Defences and Responses:
Woody species and Large Herbivores in African Savannas

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Abstract

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Plant-herbivore interactions influence both plant characteristics and feeding patterns of herbivores. In this thesis, I focus on patterns of defence in a wide range of woody species in semi-arid savannas in Botswana, and on responses in woody species to different types of browsing. Methods included both surveys of plant traits relevant to herbivory, and controlled experiments using simulated browsing.

On a regional scale gradient, the proportion of spinescent and evergreen species decreased, while fast-growing species increased, with increasing resource availability. On smaller landscape scale gradients, the proportion of spinescent and evergreen species increased, fast-growing species either increased or decreased, while C-based defences generally decreased and nutrients increased, with increasing resource availability. In some species, levels of defence (phenolics) were higher in young trees, compared to mature trees. However, contrary to our predictions, shoots within, compared to above, browsing height on mature trees were less defended (phenolics and tannins).

Simulated twig-biting during the dry season (on five species) induced compensatory growth responses in annual shoots during the growth season, and cutting at ground level induced sprouting. Leaf removal late in the growth season (on one species) induced foliage re-growth, although total leaf production during the season did not increase. The application of saliva had a small, but significant, effect on shoot growth following clipping. No evidence was found for a connection between patterns in allocation to defensive traits in shoots of different exposure to browsers, or strength of responses to clipping, and other species characteristics, *e.g.* leaf fall patterns, growth rate, presence of spines or natural browsing pressure.

I suggest further studies focusing on both chemical and structural defences, on different spatial scales and resource gradients. I also conclude that additional studies are needed on potential effects of different traits on different types of herbivores, and stress the advantage of studying a wider range of plant species to obtain more general results.

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Appendix

Papers I-V

I base my thesis on the following papers to which I refer to by their roman numerals.

I. Skarpe, C., Rooke, T., Bergström, R., Danell, K., Marokane, W. and Ramotadima, M. Herbivore-related traits of woody plants along resource gradients in southern Africa. (Manuscript).

II. Rooke, T., Danell, K., Bergström, R., Skarpe, C. and Hjältén, J. Defensive traits of savanna trees: the role of shoot exposure to browsers. (Submitted manuscript).

III. Rooke, T., Bergström, R., Skarpe, C. and Danell, K. Morphological responses of woody species to simulated twig-browsing in Botswana. (Accepted for publication in *Journal of Tropical Ecology*).

IV. Rooke, T. and Bergström, R. Growth, chemical responses and herbivory after simulated leaf browsing in *Combretum apiculatum*. (Manuscript).

V. Rooke, T. Growth responses of a woody species to clipping and goat saliva. (Accepted for publication in *African Journal of Ecology*).

Paper III and Paper V are printed in this thesis with kind permission from the publishers of the journals concerned.

Introduction

"That large animals require a luxuriant vegetation, has been a general assumption [...]; but I do not hesitate to say that it is completely false. [....] Dr Andrew Smith informs me that, taking into consideration the whole of the southern part of Africa, there can be no doubt of its being a sterile country. [...] Now if we look to the animals inhabiting these wide plains, we shall find their numbers extraordinarily great, and their bulk immense. [...] I confess it is truly surprising how such a number of animals can find support in a country producing so little food. The larger quadrupeds no doubt roam over wild tracts in search of it; and their food chiefly consists of underwood, which probably contains much nutriment in small bulk. Dr. Smith also informs me that the vegetation has rapid growth; no sooner is a part consumed, than its place is supplied by a fresh stock."
- Darwin's Journal during the Voyage of the Beagle (Darwin, 1889, p. 61-63)

As Darwin remarked, herbivores in this environment surely exert a heavy pressure on the vegetation, which plants have been forced to adapt to. Woody species constitute an important food resource for many large savanna herbivores, especially during the dry seasons and droughts (Pellew, 1983; Bergström, 1992; Topps, 1997). Furthermore, herbivory is one of the principal factors influencing and shaping African savanna ecosystems, alongside water supply, nutrient availability, fire regimes and frequency of night frosts (Werger, 1983; Scholes & Walker, 1993). In evolutionary time, plant-herbivore interactions have influenced both plant characteristics and feeding patterns of herbivores. Structural, chemical and phenological adaptations in plants can influence selectivity and food choice by large herbivores, and some of these plant traits may have developed primarily as responses to herbivory.

