.337$, $P = 0.001$), as well as spine length and condensed tannins ($r = -0.326$, $P < 0.001$). A significantly positive correlation was found between spine abundance and spine length in arid species ($P < 0.001$), while there was no significant correlation between spine abundance and spine length in humid species. There was however, a moderately strong positive correlation between spine abundance and condensed tannins ($r = 0.454$, $P < 0.001$). Arid species with prickles demonstrated a significantly strong positive relationship between prickles abundance and length only ($r = 0.713$, $P < 0.001$), while humid species with prickles did not exhibit any significant correlations between defence traits.

Fire

Overall, arid species with spines displayed a positive correlation between stem length and spine abundance ($r = 0.469$, $P < 0.001$) (Table 2). *A. erioloba* displayed a significant positive correlation between stem growth and spine length, while *A. karroo* and *A. tortilis* did not show any significant relationship between stem growth and spine length or stem growth and condensed tannins (Table 2). Overall, only humid ($r = -0.331$, $P = 0.001$) species with spines exhibited a significant trade-off between new stem growth and condensed tannins. Humid species with spines displayed a significant negative correlation between stem length and spine abundance ($r = -0.416$, $P < 0.001$). *A. gerrardii* was the only species that displayed a significant positive correlation between stem length and spine length (Table 2). Arid species with prickles only displayed a significant trade-off between stem length and condensed tannins ($r = -0.493$, $P < 0.001$) overall. *A. schweinfurthii* showed a positive correlation between stem length and prickles length (Table 2).

Overall, arid species with spines displayed a positive correlation between spine abundance and length ($r = 0.298$, $P = 0.003$), while a trade-off was shown between spine abundance and condensed tannins ($r = -0.293$, $P = 0.004$), and spine length and condensed tannins ($r = -0.400$, $P < 0.001$) in arid species. Humid species, conversely, displayed a positive correlation

between spine abundance and condensed tannins ($r= 0.468$, $P< 0.001$). Humid species with prickles did not exhibit any significant correlations between defence traits. Arid species displayed a positive correlation between prickle abundance and length ($r= 0.512$, $P< 0.001$) (Table 3).

Discussion

The RAH predicts that the optimal defence of woody plant species will increase as the potential cost of the loss of plant tissue increases (Coley et al. 1985; Herms & Mattson 1992; Stamp 2003), i.e. easily replaced, high-turnover plant tissues will be less well-defended than slow turn-over, less easily replaced tissues, because the physiological cost is lower than slow turn-over tissues that are difficult to replace. Moreover, as long-lived species, acacias are likely to be exposed to frequent herbivory or fire events. Thus, as the probability of biomass loss through herbivory/fire increases, so should the investment into defence to defend new photosynthetic biomass growth.

Overall, arid species (slow growth rates, slow turn-over of plant tissues) increased all attributes of their defence, both physical and chemical. In all instances, spine and prickle abundance and length increased subsequent to herbivory, while tannin concentration also showed a significant increase after the herbivory treatment. Humid species (fast growth rates, rapid turn-over) on the other hand grew bigger than their arid counterparts and generally exhibited an increase in only one attribute of their defence, with either only spine or prickle abundance (e.g. *A. nilotica*, *A. xanthophloea* and *A. sieberiana*), and spine and prickle length (e.g. *A. nilotica*, *A. ataxacantha*, *A. caffra* and *A. schweinfurthii*) increasing, or displaying an increase in tannin concentration (e.g. *A. sieberiana*). Thus, our findings support the predictions of the RAH, and support our first prediction that arid-adapted species will invest a greater amount of carbohydrate reserves in defence, while humid-adapted species will invest a greater proportion of carbohydrate reserves to increased biomass growth, as indicated by the significant differences in stem and roots lengths of humid relative to arid species. Hean & Ward (2011) in their experiment on *Acacia* seedlings, however, did not find any evidence that supported the predictions of the RAH. This may be attributed to the small sample size of arid-adapted *Acacia* species used (i.e. only two species in each category), including the low replication, thus not allowing a clear trend to be

observed. This current investigation however, contains a more substantial sample size of both humid- and arid-adapted *Acacia* species (seven species in each category), allowing a more concise and clear trend to be observed into the regrowth patterns of *Acacia* seedlings post disturbance.

Seedlings are left with the dilemma of limited remaining resources that need to be divided between regrowth of new stems and photosynthetic material, while also being required for the development of new defences (Hanley et al. 2007). Because physical and chemical defences both rely on the allocation of nitrogen and carbohydrate resources by the plant, classical plant defence theory suggests that plants experience an allocation dilemma with regard to which type of defence to invest in (Rhoades 1979; Coley et al. 1985; Herms & Mattson 1992; Ward & Young 2002; Stamp 2003). Although not necessarily mutually exclusive, species that adopt a physical defence strategy might be expected to possess more limited chemical defences, and *vice versa* (Hanley et al 2007, Ward & Young 2002; Koricheva et al. 2004). Analysis of trade-offs between defensive traits of all *Acacia* seedlings in this experiment revealed that *A. tortilis* was the only species that exhibited any trade-off between defensive traits (negative correlation between prickle length and condensed tannins). Hence, these 14 *Acacia* species do not support the notion that there should be a trade-off in allocation to defensive traits.

Humid-adapted species with spines generally displayed only a single increase in a defensive trait, whether it was an increase in spine abundance or length, or an increase in tannin concentration. Nevertheless while a trade-off between these two main types of plant defence may exist for some species/genera, a trade-off is neither ubiquitous nor does it imply that one defence is gained at the expense of the other (Rohner & Ward 1997; Ward & Young 2002; Schindler et al. 2003; Koricheva et al. 2004). The regrowth patterns of plant defences are affected by two likely outcomes: 1) the probability of attack by vertebrate and invertebrate herbivores changes substantially as the plant develops (Boege & Marquis 2005), and 2) the effectiveness of some structures depends on the type of herbivore (see e.g. Hanley et al 2007).

It appears that local site adaptation is key to our understanding of regrowth patterns of *Acacia* seedlings, not only in terms of photosynthetic biomass, but in terms of physical and chemical defences as well. Seedlings in resource-poor environments, such as arid savannas, have a low tolerance and high susceptibility to fire, and can be killed years after initial establishment

(Bond 2008, but see Meyer et al. 2005). Removal of plant material through herbivory or fire may result in a reallocation of available resources to defence production at the expense of storage and reproduction (Coley et al. 1985), without compromising growth (Rohner & Ward 1997; Ward & Young 2002; Ward 2010). As a general hypothesis, due to these limited resources subsequent to fire and herbivory, plants must either invest in defensive traits or increased regrowth capacity (van Der Meijden et al. 1988; Herms & Mattson 1992). Physical and chemical defences have traditionally been perceived to be present at fixed (i.e. constitutive) levels as an evolutionary response to herbivore interactions (Karban & Baldwin 1997; Hanley et al 2007). Numerous preceding studies have shown that both physical and chemical plant defences are able to increase in magnitude in response to biomass removal, i.e. plant defences are inducible (Karban & Baldwin 1997; Milewski et al. 1991; Rohner & Ward 1997; Ward & Young 2002; Zinn et al. 2007). However, the effectiveness of plant spinescence structures as antiherbivore traits has been questioned, but are considered to be constrained by co-evolution of spines with herbivores (Ito & Sakai 2009). Several studies have shown a significant correlation between increased plant defence and a decrease in herbivore attack (e.g. Cooper & Owen-Smith 1986; Gowda 1997; Young & Okello 1998).

Studies have highlighted the fact that the responses of woody species are affected by TNC stores (Hendry 1993; El Omari et al. 2003; Dube et al 2010; Kobe, Iyer & Walters 2010), while several preceding studies have expressed uncertainty towards the role of roots as storage organs of TNC. However, many researchers believe that cotyledons are a primary source of carbohydrate stores in woody seedlings (e.g. Du Toit et al. 1990; Kabeya & Sakai 2003; Dube et al. 2010). The role that roots play as sources of reserve carbohydrates is however, undeniable, as seedlings in this experiment were treated long after cotyledons had fallen off the individual plants. Hean & Ward (2011) suggested that the regrowth patterns of *Acacia* seedlings could be underpinned by TNC mobilization from reserves in roots (see also Schutz et al. 2009 and Wigley et al. 2009 for *A. karroo*, and Kgope et al. 2010 for similar results for *A. karroo* and *A. nilotica*). The significant reduction in TNC in roots subsequent to herbivory and fire treatments in this study confirm that *Acacia* seedlings are indeed drawing upon and reliant on their roots as carbohydrate reserves, thus supporting the suggestions of Hean and Ward (2011). Both arid and humid species displayed significant reductions in their root TNC, while arid species invested

more into physical and chemical defence. There are several factors that determine the kind of resource investment into plant defence, including herbivore pressure, resource availability and ecophysiological/ ontogenic (growth and reproduction) constraints (Grubb 1992; Herms & Mattson 1992, Stamp 2003; Boege & Marquis 2005). The inherent costs of anti-herbivore defence (physical and chemical), together with the unpredictability of herbivore attack are thought to impose constraints on plant metabolism, leading to a plant resource allocation dilemma: to grow or to defend? (Herms & Mattson 1992). It appears that the humid species we studied are investing more of these stored carbohydrate reserves back into the growth of photosynthetic material, because physical and chemical defences did not show the same increase as for the arid species. Also, the fact that stems appeared to have higher TNC after fire compared to herbivory suggests that fire does not completely destroy all nutrients and plant tissues in the stem of *Acacia* seedlings. Balfour & Midgley (2006) suggested that stem death may be caused by xylem malfunction subsequent to fire, leading to the loss of hydraulic conductivity in particularly affected plant tissues, and does not necessarily lead to death of the entire plant. This study observed similar trends in *Acacia* seedling responses to fire, whereby stem death was evident, but seedlings themselves were not killed by the fire treatment. Thus, the elevated levels of TNC in roots subsequent to fire compared to herbivory may be due to the assimilation of unaffected TNC after xylem malfunction in stem tissues. Despite the fact that simulated herbivory has often been substituted for fire (e.g. Bond & Keeley 2005; Levick et al 2009; Kgope et al. 2010), our experiment has clearly defined the differences that simulated herbivory and fire have on plant tissue and defence regrowth. The pressures that herbivory and fire exert on *Acacia* seedlings is unique. Coupled with the significantly different effects that herbivory and fire have on TNC stores in stems and roots post-disturbance, we thus support the notion of herbivory and fire are not substitutable, as highlighted by Hean & Ward (2011).

Conclusions

This study supports the RAH, as indicated by increased defence in arid *Acacia* species and regrowth in humid *Acacia* species. This study also provides evidence that prickles (epidermal

defences) are indeed inducible, as illustrated by *A. fleckii*, *A. mellifera*, and *A. erubescens*. Mobilization and distribution of TNC stored in the roots of *Acacia* seedlings underpin regrowth patterns after above-ground biomass removal, although further research on species-specific resource adaptation is vital to our understanding of plant responses to major disturbance events. Woody seedlings have often been cited as being vulnerable due to their size and morphological traits (Palo et al. 1985). This study has also shown that *Acacia* seedlings are highly resilient to even very high degrees of tissue damage and loss, while still being able to regrow new photosynthetic shoots and adequately defend them through various defence mechanisms. The findings of this study could provide some insight into the mechanisms of woody plant encroachment (see e.g. Wiegand et al. 2005; Wiegand et al. 2006; Kraaij & Ward 2006; Balfour & Midgley 2008). Despite the resilience of *Acacia* seedlings to disturbance, they should still be considered to be vulnerable to attack. By better understanding the mechanisms of woody plant encroachment from a seedling stage, land managers and plant ecologists will be provided insight into ways that may help to curb the invasion of woody species into native grasslands.

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Figure legends

Fig. 1. Mean ($\pm 95\%$ confidence intervals) stem length. Treatments are represented by NF+NC (control), NF+C (herbivory), F+NC (fire), and F+C (herbivory*fire interaction). Species abbreviations: heb (*A. hebeclada*), eriol (*A. erioloba*), karr (*A. karroo*), tort (*A. tortilis*), gerr (*A. gerrardii*), nil (*Acacia nilotica*), xan (*A. xanthophloea*), sieb (*A. sieberiana*), fleck (*A. fleckii*), mell (*A. mellifera*), erub (*A. erubescens*), atax (*A. ataxacantha*), caff (*A. caffra*), schw (*A. schweinfurthii*).

Fig. 2. Mean ($\pm 95\%$ confidence intervals) root weight. Treatments are represented by NF+NC (control), NF+C (herbivory), F+NC (fire), and F+C (herbivory*fire interaction). Species abbreviations: heb (*A. hebeclada*), eriol (*A. erioloba*), karr (*A. karroo*), tort (*A. tortilis*), gerr (*A. gerrardii*), nil (*Acacia nilotica*), xan (*A. xanthophloea*), sieb (*A. sieberiana*), fleck (*A. fleckii*), mell (*A. mellifera*), erub (*A. erubescens*), atax (*A. ataxacantha*), caff (*A. caffra*), schw (*A. schweinfurthii*).

Fig. 3. Mean ($\pm 95\%$ confidence intervals) spine and prickles abundance. Treatments are represented by NF+NC (control), NF+C (herbivory), F+NC (fire), and F+C (herbivory*fire interaction). Species abbreviations: heb (*A. hebeclada*), eriol (*A. erioloba*), karr (*A. karroo*), tort (*A. tortilis*), gerr (*A. gerrardii*), nil (*Acacia nilotica*), xan (*A. xanthophloea*), sieb (*A. sieberiana*), fleck (*A. fleckii*), mell (*A. mellifera*), erub (*A. erubescens*), atax (*A. ataxacantha*), caff (*A. caffra*), schw (*A. schweinfurthii*).

Fig. 4. Mean ($\pm 95\%$ confidence intervals) spine and prickles length. Treatments are represented by NF+NC (control), NF+C (herbivory), F+NC (fire), and F+C (herbivory*fire interaction). Species abbreviations: heb (*A. hebeclada*), eriol (*A. erioloba*), karr (*A. karroo*), tort (*A. tortilis*), gerr (*A. gerrardii*), nil (*Acacia nilotica*), xan (*A. xanthophloea*), sieb (*A. sieberiana*), fleck (*A. fleckii*), mell (*A. mellifera*), erub (*A. erubescens*), atax (*A. ataxacantha*), caff (*A. caffra*), schw (*A. schweinfurthii*).

Fig. 5. Mean ($\pm 95\%$ confidence intervals) tannin concentration in quebracho tannin equivalents (Q. E). Treatments are represented by NF+NC (control), NF+C (herbivory), F+NC (fire), and F+C (herbivory*fire interaction). Species abbreviations: heb (*A. hebeclada*), eriol (*A. erioloba*), karr (*A. karroo*), tort (*A. tortilis*), sieb (*A. sieberiana*), fleck (*A. fleckii*), mell (*A. mellifera*), erub (*A. erubescens*).

Fig. 6. Mean ($\pm 95\%$ confidence intervals) root total non-structural carbohydrates. Treatments are represented by NF+NC (control), NF+C (herbivory), F+NC (fire), and F+C (herbivory*fire interaction). Species abbreviations: heb (*A. hebeclada*), eriol (*A. erioloba*), karr (*A. karroo*), tort (*A. tortilis*), gerr (*A. gerrardii*), nil (*Acacia nilotica*), xan (*A. xanthophloea*), sieb (*A. sieberiana*), fleck (*A. fleckii*), mell (*A. mellifera*), erub (*A. erubescens*), atax (*A. ataxacantha*), caff (*A. caffra*), schw (*A. schweinfurthii*).

Table 1. Differentiation of *Acacia* species between native habitat type and forms of physical defence

Species with spines	Species with prickles
Arid	
<i>Acacia hebeclada</i>	<i>Acacia fleckii</i>
<i>Acacia erioloba</i>	<i>Acacia mellifera</i>
<i>Acacia karroo</i>	<i>Acacia erubescens</i>
<i>Acacia tortilis</i>	
Humid	
<i>Acacia gerrardii</i>	<i>Acacia ataxacantha</i>
<i>Acacia nilotica</i>	<i>Acacia caffra</i>
<i>Acacia xanthophloea</i>	<i>Acacia schweinfurthii</i>
<i>Acacia sieberiana</i>	

Table 2. Relationships between new stem growth, physical defences and condensed tannins using Pearson's product-moment correlation (r ; Bonferoni correction-bold = significance <0.01). Negative r values indicate a trade-off. Significant values are in bold. ¹=species with spines and ²=species with prickles.

Species	Herbivory			Fire		
	Stems vs. thorn abundance	Stems vs. thorn length	Stems vs. condensed tannins	Stems vs. thorn abundance	Stems vs. thorn length	Stems vs. condensed tannins
<u>Arid Species</u>						
<i>A. hebeclada</i> ¹	-0.789	-0.467	-0.510	0.928	0.440	0.078
<i>A. erioloba</i> ¹	-0.513	-0.737	-0.590	0.510	0.773	-0.157
<i>A. karroo</i> ¹	-0.693	-0.689	-0.599	0.673	0.069	-0.099
<i>A. tortilis</i> ¹	-0.854	-0.806	-0.577	-0.555	0.374	-0.085
<i>A. fleckii</i> ²	-0.228	-0.534	-0.608	0.428	0.485	-0.535
<i>A. mellifera</i> ²	-0.508	-0.434	-0.663	-0.254	0.369	-0.616
<i>A. erubescens</i> ²	-0.613	-0.334	-0.629	-0.111	0.330	-0.256
<u>Humid Species</u>						
<i>A. gerrardii</i> ¹	0.297	0.151	0.215	0.489	0.843	0.136
<i>A. nilotica</i> ¹	-0.772	0.146	-0.023	-0.582	-0.471	-0.004
<i>A. sieberiana</i> ¹	-0.801	0.576	-0.757	-0.638	0.724	0.174
<i>A. xanthophloea</i> ¹	-0.795	0.219	-0.067	-0.407	0.694	0.048
<i>A. ataxacantha</i> ²	0.458	-0.776	-0.001	0.356	-0.386	0.274
<i>A. caffra</i> ²	0.413	0.479	0.017	0.133	-0.495	0.016
<i>A. schweinfurthii</i> ²	-0.122	-0.560	-0.064	0.245	0.524	-0.056

Table 3. Relationships between physical defences and condensed tannins using Pearson's product-moment correlation (r; Bonferoni correction, bold = significance <0.01). Negative r values indicate a trade-off. ¹ = species with spines and ² = species with prickles.

Species	Herbivory			Fire		
	Thorn abundance vs. thorn length	Thorn abundance vs. condensed tannins	Thorn length vs. condensed tannins	Thorn abundance vs. thorn length	Thorn abundance vs. condensed tannins	Thorn length vs. condensed tannins
<u>Arid Species</u>						
<i>A. hebeclada</i> ¹	0.443	0.319	0.310	0.452	0.141	-0.070
<i>A. erioloba</i> ¹	0.361	0.213	0.694	0.492	-0.211	0.127
<i>A. karroo</i> ¹	0.643	0.519	0.411	0.224	0.175	-0.120
<i>A. tortilis</i> ¹	0.794	0.489	0.342	-0.058	-0.352	0.074
<i>A. fleckii</i> ²	0.499	0.177	0.233	0.432	-0.600	-0.298
<i>A. mellifera</i> ²	0.601	0.399	0.219	0.212	0.367	-0.009
<i>A. erubescens</i> ²	0.535	0.430	0.173	-0.013	0.487	-0.123
<u>Humid Species</u>						
<i>A. gerrardii</i> ¹	0.209	0.082	0.042	0.525	-0.039	0.046
<i>A. nilotica</i> ¹	-0.066	0.047	-0.142	-0.024	0.409	-0.058
<i>A. sieberiana</i> ¹	-0.392	0.539	-0.304	-0.345	-0.263	0.434
<i>A. xanthophloea</i> ¹	-0.304	-0.152	-0.255	-0.438	0.015	0.224
<i>A. ataxacantha</i> ²	-0.117	-0.032	0.010	-0.073	0.062	-0.142
<i>A. caffra</i> ²	0.162	-0.051	-0.031	0.132	-0.048	0.035
<i>A. schweinfurthii</i> ²	0.181	0.009	0.073	0.250	-0.034	0.126

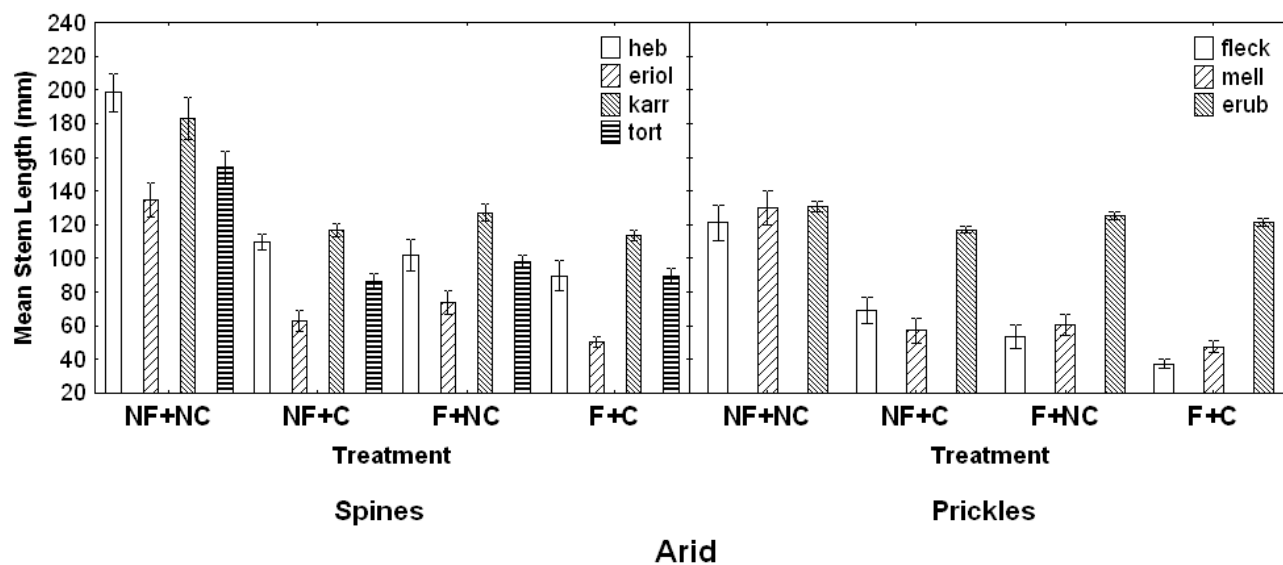


Fig. 1a

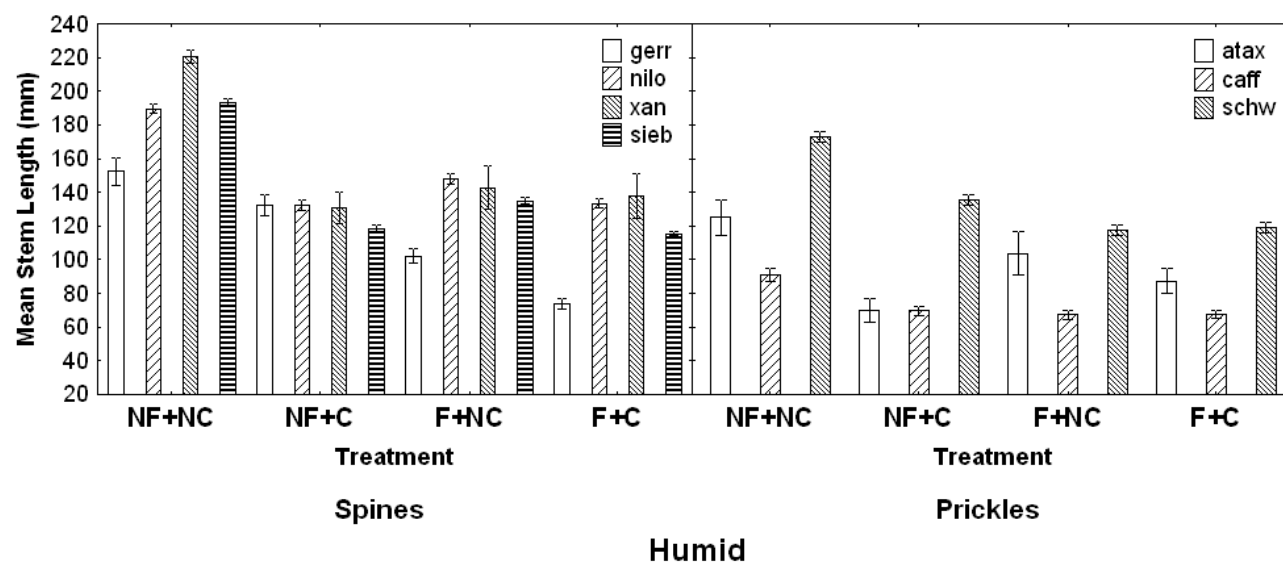


Fig. 1b

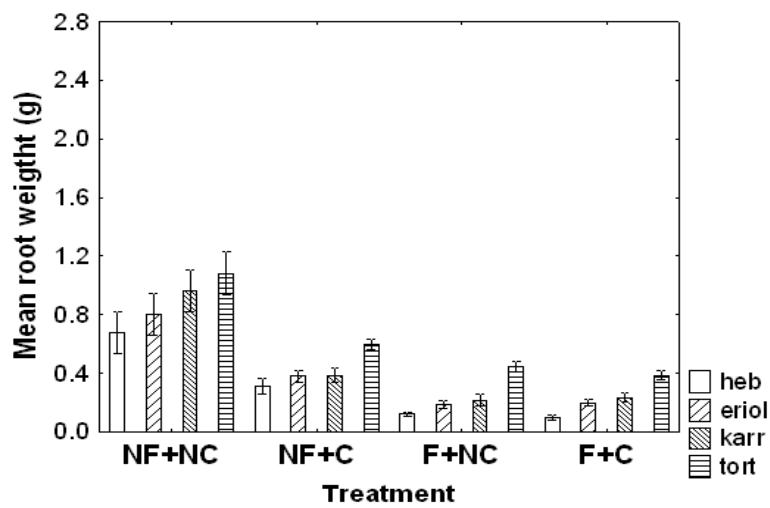


Fig. 2a

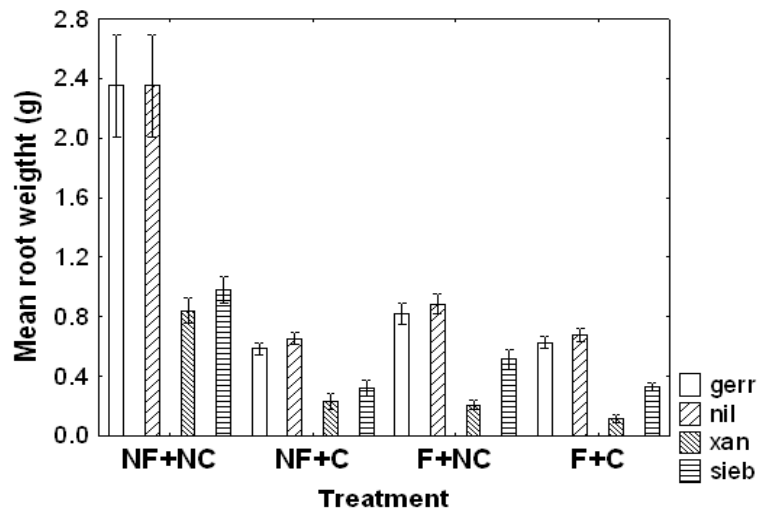


Fig. 2b

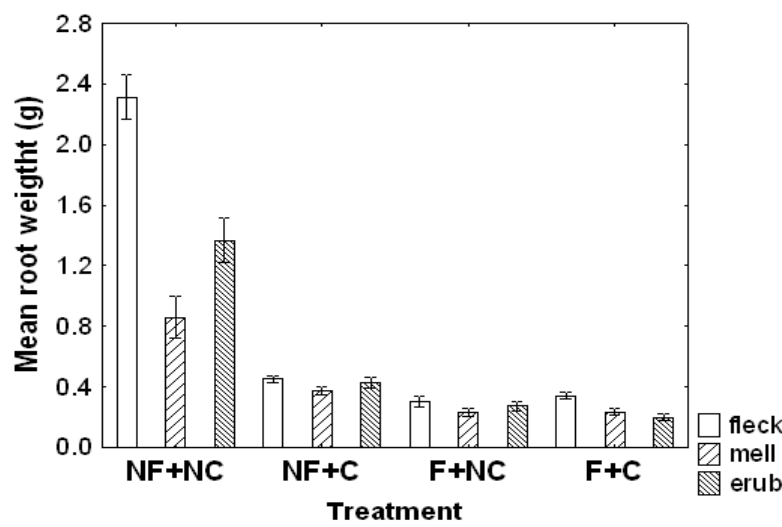


Fig. 2c

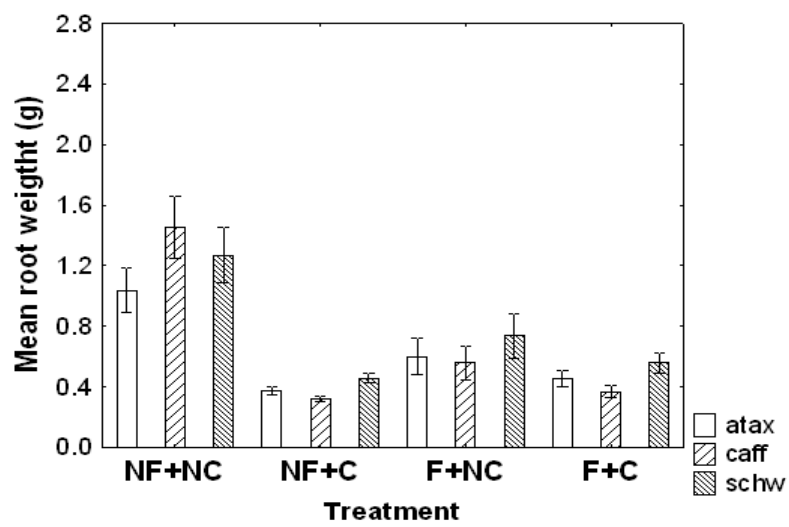
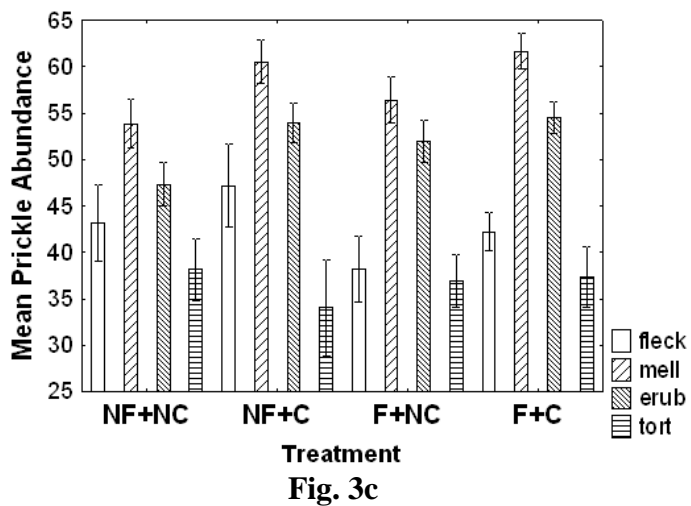
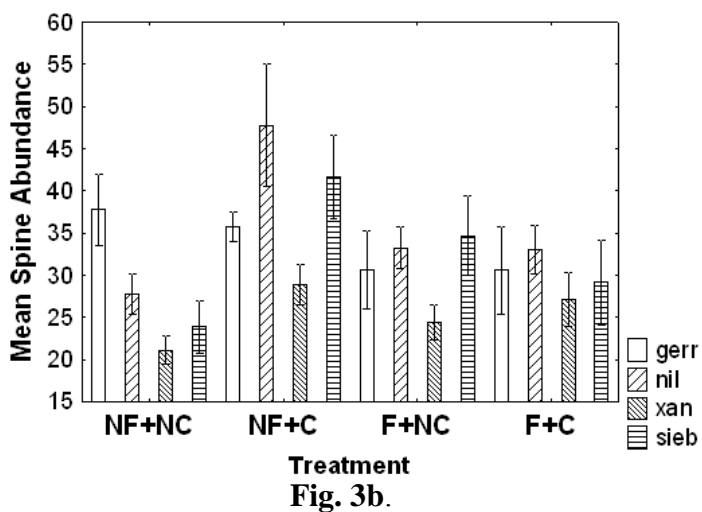
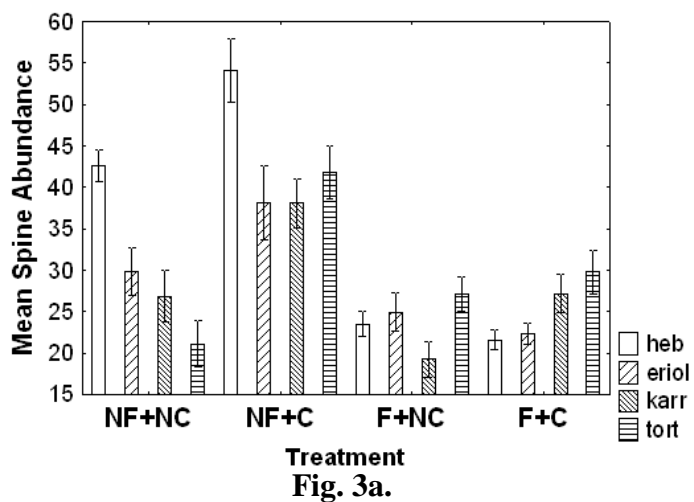


Fig. 2d



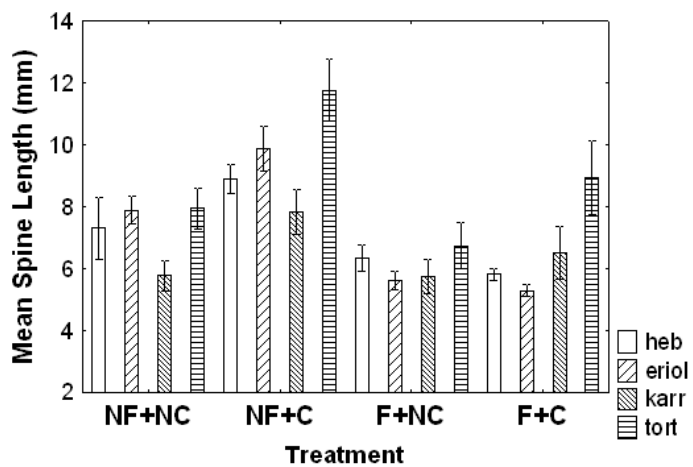


Fig. 4a.

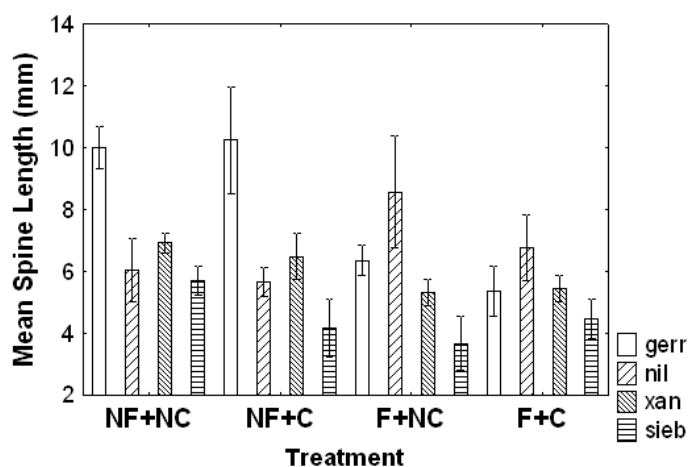


Fig. 4b.

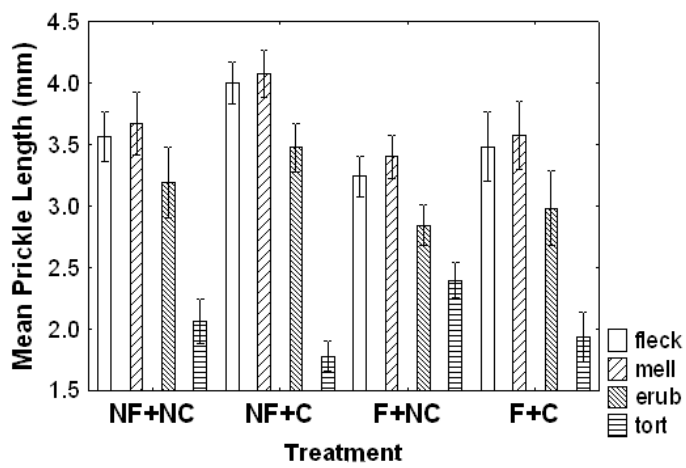


Fig. 4c.

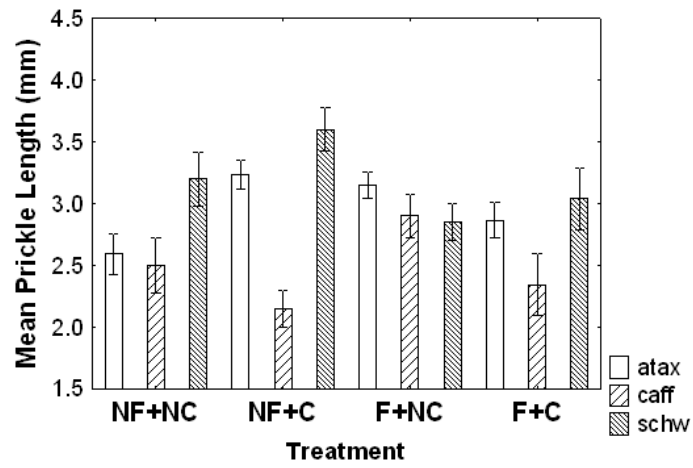


Fig. 4d.

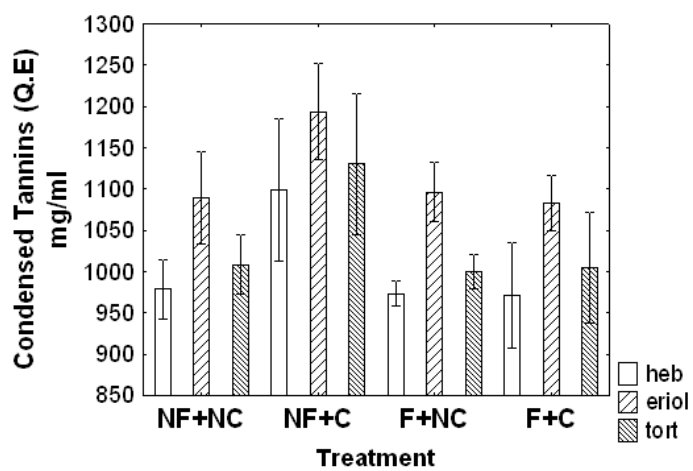


Fig. 5a.

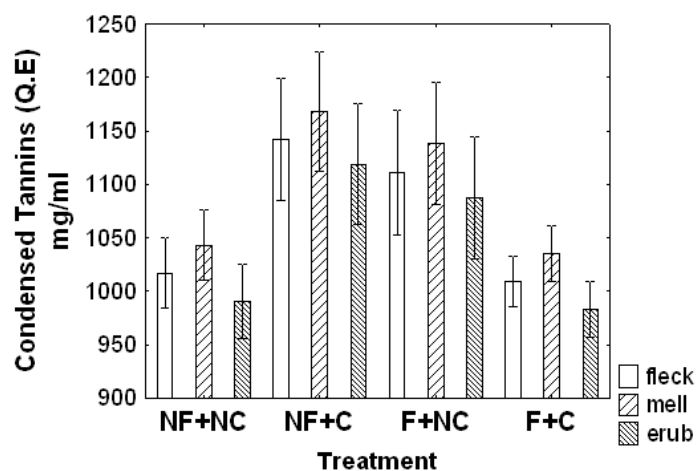


Fig. 5b.

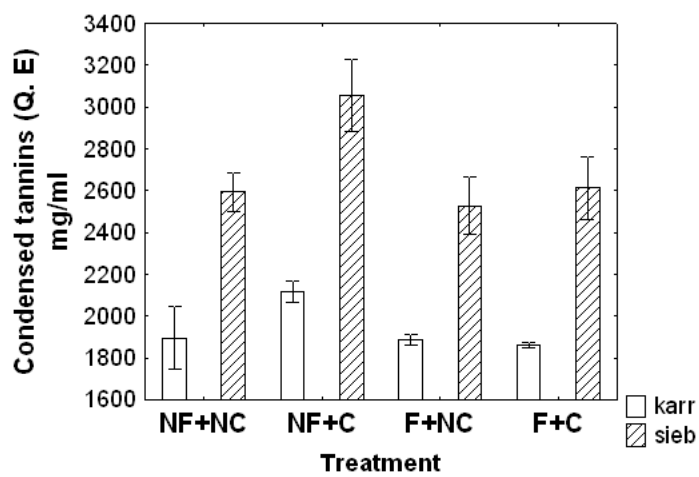
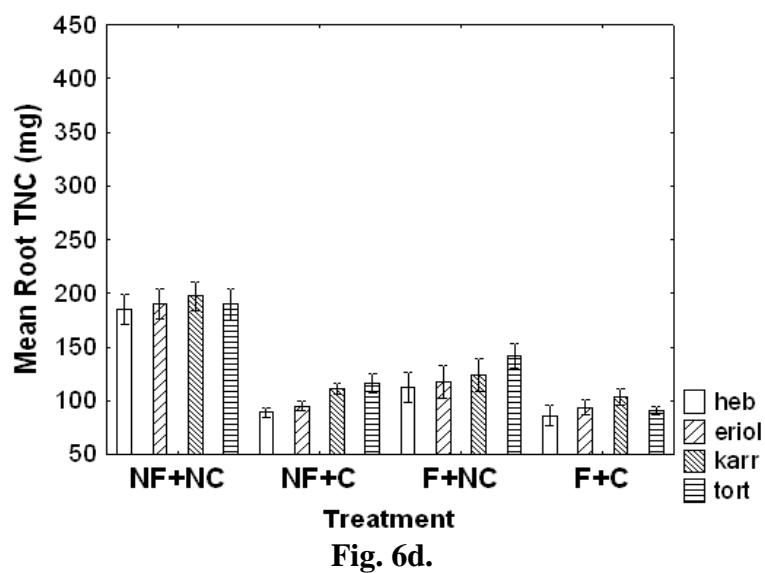
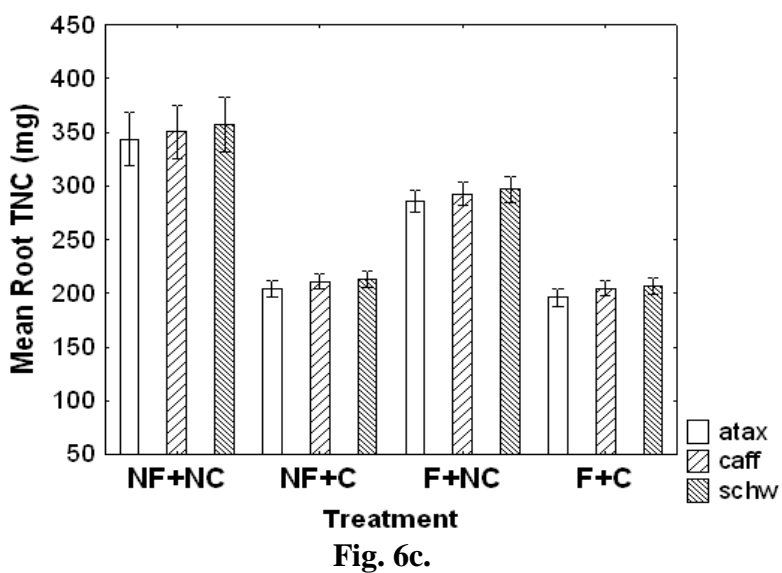
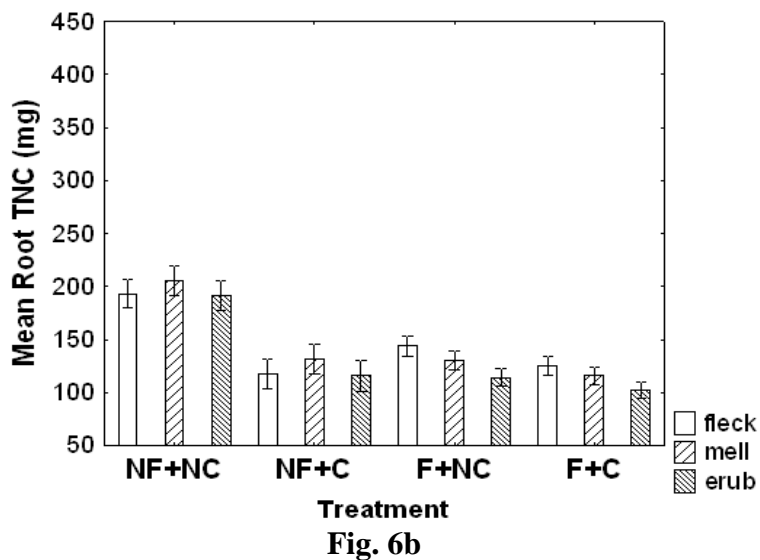
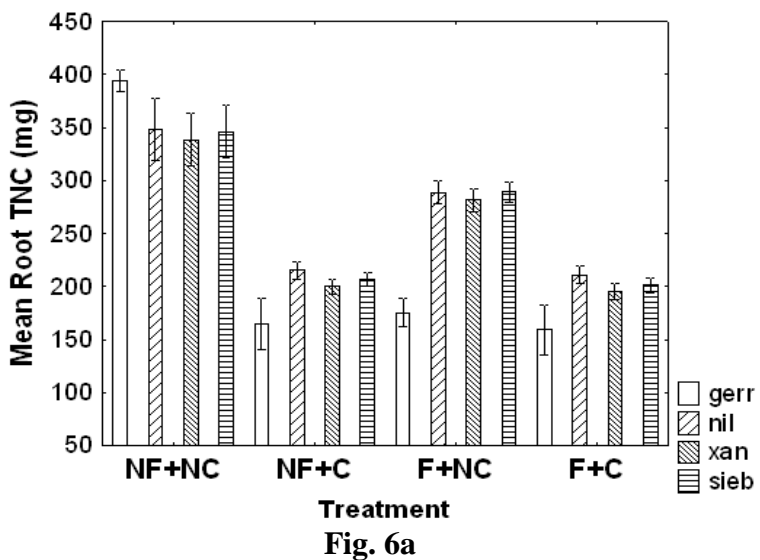


Fig. 5c.



Chapter 3

The effects of nutrient availability on growth, defence and mobilization of total non-structural carbohydrates after herbivory and fire in seedlings of three *Acacia* species.

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Summary

1. Plants are often subjected to many disturbance events that inevitably lead to tissue damage or even mortality. Nutrient availability has been pointed out as being fundamental to our understanding of plant regrowth patterns subsequent to tissue loss.
2. Several resource availability hypotheses have been formulated that attempt to explain the regrowth patterns of plants after tissue loss. The Growth –Differentiation Balance Hypothesis (GDBH) assumes that plants living in resource-poor environments have slow growth rates, thus invest more in defences after tissue loss in order to defend new biomass. Resource-rich species exhibit less investment in defence, as they have high growth rates and thus can easily replace lost tissues. Using five levels of nutrient availability, we determined the effects of simulated herbivory and fire on shoot regrowth, total non-structural carbohydrates (TNC) and defence production in the seedlings of *Acacia erioloba*, *Acacia karroo*, and *Acacia nilotica*.
3. Overall, the growth of roots and stems of all species and TNC increased with an increase in nutrient availability. Spine abundance and spine length displayed a unimodal trend in all three species, with spine abundance and spine length being greatest at a nutrient level of 800 mg/ ℓ. Spine abundance, spine length and condensed tannins increased significantly after herbivory, while fire generally had the opposite effect.
4. *Acacia* seedlings exhibited mixed support for the assumptions and predictions of the expanded GDBH, with stem growth and spine defences consistent with the patterns predicted by the GDBH. These seedlings were shown to be highly resilient to disturbance events, while the growth responses of *Acacia* seedlings are underpinned by TNC stores in roots. Herbivory and fire were shown to not be substitutable in their effects on *Acacia* seedlings.

Key-words: arid, condensed tannins, Growth-Differentiation Balance Hypothesis, humid, spines.

Introduction

Disturbances, such as herbivory and fire, and resources interact to affect overall plant growth, reproduction and survival (Gao et al. 2008). A plant's ability to compensate for tissue loss due to herbivore attack or fire is influenced by several factors, including timing and degree of damage (Canham, McAninch & Wood 1994; Danel, Bergström & Edenius 1994), competition and nutrient availability (Mashinski & Whitham 1989; Hjalten, Danell & Lundberg 1993) and plant intrinsic factors (Bryant, Chapin & Klein 1983). Plants, due to limited resources, either invest in defensive traits or greater regrowth capacity (Herms & Mattson 1992), with some plants even compensating (McNaughton 1983) or even overcompensating in terms of growth after a disturbance event (Paige & Whitham 1987). Disturbances are assumed to have an overall negative effect on plants (Gao et al. 2008), although it has been shown that plants may actually benefit from disturbances, by increasing biomass production, seed and fruit production or shoot production (Paige & Whitham 1987; Lennartsson, Nilsson & Tuomi 1998; Christel & Rodger 2006).

There are many theories that have been developed over the past four decades that attempt to explain the responses of plant defences to disturbance events (see Herms & Mattson 1992; Stamp 2003 for reviews). While the concepts behind plant defence theories have been supported on several occasions (e.g. Koricheva, Nyaken & Gianolis 2004; Endara & Coley 2011), the reasons why there is such broad variation in plant defences among species still remains an enigma (Gao et al 2008). The Limiting Resource Model (Gao et. al. 2008) demonstrated that interactions between varying water levels and clipping intensities aggravate the detrimental impacts of herbivores on plant growth and reproduction (Gao et al. 2008). However, the antagonistic interactions between nutrient availability and herbivory can alleviate the negative effects of tissue loss to herbivory, while biomass compensation and density compensation were identified as important mechanisms of tolerance of herbivory (Gao et al 2008). Most of the synthetic theories addressing inter-specific differences in defence (e.g. Optimal Defence Hypothesis, Resource Availability Hypothesis and Growth-Differentiation Balance Hypothesis – reviewed by Stamp 2003) assume that selection has optimized investments, such that the benefits outweigh the costs (Feeney 1976; McKey 1979; Coley, Bryant & Chapin 1985; Crawley 1985). Resource availability is one factor that may significantly influence the degree to which plants

may recover from disturbances (Hawkes & Sullivan 2001; Schumacher et al 2009). For growth, maintenance and reproduction, plants require a relatively small number of basic resources from their external environments, including light, carbon dioxide, water, and inorganic nutrients (e.g. N, P, K, and various trace elements) (Schumacher et al 2009). Prevailing ecological wisdom has long held that plants growing in relatively resource-rich, low-competition, or otherwise benign environments should be better able to tolerate (i.e. compensate for) herbivory than plants growing in more stressful environments (Whittaker 1979; Coley et al 1985; Oesterheld & McNaughton 1991). Replacing a fixed amount of removed tissue requires a larger fraction of the net production of a plant growing in a resource-poor environment than that of a plant growing in a resource-rich environment (Coley et al 1985; Herms & Mattson 1992; Gao et al 2008). This prediction of lower resistance to herbivory in stressful environments possesses strong intuitive appeal, and it is an important assumption of some popular general theories of plant resistance, including the resource availability hypothesis and the carbon nutrient balance hypothesis (Bryant, Chapin & Klein 1983; Coley et al. 1985; Coley 1987; Herms & Mattson 1992; Stamp 2003).

Plant species are adapted to thrive in environments with different relative levels of these essential resources (Chapin et al. 1987). Some argue that in any given environment, one resource is generally likely to have a greater potential to limit plant fitness than any other (Wise & Abrahamson 2005), but Chapin et al. (1987) showed that several resources may end of being co-limiting. Thus, in either instance roots (acting as carbohydrate stores) allow plants to draw on stored nutrients that may not be available or accessible subsequent to disturbance events. Even in the face of potential resource inadequacy, plants possess a remarkable degree of developmental plasticity that allows them to balance their resource acquisition to maximize their fitness (McConnaughay & Coleman 1999; Wise & Abrahamson 2005). Damage by disturbance events, such as herbivory and fire, have the potential to disrupt this balance, thereby exacerbating an existing limitation or causing a different resource to become limiting (Tilman 1982, 1988; Wise & Abrahamson 2005).

The expanded Growth-Differentiation Balance hypothesis (GDBH) (Loomis 1932, 1953; reviewed by Stamp 2003) has commonly been used to explain the differentiation of plant tissue and defence regrowth post-disturbance. The GDBH proposes that species that are adapted to

growing in resource-rich environments are inherently fast growing, and have high foliar nutrient concentrations, high tissue turnover rates, low investment in anti-herbivore defences and, consequently, high levels of herbivore damage (Coley et al. 1985; Stamp 2003). By contrast, species that are adapted to growing in resource-poor environments will have inherently slow growth-rates, resulting in lower tissue nutrient concentration, greater tissue longevity and higher investment in anti-herbivore defences, leading consequently to lower levels of herbivory (Coley et al. 1985; Stamp 2003). Replacement of tissue lost to herbivores is relatively more costly for slow-growing plant species with long-lived tissues than for fast-growing species, whereas diverting resources to defence rather than growth will incur high costs for fast-growing species in highly competitive environments. With regard to predicted responses to resource-poor and resource-rich environments, the GDBH is very similar to the Resource Availability hypothesis (also known as the Growth Rate hypothesis – Stamp 2003). However, the GDBH recognizes the physiological tradeoffs between growth and differentiation at the cellular and tissue levels relative to the selective pressures of resource availability (Stamp 2003). In particular, the GDBH predicts a monotonic increase in growth rate with increasing resources but a unimodal response (peaking at intermediate levels) for secondary metabolites (Stamp 2003). Although theories of defence allocation have been the subject of debate, the GDBH has gained significant support (Stamp 2003), and is classified as “the most theoretically mature” (Stamp 2003) among the plant defence theories; its relationship to herbivore feeding has been extensively tested for woody plant species (Matsuki & Koike 2006).

AFRICAN ACACIA TREES

Acacia trees are common and highly abundant throughout Africa and the Middle East (Rohner & Ward 1997), and the presence of *Acacia* species is known to drive various ecosystem functions (Munzbergova & Ward 2002). Increased soil-water infiltration (Dougill, Heathwaite & Thomas 1998), nutrient pool size and availability (Hudak, Wessman & Seastedt 2003), species diversity (Dean, Milton & Jeltsch 1999) and ecosystem productivity (Belsky 1994) are all closely linked to the presence and abundance of *Acacia* trees.

We attempt to apply the expanded GDBH to the explain growth and defence responses of three *Acacia* species (*A. erioloba*, *A. karroo* and *A. nilotica*) that were subjected to simulated herbivory and fire across an increasing nutrient gradient. We note that Stamp (2003) indicated that at least five levels of fertilizer are needed to effectively test the GDBH, because a unimodal response of secondary metabolites to herbivory is predicted (Fig 1). We make the following predictions: 1) According to the GDBH, arid-adapted *Acacia* species will invest more in defence to defend new regrowth because they have relatively low growth rates compared to humid-adapted *Acacia* species, 2) Net stem and root length will increase with an increase in nutrient availability, 3) An increase in nutrient availability will induce a unimodal response for defences such as spine abundance and length, and condensed tannin concentration (Fig 1). 4) *Acacia* species will display an increase in total stored non-structural carbohydrates in roots with an increase in nutrient availability, as a means to accumulate carbohydrate stores that can be drawn upon to resist future damage.

Methods and Materials

STUDY SPECIES

Acacia erioloba is a tall (up to 15 m) tree or shrub, with a rounded crown, and dark, rough and fissured bark (Ross 1979). Physical defences are usually in the form of long spines (± 10 cm long) in pairs (Ross 1979), but also exhibits condensed tannins as a form of chemical defences (Hean & Ward 2011). *A. erioloba* generally occurs in dry woodland, and frequently in arid Kalahari sands (Ross 1979). *A. karroo* is a flat crowned, several stemmed tall tree that grows up to 12 m in height (Ross 1979). They exhibit long spines (± 7 cm, but up to 25 cm in some instances) that occur in pairs (Ross 1979), while also exhibit condensed tannins as chemical defences (Hean & Ward 2011). *A. karroo* is the most widespread and common *Acacia* species in southern Africa (Ross 1979; Ward 2011). *A. nilotica* has a flattened, rounded crown with dark, deeply fissured bark. Spines (± 8 cm) occur in pairs with a gland at the base of each pair of spines (Ross 1979). *A. nilotica* is common and widespread throughout tropical and sub-tropical Africa, occurring on a variety of soils (Ross 1979). *Acacia erioloba* is considered to be a resource-poor

(arid species) while *A. karroo* and *A. nilotica* have evolved in resource-rich (mesic-humid) environments.

EXPERIMENTAL DESIGN

A three factor, fully randomized design was utilized, where treatments were simulated burning and herbivory, coupled with five levels of fertilizer (3: 2: 1 NPK), viz. 200 mg/ ℓ (1), 400 mg/ ℓ (2), 800 mg/ ℓ (3), 1500 mg/ ℓ (4), 3000 mg/ ℓ (5). Factors were control (no burning and no cutting), burning only, cutting only, and burning and cutting. These species were laid in blocks and planted in 20 l pots (2:1 potting medium, sand to wood mulch ratio). Seeds from each species were manually scarified, and germinated on agar plates. Germination (radicle length of 3-4 cm) took 3 d. Thereafter, one seedling of each was planted in each pot, with treatments being replicated 15 times and randomized within species blocks. Pots were laid on an automated mist bed, with watering taking place every three hours for 1 min. Seedlings were allowed to grow for a further 15 weeks. Burning was achieved through the use of a butane-fuelled blow torch, using a flame of approximately 15 cm held at a distance of 20 cm (approximately 1000° C) from the seedling for 8 s. Seedlings were burnt from the first node (point of attachment for cotyledons) to the apex of the seedling. Simulated herbivory was achieved with the use of hand-held garden scissors and cut at the first node (Dube, Mlambo, Sebata 2010). Seedlings that were exposed to the interaction treatment (fire and simulated herbivory) were burned first, allowed to grow for 10 days, and then cut at the first node (Dube, Mlambo & Sebata 2010). All seedlings were then allowed to grow for a further 21 d, for a total growing time of 31 d for all seedlings after treatments.

Above- and belowground biomass

The effect of the various treatments on biomass re-growth for each species was determined by measuring and comparing dry weight (DW) of roots and stems (Trlica & Cook 1971). Individual seedlings were carefully removed from their allocated pots and all soil washed off the roots. Stems and roots were separated by cutting at the first node, and dried in a 60° C oven for 48h. Photosynthetic material and roots were directly compared for each individual seedling by weighing the DW material.

Spinescence

The effect of simulated burning and herbivory on spine abundance and spine length was also measured. Total spine abundance was recorded for each seedling and spine length was recorded using vernier calipers. Mean spine length was calculated for each seedling.

Condensed tannin analysis

Leaf material was used in the determination of tannin concentration. The photosynthetic material was dried in a 60 °C for 48 h, and then ground in a Wiley mill to pass through a 40 mesh sieve (Van Der Heyden & Stock 1996). Using 0.25 g of dried leaf material, condensed tannin analysis was performed using the acid butanol assay for condensed tannins, as described by Hagerman (2002) using quebracho tannin as a standard.

Non-structural carbohydrates

Using a 0.5 g sample of aboveground biomass, and 0.2 g of belowground biomass, total non-structural carbohydrates were determined and analysed. As with the determination of tannin concentrations, all plant material was dried in an oven for 48 h at 60° C, then ground with the aid of a Wiley mill to allow the material to pass through a 40 mesh sieve (Van Der Heyden & Stock 1996). Total non-structural carbohydrates were extracted for each species in a two-step process according to Tolsma, Read & Tolhurst (2010). Total concentration of starch was determined through the modified quantification method as first outlined by Knutson and Grove (1994). The sample was standardized by calculating $\% \text{ amylose}_{\text{corrected}} = (\% \text{ amylose}_{\text{uncorrected}} - 6.2) / 93.8$, where $\% \text{ uncorrected amylose} = \% \text{ calculated from the standard curve}$. The samples were repeated in triplicate to avoid contamination and biased readings from cellulose lint (Tolsma et al. 2007).

STATISTICAL ANALYSIS

SPSS version 18 for Windows was used for all analyses. All variables conformed with the requirements of normality and homogeneity of variance. Using simulated herbivory, fire and fertilizer levels as independent factors, a three-factor ANOVA was used to calculate significant

differences. For significant effects, a Scheffe's *post hoc* test was performed. The effects of simulated herbivory and fire were tested by comparing the means of stem weight, root weight, spine abundance, spine length, condensed tannin concentration and the overall total non-structural carbohydrates in stems and roots. Pearson's product moment correlations (with Bonferroni correction for multiple comparisons) between stem length and spine abundance, spine length and condensed tannins were conducted to determine whether there were any trade-offs between growth and defence traits, and between the various defence traits.

Results

ABOVE- AND BELOW-GROUND BIOMASS

Stem and root length/weight of all three species displayed a significant increase with an increase in nutrient availability for *A. erioloba* ($F= 1079$, $p<0.001$, error d.f.=220), *A. nilotica* ($F= 3313$, $p< 0.001$, error d.f.=220) and *A. karroo* ($F= 3661$, $p< 0.001$, error d.f.=220) with stem length at each level of nutrients being significantly longer than the previous nutrient level after simulated herbivory ($p< 0.001$) and fire ($p< 0.001$). Overall, root length of each species displayed a similar trend to stem growth, whereby root length increased significantly with an increase in nutrient availability (*A. erioloba* ($F= 1204$, $p< 0.001$, error d.f.=220, Fig 3a); *A. nilotica* ($F= 308$, $p< 0.001$, error d.f.=220, Fig 3b); *A. karroo* ($F= 3573$, $p< 0.001$, error d.f.=2201, Fig 3c). Root length was significantly longer per nutrient level than the previous for all three species after simulated herbivory ($p< 0.001$) and fire ($p< 0.001$). The stem length, stem weight, root length, and root weight of *A. erioloba* and *A. nilotica* were not significantly different between nutrient levels 1 and 2 after simulated herbivory or fire (see Fig 2-5).

PHYSICAL AND CHEMICAL DEFENCES

Spine abundance of *A. erioloba* and *A. karroo* displayed a unimodal response to herbivory and fire across the nutrient gradient. *A. nilotica* did not exhibit a significant difference in spine abundance as nutrient availability increased. *A. erioloba* and *A. karroo* displayed the highest

spine abundance at 800mg/ ℓ nutrient availability (Fig 4a, b) after herbivory and fire. *A. erioloba* and *A. karroo* displayed a similar trend in that spine abundance was not significantly different between nutrient levels 1 and 5, and 2 and 4 after herbivory (Table 1).

Spine length was shown to be significantly different between the upper and lower levels of nutrient availability. *Acacia erioloba* had significantly longer spines at nutrient availability level 5, compared to levels 1 and 2, subsequent to both herbivory and fire (Fig 5a, Table 1 and 2). *A. nilotica* (Fig 5c) and *A. karroo* (Fig 5b) had longer mean spine lengths between nutrient level 1 and 5 subsequent to herbivory and fire treatments, while there was no significant difference in spine length between any other nutrient levels.

Increased nutrient availability resulted in an increase in condensed tannin concentration in *Acacia erioloba* and *A. nilotica* only. Condensed tannin concentration for both species was significantly higher between the upper and lower ends of nutrient availability after herbivory in both species (Fig 6a,b, Table 1 and 2). *A. erioloba* exhibited an elevated tannin concentration at nutrient levels 4 and 5 which was significantly higher than levels 1 and 2 (Fig 6a, Table 1) *A. nilotica* exhibited elevated tannin concentrations at nutrient level 4 compared to nutrient level 1 (Table 1), and level 5 compared to levels 1 and 2 (Table 1) after herbivory. All other nutrient levels were found not to be significantly different in condensed tannin concentration subsequent to simulated herbivory (Table 1). *A. erioloba* and *A. nilotica* also exhibited increased tannin concentration with increased nutrient availability after fire (Fig 6a, b, Table 2).

TOTAL NON-STRUCTURAL CARBOHYDRATES (TNC)

An increase in nutrient availability resulted in an increase in the overall TNC concentration in both stems and roots of all three species. Overall, simulated herbivory had a greater negative effect on TNC in stems and roots of all three species ($p < 0.001$, error d.f.=220, Fig 7 and 8). *A. erioloba*, *A. nilotica* and *A. karroo* all exhibited the highest TNC concentration at nutrient availability 5, and was shown to be significantly higher than all other nutrient levels after herbivory and fire ($p < 0.001$ for all). Generally, TNC were significantly higher in stems and roots at each nutrient level after both herbivory and fire, while there was no significant difference

in TNC between nutrient availability level 3 and 4 ($p > 0.990$ for all species), and level 1 and 2 ($p > 0.990$ for all species) (Fig 7 and 8).

TRADE-OFFS

Overall, *Acacia erioloba* did not exhibit any trade-offs between new stem growth and physical and chemical defences, with the exception of a weak, but significant negative correlation between stem growth and condensed tannins when nutrient availability was 1500 mg/l ($r = -0.461$, $p = 0.001$). There was no significant correlation between stem growth and spine length at nutrient availability 3 ($r = 0.319$, $p = 0.027$), 4 ($r = 0.329$, $p = 0.022$), and 5 ($r = 0.304$, $p = 0.035$). There was also no trade-off between spine abundance, spine length or condensed tannins for *A. erioloba*, but with strong positive correlations existing between spine abundance and length ($r > 0.5$, $p < 0.001$), spine abundance and condensed tannins ($r > 0.3$, $p = 0.002$), as well as spine length and condensed tannins ($r > 0.7$, $p < 0.001$).

A trade-off between stem growth and spine abundance was shown in *A. nilotica* across all nutrient levels ($r > -0.5$, $p < 0.001$). There was no significant correlation between spine abundance and condensed tannins at nutrient level 3 ($r = 0.292$, $p = 0.044$) and 5 ($r = 0.290$, $p = 0.045$), and no significant correlation between spine abundance and spine length at nutrient level 3 ($r = -0.293$, $p = 0.43$).

A. karroo displayed a strong positive correlation was seen between spine abundance and length ($r > 0.585$, $p < 0.001$), and spine abundance and condensed tannins ($r > 0.426$, $p < 0.001$) throughout all nutrient levels.

Discussion

Our findings have provided evidence that supports the assumptions of the expanded GDBH. *A. erioloba* is an arid-adapted species where resource availability is low, thus growth rate and tissue turn-over rate is low. Hence, it this species was predicted to invest more carbohydrate reserves into differentiation in order to defend new photosynthetic material. *A. nilotica* and *A. karroo*, which occur in resource-rich environments, should conversely, invest more into growth than defence, because they have high growth and tissue turn-over rates. *A. nilotica* and *A. karroo* both

displayed an increase in spine length, but the only significant difference was between the upper and lower levels of the nutrient gradient, while *A. erioloba* increased all defence traits. Thus, these data are consistent with our first prediction. All species grew with increased nutrients, supporting our second prediction.

Repeated disturbance and biomass removal will select for greater storage of carbohydrate reserves below-ground to support rapid shoot growth after biomass loss through herbivory or fire (Van Der Heyden & Stock 1995, 1996). Disturbance-adapted plants can be expected to achieve some resistance to browsing because of their inherently rapid vertical growth rate and below-ground storage (Bryant et al. 1983). The GDBH is better equipped to explain the growth trends of plants across varying nutrient availability levels than other plant defence hypotheses, predominantly because the GDBH provides a model that shows how investment into growth and differentiation varies with an increase in nutrient availability (Fig 1). According to this model, the net assimilation rate (NAR) should increase until an asymptote, while secondary metabolite defences (secondary metabolites) should follow a unimodal trend. Using this model, we are able to determine the trends in growth and defence that were observed for the three *Acacia* species tested in this experiment, while also being able to effectively test the hypothesis.

While tannin concentration of all three species increased linearly and thus does not support the assumptions of the model, the spine abundance of *A. erioloba* and *A. karroo* followed the trend, with spine abundance in both species reaching a maximum at a nutrient level of 800mg/ℓ. Our findings partially support our third prediction, because spine length and condensed tannin concentration continued to increase as nutrient availability increased. Total non-structural carbohydrates in stems and roots both followed the trend of net assimilation in the model. However, the increase of nutrient assimilation did not perfectly follow the trend outlined by the GDBH model (Fig 1). The model predicts an asymptote in NAR; perhaps if higher levels of nutrients were used, the asymptote might have been reached. This increased uptake of nutrients may be converted to stored TNC in stems and roots. Therefore, the conversion of assimilated nutrients into TNC becomes an important issue in understanding how woody plants may respond to disturbance events. These data therefore support our fourth prediction that TNC in roots will increase with an increase in nutrient availability, allowing the plant to better recover from future tissue damage or loss. Depending on the nutrient level, a plant may switch from a

defensive strategy to an increased growth strategy to avoid future biomass loss (Coley et al. 1985; Bond & Midgley 2001). Our results have provided evidence that partially supports the GDBH, because an increase in nutrient availability did not induce a switch from a defensive strategy to increased growth. All three species of *Acacia* used in this experiment exhibited an increase in their overall growth of above- and below-ground tissues, as indicated by length and dry weight. This also indicates that accumulated carbon stores in roots allow woody seedlings to minimize the cost of removal of photosynthetic material. Our results also support the findings of Hean & Ward (2011), whereby TNC have been shown to be the underlying mechanism that determines the regrowth responses of woody plants after disturbance events.

These data show that there is not necessarily always a trade-off (e.g. *A. erioloba*) between growth processes and differentiation (i.e. investment in defensive traits) with an increase in nutrient availability. *Acacia* seedlings, in this instance, may be taking advantage of the increased availability of elemental nutrients and maximizing growth of photosynthetic tissues, along with defensive traits, thus optimizing their fitness (e.g. Mboumba & Ward 2008, Ward 2010). Why condensed tannin concentration continued to increase with an increase in nutrient availability remains an enigma, but could indicate that the nutrient levels used in this experiment were too low to see the expected response.

Conclusion

Woody seedlings are highly resilient in the face of disturbance, and have the ability to not only display increased growth of photosynthetic material, but to also increase their defensive capabilities in order to protect new photosynthetic material from future attack. This experiment also supports the notion that herbivory and fire are not substitutable in their effects on the regrowth patterns of *Acacia* species, as suggested by Hean & Ward (2011). The expanded GDBH is a useful plant defence hypothesis in explaining the regrowth trends of *Acacia* seedlings, although it was not fully supported in some instances. However, many of the assumptions and predictions of the GDBH can be accepted from our experimental evidence, while the resource availability model of the GDBH is key to our understanding of the growth-

differentiation balance that plants display subsequent to biomass loss, and consequently furthering our knowledge of savanna ecosystem functioning.

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Figure Legends

Fig 1. Relationship of net assimilation rate (NAR), relative growth rate (RGR) and differentiation (secondary metabolism), across a resource gradient for which the resource affects growth more than it does photosynthesis. At point A, both growth and photosynthesis are constrained by low resource availability. At point B, growth is more constrained than photosynthesis and thus there is more allocation to differentiation. At point C, growth is less constrained and thus there is more allocation to growth. Stamp (2003) noted that a minimum of five resource levels spread along the gradient are necessary in order to determine this pattern. (adapted from Herms and Mattson (1992)).

Fig 2 Mean (± 95 % confidence intervals) stem length. Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5). Dark triangles= control; empty squares= response to herbivory, empty circles= response to fire. Herb=Simulated herbivory.

Fig 3 Mean (± 95 % confidence intervals) root length. Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5). Dark triangles= control; empty squares= response to herbivory, empty circles= response to fire. Herb=Simulated herbivory.

Fig 4. Mean (± 95 % confidence intervals) stem weight. Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5). Dark triangles= control; empty squares= response to herbivory, empty circles= response to fire. Herb=Simulated herbivory.

Fig 5. Mean (± 95 % confidence intervals) root weight. Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5). Dark triangles= control; empty squares= response to herbivory, empty circles= response to fire. Herb=Simulated herbivory.

Fig 6. Mean (± 95 % confidence intervals) spine abundance. Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5). Dark triangles= control; empty squares= response to herbivory, empty circles= response to fire. Herb=Simulated herbivory. Letters infer similarity to , or significant difference from other levels of nutrients within treatments.

Fig 7. Mean (± 95 % confidence intervals) spine length. Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5). Dark triangles= control; empty squares= response to herbivory, empty circles= response to fire. Herb=Simulated herbivory. Letters infer similarity to , or significant difference from other levels of nutrients within treatments.

Fig 8. Mean (± 95 % confidence intervals) condensed tannin concentration (quebracho tannin equivalents). Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5). Dark triangles= control; empty squares= response to herbivory, empty circles= response to fire. Herb=Simulated herbivory. Letters infer similarity to , or significant difference from other levels of nutrients within treatments.

Fig 9. Mean (± 95 % confidence intervals) stem total non-structural carbohydrates (TNC). Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5). Dark triangles= control; empty squares= response to herbivory, empty circles= response to fire. Herb=Simulated herbivory. Letters infer similarity to , or significant difference from other levels of nutrients within treatments.

Fig 10. Mean (± 95 % confidence intervals) root total non-structural carbohydrates (TNC). Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5). Dark triangles= control; empty squares= response to herbivory, empty circles= response to fire. Herb=Simulated herbivory. Letters infer similarity to , or significant difference from other levels of nutrients within treatments.

Table 1. ANOVA *post-hoc* output for defensive traits after simulated herbivory across all nutrient levels. Bold values signify significance ($p < 0.05$). Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5).

Fertilizer levels	Herbivory									
	1 vs. 2	1 vs. 3	1 vs. 4	1 vs. 5	2 vs. 3	2 vs. 4	2 vs. 5	3 vs. 4	3 vs. 5	4 vs. 5
<i>A. erioloba</i>										
Spine Abundance	1.000	<0.001	0.338	0.998	0.724	0.991	<0.001	0.017	<0.001	<0.001
Spine length	0.997	0.232	<0.001	<0.001	0.925	0.082	<0.001	0.998	0.361	0.997
Condensed Tannins	0.996	0.070	<0.001	<0.001	0.818	0.029	<0.001	0.992	0.436	0.998
<i>A. nilotica</i>										
Spine Abundance	0.986	0.989	0.998	0.998	0.998	0.997	0.619	0.001	0.617	0.987
Spine length	0.997	0.997	0.721	0.026	0.998	0.979	0.224	0.996	0.835	0.995
Condensed Tannins	0.997	0.371	0.004	<0.001	0.993	0.337	<0.001	0.998	0.633	0.997
<i>A. karroo</i>										
Spine Abundance	0.250	<0.001	0.006	0.998	0.231	0.998	0.031	<0.001	<0.001	<0.001
Spine length	0.998	0.980	0.443	0.016	0.997	0.955	0.292	0.988	0.960	0.995
Condensed Tannins	0.990	0.371	0.004	<0.001	0.998	0.338	<0.001	0.996	0.633	0.977

Table 2. ANOVA *post-hoc* output for all defence traits after fire across all nutrient levels. Bold values signify significance ($p < 0.05$). Nutrient levels are 200mg/l (1), 400mg/l (2), 800mg/l (3), 1500mg/l (4), and 3000mg/l (5).

Fertilizer levels	Fire									
	1 vs. 2	1 vs. 3	1 vs. 4	1 vs. 5	2 vs. 3	2 vs. 4	2 vs. 5	3 vs. 4	3 vs. 5	4 vs. 5
<i>A. erioloba</i>										
Spine Abundance	<0.001	<0.001	0.158	0.998	0.399	0.998	<0.001	<0.001	<0.001	0.009
Spine length	0.998	0.629	0.010	<0.001	0.989	0.235	<0.001	0.997	0.330	0.996
Condensed Tannins	0.990	0.258	0.004	<0.001	0.889	0.153	<0.001	0.993	0.731	0.990
<i>A. nilotica</i>										
Spine Abundance	0.971	0.982	0.997	0.998	0.999	0.998	0.681	0.998	0.682	0.989
Spine length	0.998	0.995	0.672	0.217	0.996	0.974	0.212	0.996	0.806	0.998
Condensed Tannins	0.960	0.693	0.073	<0.001	0.964	0.736	0.018	0.995	0.268	0.997
<i>A. karroo</i>										
Spine Abundance	0.406	<0.001	0.008	0.998	0.052	0.998	0.006	0.753	<0.001	<0.001
Spine length	0.997	0.940	0.251	<0.001	0.994	0.856	0.047	0.981	0.814	0.998
Condensed Tannins	0.989	0.693	0.073	<0.001	0.964	0.736	0.018	0.978	0.268	0.998

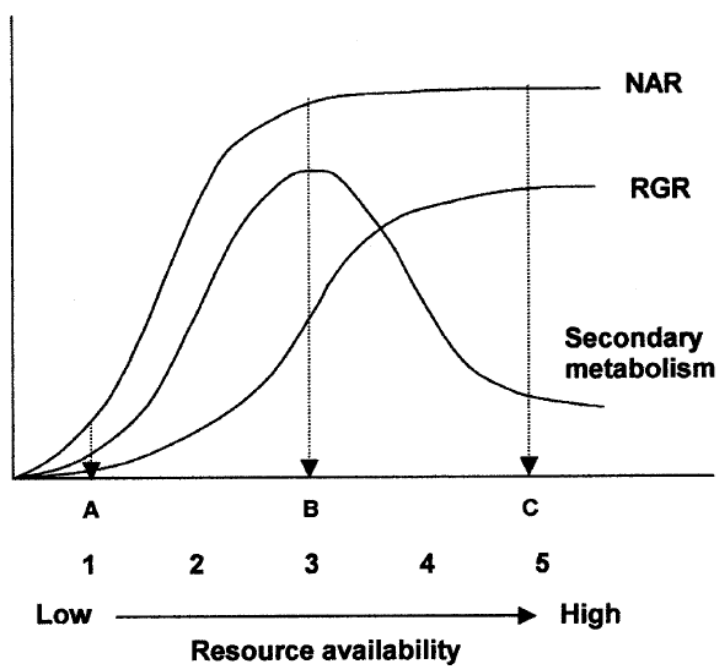
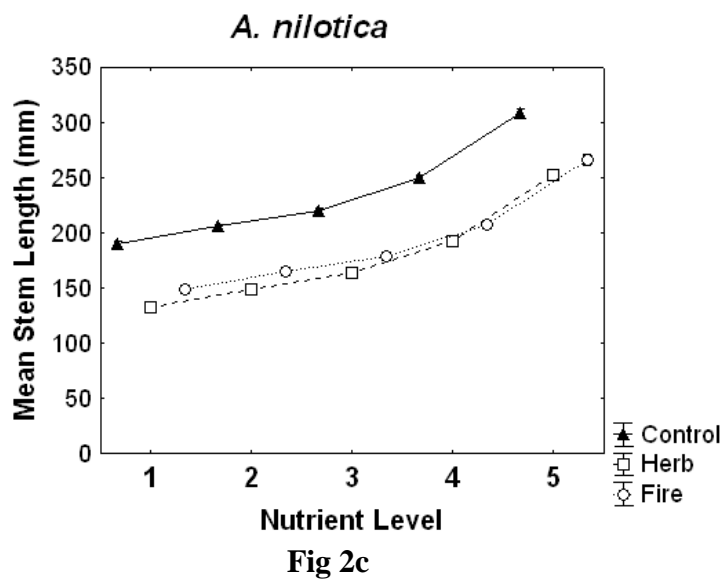
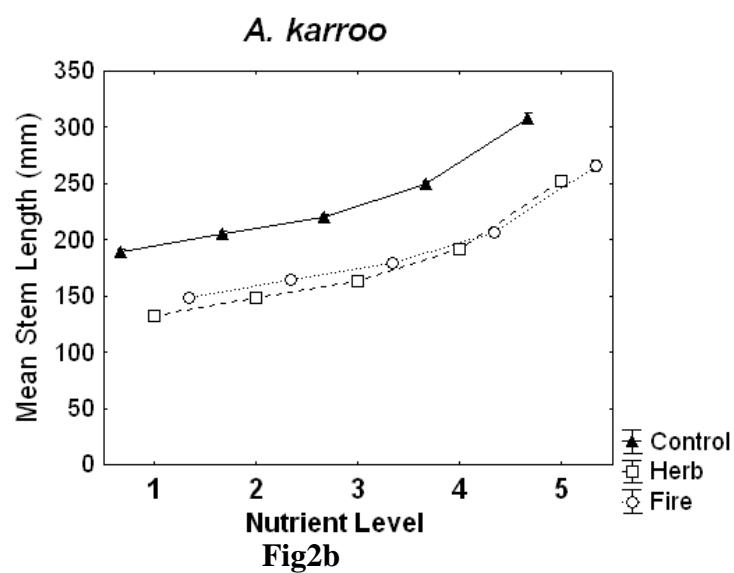
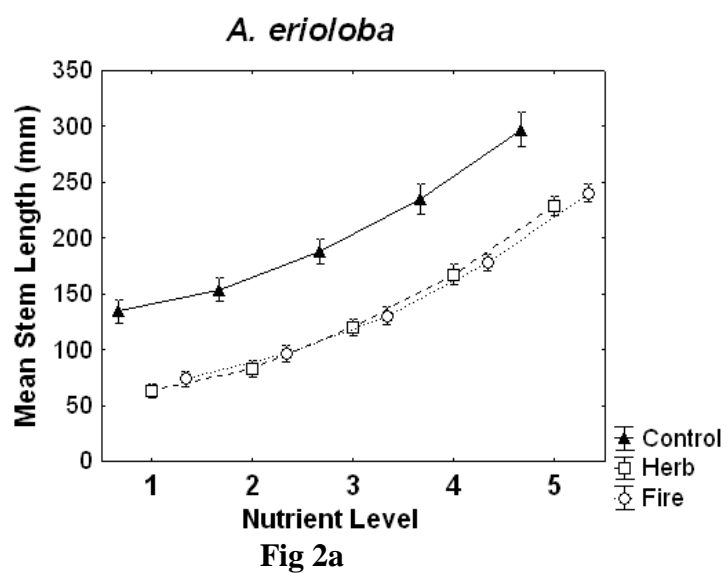


Fig 1.



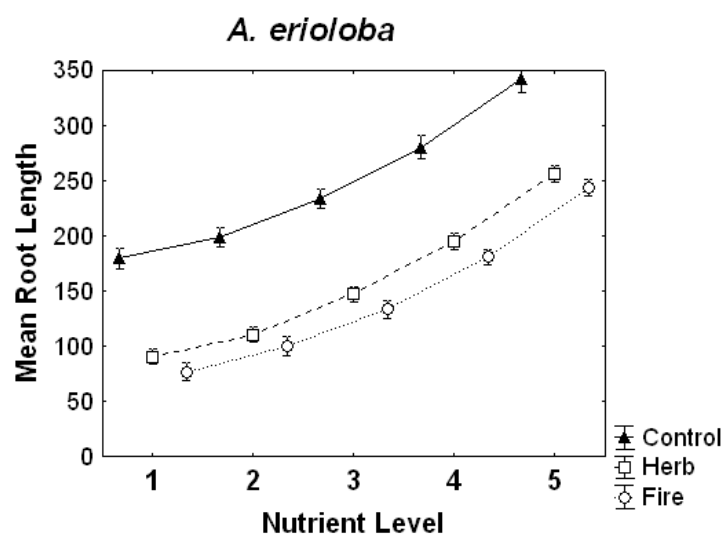


Fig 3a

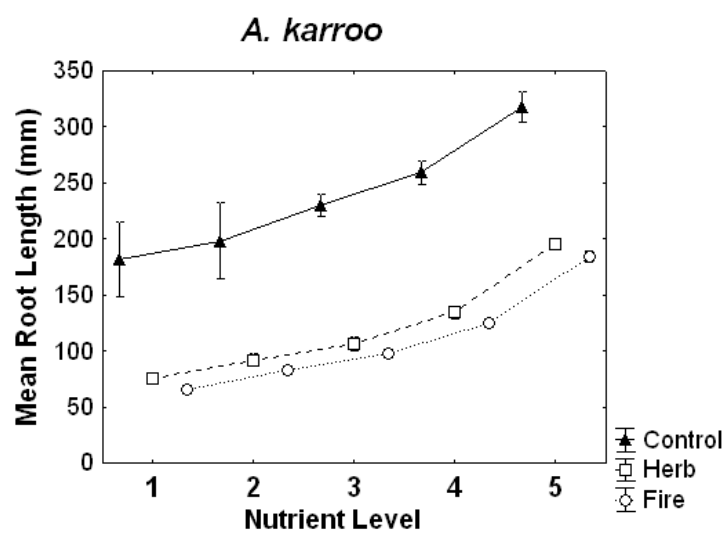


Fig 3b

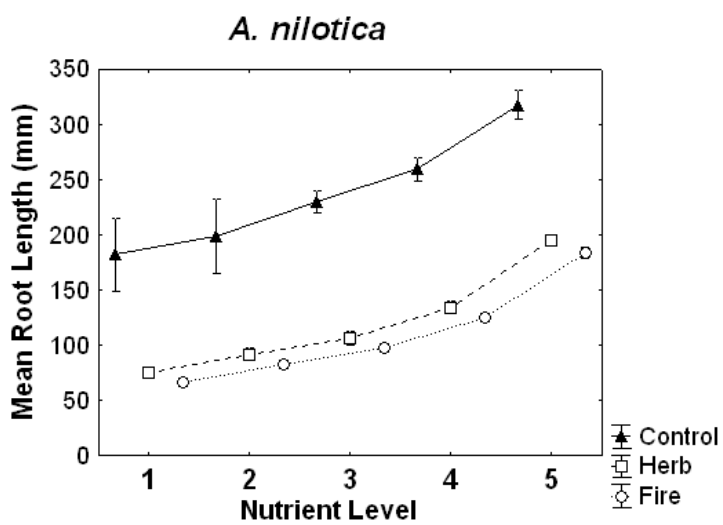


Fig3c

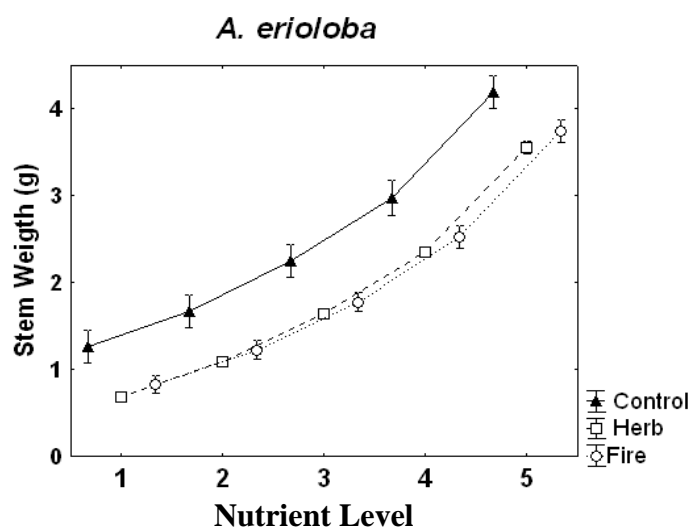


Fig 4a

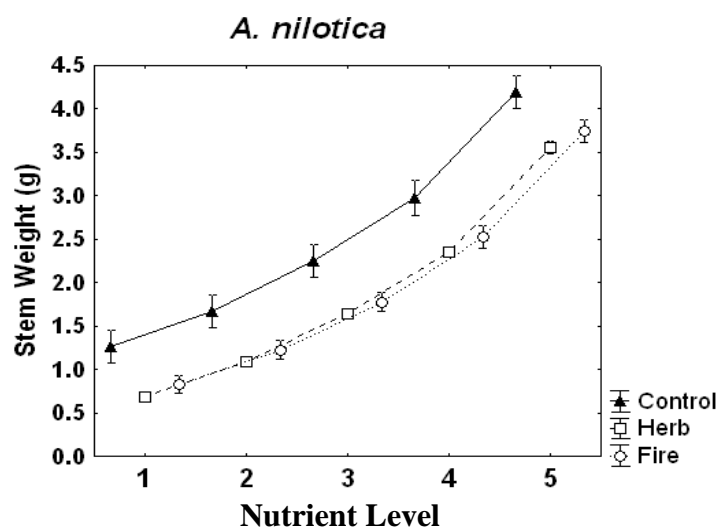


Fig 4b

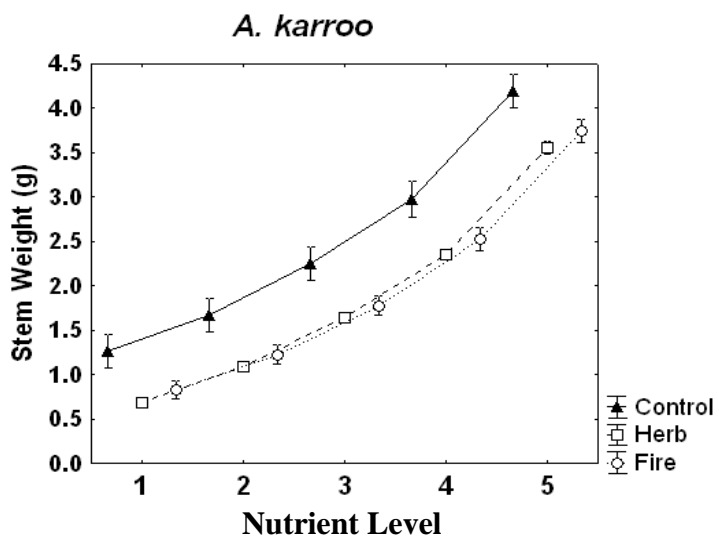


Fig 4c

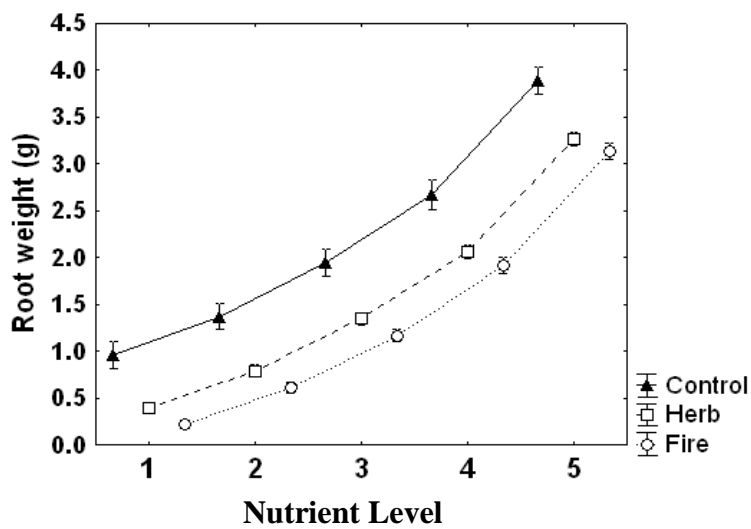
A. erioloba

Fig 5a

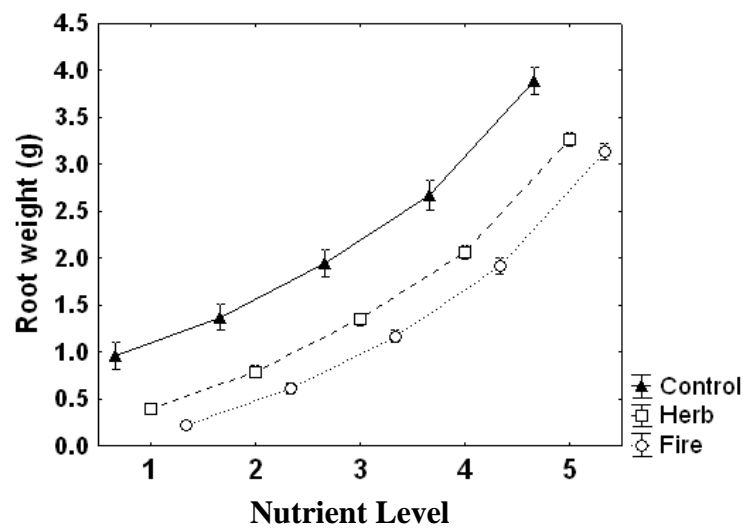
A. nilotica

Fig 5b

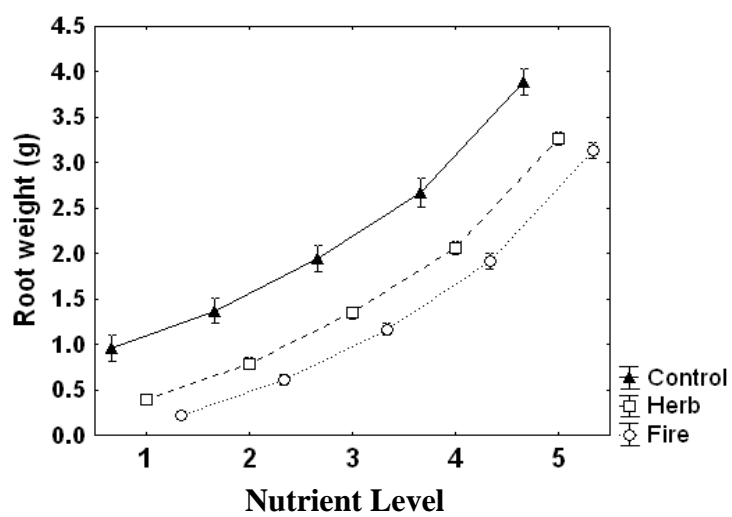
A. karroo

Fig 5c

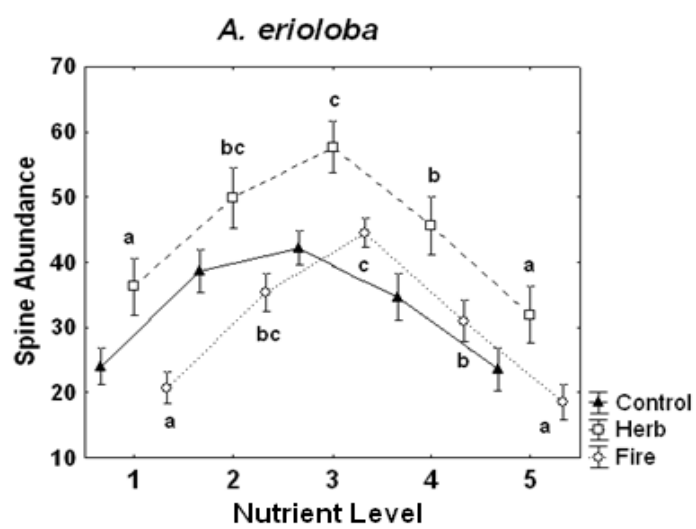


Fig 6a.

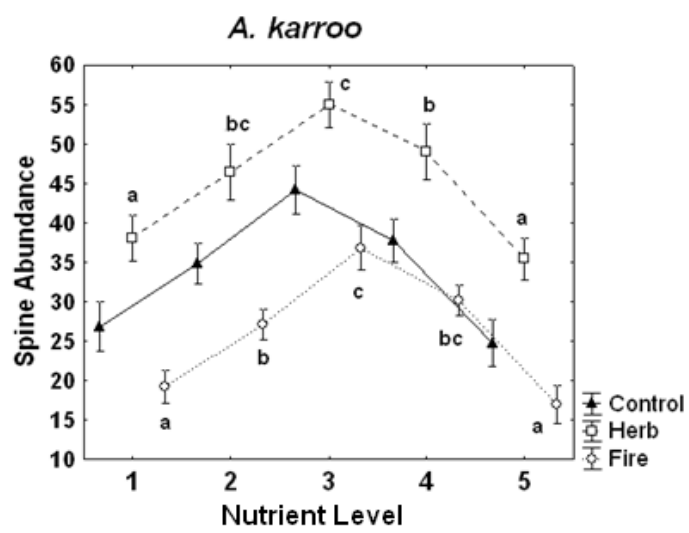


Fig 6b.

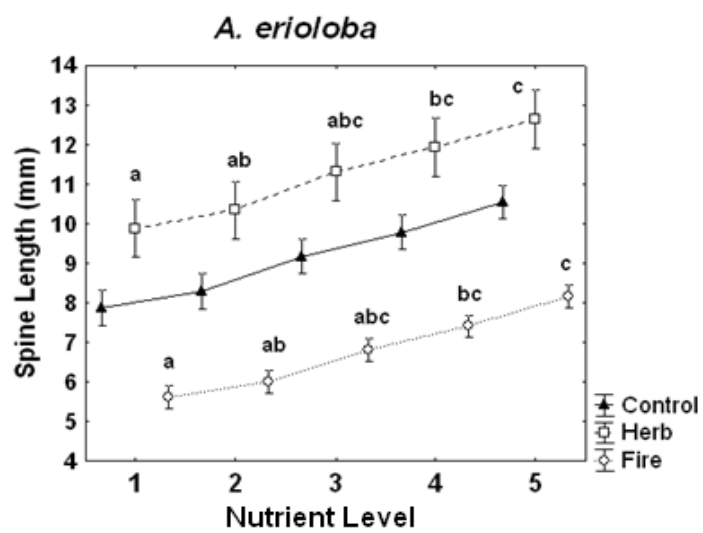


Fig. 7a.

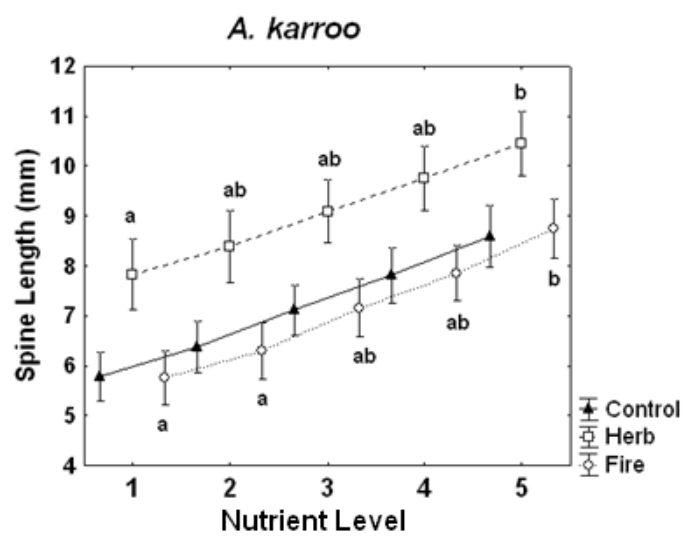


Fig. 7b.

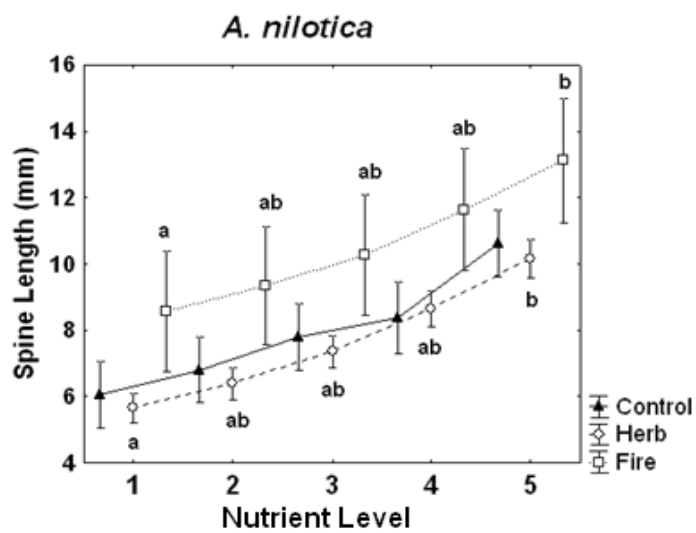
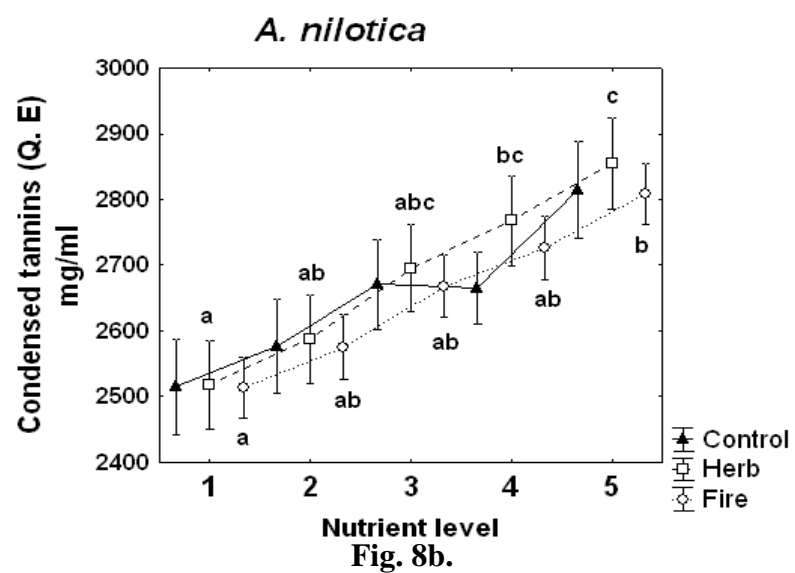
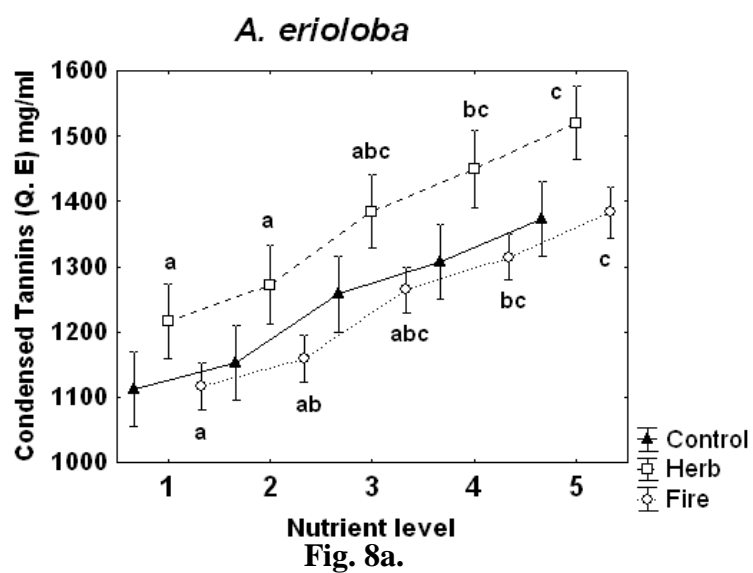


Fig. 7c.



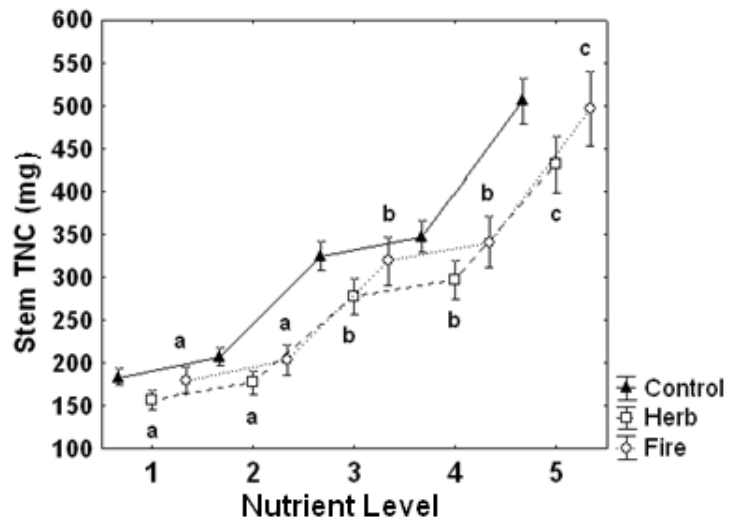
A. erioloba

Fig. 9a.

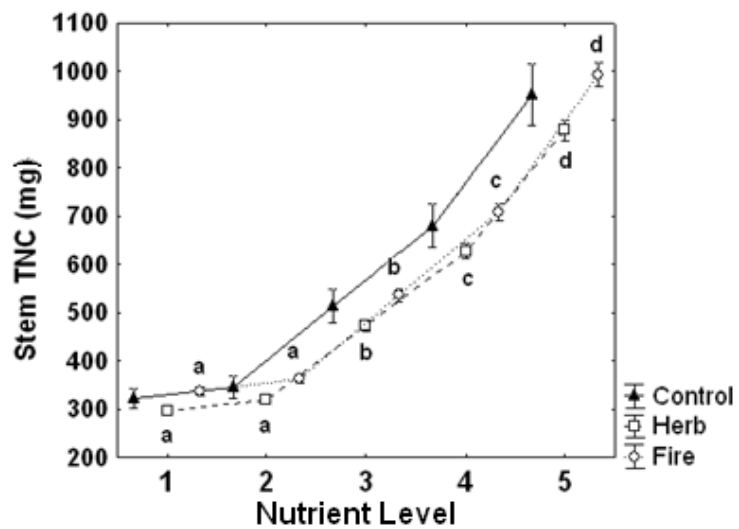
A. nilotica

Fig. 9b.

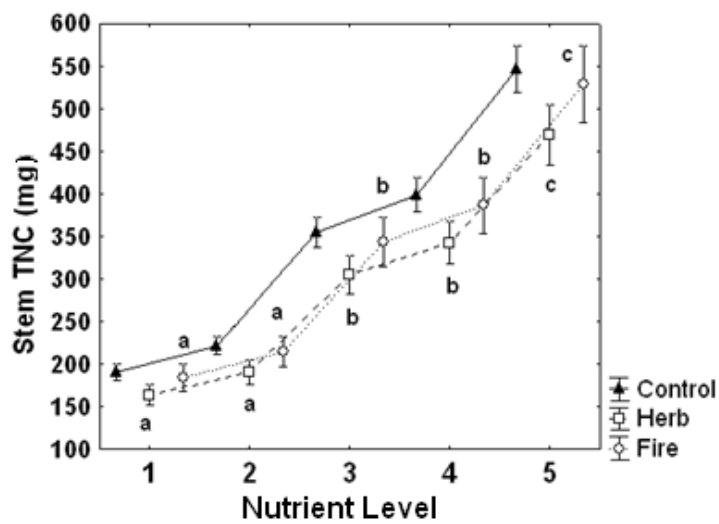
A. karroo

Fig. 9c.

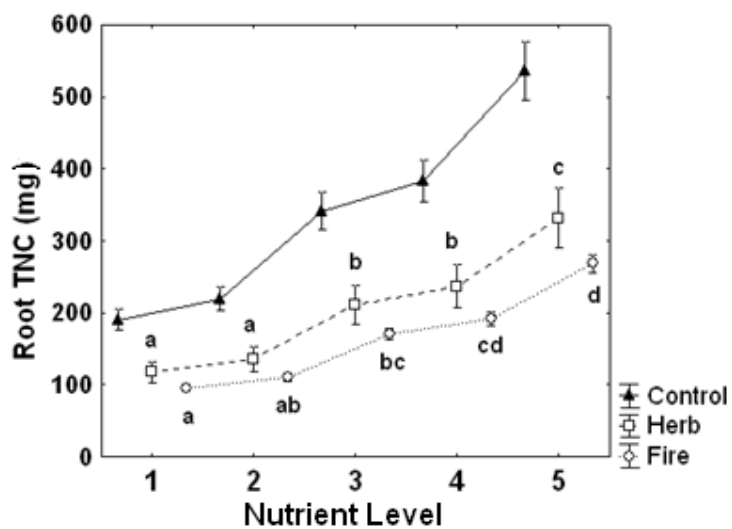
A. erioloba

Fig. 10a.

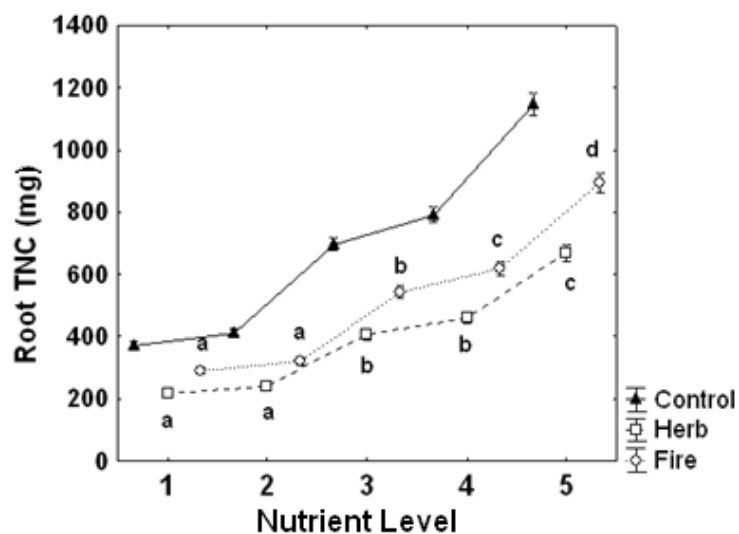
A. nilotica

Fig. 10b.

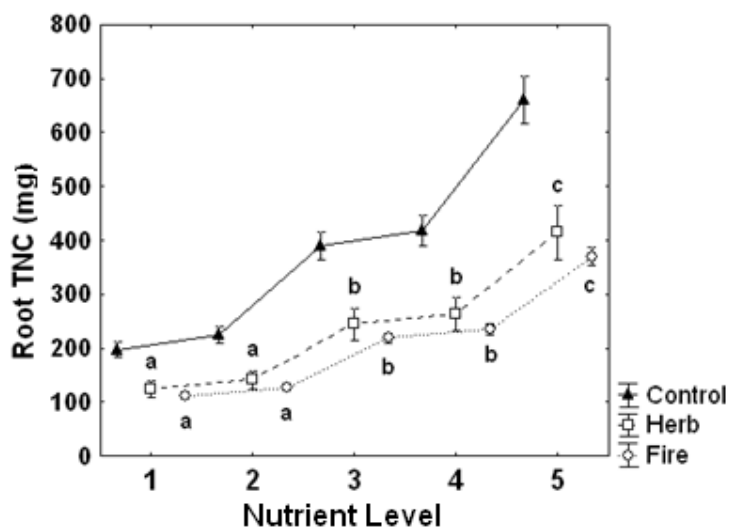
A. karroo

Fig. 10c.

Chapter 4

Conclusion and future work

Acacia trees are keystone species that play an integral role in African savanna functioning (Munzbergova & Ward 2002). They are known to increase the heterogeneity, water infiltration, and soil moisture of the surrounding savanna (Dube, Mlambo & Sebata 2010), while also acting as a key food source for numerous wild and domestic ungulates and invertebrates (Belsky, Amundson & Duxbury 1989). There is a multitude of studies concerning adult *Acacias* and their growth responses to disturbances, such as herbivory and fire (Barnes 2001; Bond & Midgley 2001), as well as their interactions with native grasses in savannas (e.g. Hanan et al. 2008; Scogings & Mopipi 2008; Moustakas et al. 2009). Several studies have highlighted the vulnerability of *Acacia* seedlings to herbivory and fire (Meyer et al. 2005; Midgley, Lawes & Chamaille-Jammes 2010). Despite their significance, there is comparatively little literature that focuses on effects of herbivory and fire on *Acacia* seedlings (Bond & Midgley 2001). Any biotic or abiotic factors that may have significant effects on the recruitment, survival and growth patterns of *Acacia* seedlings needs to be better understood, as they will have a variety of implications on savanna functioning, tree and grass species dynamics, as well as increasing our knowledge of the factors that drive bush encroachment (e.g. see Ward 2005; Wiegand et al. 2005; Wiegand et al. 2006; Meyer et al. 2009; Wigley, Bond & Hoffman 2009).

Herbivory vs. fire

Through our experimentation, we have shown that in contrast to common assumptions, *Acacia* seedlings are indeed highly resilient to severe tissue loss through herbivory and fire (see Hean & Ward 2011), with 100% survival of all seedlings used. Herbivory was shown to induce the greatest response in terms of *Acacia* seedling defence, with nearly all species exhibiting a significant increase in defence traits, both physical and chemical. Fire generally induced a negative response in seedling defences, and a significant reduction was observed in most instances. Fire generally reduced total non-structural carbohydrates (TNC) stores in roots significantly by a smaller amount than herbivory. Balfour & Midgley (2006) indicated that stem death may be caused by xylem malfunctioning and loss of hydraulic conductivity within stems of

seedlings and savanna trees after heat treatment. This could imply that some TNC residues are left behind in the stems after fire that can be re-absorbed and used in regrowth of new stems, while herbivory removed all above-ground biomass, thus leaving only TNC stores in roots to be used, resulting in a greater reduction of TNC. This recycling of carbohydrates and nutrients in undamaged cells and tissues of leaves and stems could be an underlying factor that differentiates the overall effects of burning and cutting treatments on *Acacia* seedlings.

As was the case with *Acacia* seedlings with spines, seedlings with prickles also exhibited induced defences, whereby prickle abundance and prickle length increased after herbivory. Prickles were thought to not be inducible because they are modified epidermal cells, thus making them relatively physiologically cheap to produce (Bazely, Myers & da Silva 1991). Currently, there are no studies that have documented induced defences for *Acacia* species with prickles.

The outcomes of the first experiment (chapter 2) support the assumptions and predictions of the Resource Availability hypothesis (RAH) (Coley, Bryant & Chapin 1985). I note that the RAH (also known as the Growth Rate hypothesis by Stamp (2003)) is similar in this regard to the Growth-Differentiation Balance hypothesis in that both predict the same differences in arid (resource-poor) and humid (resource-rich) environments. Arid-adapted species displayed the greatest investment in defence, as the loss of photosynthetic tissue was costly due to a slow tissue turn-over rate, whereas humid-adapted species invested more in growth than defence, as tissue turn-over was high and the loss of photosynthetic material was relatively cheap (Stamp 2003). These findings also provide support for the argument that simulated herbivory and fire are not substitutable, as first suggested by Hean & Ward (2011). The uncoupling of simulated herbivory and fire could have far-reaching implications for plant ecology. Several studies should be re-examined because they have substituted fire with a simulated herbivory treatment (e.g. Bond & Keeley 2005; Levick et al. 2009; Kgope, Bond & Midgley 2010). These studies should use herbivory and fire treatments separately to determine the effects of each on the species tested. The effects of herbivory (natural or simulated by cutting) and fire on adult trees and seedlings need to be considered as individual effects, rather than as a single associated effect for both. The role of herbivory and fire in African savannas is significant (Midgley & Bond 2001; Bond 2008; Midgley, Lawes & Chammille-Jammes 2010), and there is now evidence to show that these two factors need to be considered separately.

The differences between insect and ungulate herbivory have not been well documented. Insects have been shown to consume a significant amount of biomass (e.g. Scholes & Walker 1993) in African savanna ecosystems. The effects that insect herbivory plays on African savanna trees with bi-pinnate compound leaves, such as *Acacia* species, can be considered to be negligible (Scholes & Walker 1993). Insect herbivory is primarily focused on broad-leaved species (e.g. *Burkea africana* and *Terminalia sericea*) (Scholes & Walker 1993). Bruchid beetles appear to be the greatest form of insect herbivory on *Acacia* species; bruchid beetle larvae feed and often completely destroy the seeds of *Acacia* species (see Mucunguzi 1995; Or & Ward 2003; O'Connor, de Ridder & Hobson 2010; Ward et al. 2010; Rodriguez-Perez, Wiegand & Ward 2011). The selective removal of plants by herbivores can effectively exclude certain species from plant communities (Hanley, Fenner & Edwards 1995), and has the ability to affect plant communities directly through manipulation of succession, competitive interactions and species diversity (Hanley et al. 1995; Vasconcelos & Cherrett 1997; Wilby & Brown 2001). Even though fire has been viewed as a “global herbivore” (Bond & Keeley 2005), it does not exhibit specificity or preference for individual plants or plant species. Herbivores however, often do show preference for certain plant species. Thus, fire cannot be considered to have the same driving force on savanna plant community structure as herbivory. Furthermore, the effects of different fire “types” should also be considered in future investigations. The loss of plant tissue by a fast moving “head-burn” will significantly less than from a slow moving “back-burn” (Gibson 2009)), because the exposure time to fire in a back-burn is significantly longer than in a head-burn (Tainton 1999).

Currently, there is no comprehensive analysis of whether defensive traits are correlated consistently across a broad spectrum of co-existing species, and whether these traits are shared within common functional groups (Hanley & Lamont 2002). Endara & Coley (2011) conducted a meta-analysis on studies that tested the Resource Availability hypothesis (RAH). Overall, they found that the assumptions of the RAH are generally supported. However, the meta-analysis was comprised of studies that were conducted in temperate and tropical forests. To date, there is no meta-analysis testing the assumptions of the RAH that has focused on African savanna species. The current study has shown that seedling defence is a key characteristic that displays varied response to herbivory and fire within seedlings of a single genus. If we are to understand more

fully what governs the evolution of plant life-history traits within seedlings, a deeper insight into the development and expression of seedling defence across generic and functional group boundaries is required (Hanley & Lamont 2002). The second experiment of this thesis (chapter 3) dealt with nutrient availability in accordance with the expanded Growth-Differentiation Balance hypothesis (GDBH). Once again, herbivory was shown to induce a significantly positive effect on plant defences, while fire induced a negative effect on plant defences. Thorn abundance of *Acacia erioloba* and *A. karroo* followed the predicted pattern of plant defence, which increased to a maximum at the modal nutrient value of 800 mg/l, then decreased as nutrient availability increased. The decrease in plant defences above the modal nutrient concentration suggests a switch from differentiation to increased growth.

Our data only partially supported the expanded GDBH, as thorn length and tannin concentration did not follow the trend, and continued to increase as nutrient availability increased. However, in *A. erioloba*, an arid-adapted species, there was increased investment in defences, while *A. nilotica* and *A. karroo* appeared to invest more in growth. These results support the predictions of the GDBH; plants with low maximal growth rates (i.e. *A. erioloba*) mobilized more TNC reserves into defensive traits, while plants with high maximal growth rates (*A. nilotica* and *A. karroo*) mobilized more TNC into increased growth. The resource environment of plants is only one of many variables known or predicted to influence plant recovery from herbivory (e.g., McNaughton 1983, Richards 1993, Trumble, Kolodny-Hirsh & Ting 1993), and hypotheses which rely solely on plant resources to predict these responses are likely to have limited predictive power. High- and low-resource environments will certainly play a role in how plants respond to damage of any sort, but a better understanding of this role will only come from integrating water availability, nutrients, fire and herbivory into the GDBH model (see e.g. Cramer 2011). Once this has been effectively done, we need to go beyond the study of patterns and general mechanisms to attempt to elucidate the fundamental physiological and developmental processes responsible for the observed plant responses (Hawkes & Sullivan 2001).

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