Defensive traits

Although spinescent woody species do not entirely escape being browsed, removal of thorns increases herbivory by large browsing mammals (Milewski, Young & Madden, 1991; Bergström *et al.*, In prep.). Thorns may penetrate mouthparts or restrict lateral mouth movements of giraffe (Milewski, Young & Madden, 1991), limit total intake by smaller herbivores by restricting bite size (Cooper & Owen-Smith, 1986; Belovsky *et al.*, 1991; Gowda, 1996; Bergström *et al.*, In prep.), and encourage leaf-picking over the possibly more detrimental twig-biting (Gowda, 1996). Goats also get their hanging ears caught on thorns while feeding (Cooper & Owen-Smith, 1986). In addition, thorns may reduce browsing by small climbing mammals, such as rodents (Cooper & Ginnett, 1998).

Investigations into the role of secondary plant metabolites as repellents to herbivores started with Fraenkel (1959). Chemical defences in plants are now known to deter feeding by herbivores by being bitter tasting, poisonous or by having anti-nutritional effects (Harbourne, 1991). Feeny (1976) introduced a distinction between quantitative defences, with dosage dependent effects (*e.g.* tannins), and qualitative defences, effective even in small concentrations (*e.g.*

glucosinolates). Based on biosynthetic origin the secondary metabolites are divided into three main classes: phenolics, nitrogen-containing compounds and terpenoids (Harbourne, 1991).

Phenolic metabolites can affect food selection, eating and digestion of mammalian herbivores (Harbourne, 1991), and some are powerful fungicidal and bacteriocidal agents (Noggle & Fritz, 1976). Among these, tannins have received considerable attention as potential plant defences. Browsers, which are adapted to tanniferous forage, can produce salivary tannin-binding proteins (Robbins *et al.*, 1987b; McArthur, Hagerman & Robbins, 1991), and it has been argued that tannins may have evolved for purposes other than defence against herbivory (Bernays, Cooper Driver & Bilgener, 1989). As an example, plant phenolics may protect leaves from photodamage by acting as antioxidants (Close & McArthur, 2002). Even so, tannins can still reduce protein availability to ruminants, including browsers, and intake of tanniferous forage may be limited by the animals ability to detoxify and eliminate the phenolic components (Robbins *et al.*, 1987a), wherefore they must still be considered as potential defences. In accordance, negative correlations between feeding preferences of mammalian herbivores and concentrations of condensed tannins in different forage species have been reported (Cooper & Owen-Smith, 1985; Cooper, Owen Smith & Bryant, 1988; Woodward & Coppock, 1995). Nitrogen-containing metabolites have a more restricted distribution in plants compared to phenolics and are usually produced in low quantities, but are mobile within a plant and highly toxic to mammalian herbivores (Harbourne, 1991). Some plant terpenoids are also highly toxic to mammals (Harbourne, 1991).

Apart from avoidance of chemical defences, herbivores also have other requirements on food quality and quantity. In a broad sense, relatively low concentrations of nitrogen or high concentrations of fibre may function as plant defences, as long as herbivores do not compensate the lower nutritional value by increasing intake of the species (Lundberg & Åström, 1990; Augner, 1995). Plant architecture may serve as a type of defence by affecting the accessibility of plant tissues and the amount of food a herbivore can collect in one bite (Owen Smith & Danckwerts, 1997).

General hypotheses concerning plant defence

According to the basic rules of natural selection, the benefit must be greater than the cost if a defensive trait is to be favoured. Apart from defence, other demands on resources (*i.e.* costs) include growth, reproduction, competition, and protection from abiotic stresses (*e.g.* light or temperature extremes). The Growth-Differentiation Balance hypotheses (Herms & Mattson, 1992) addresses opportunity costs of defence and is based on the physiological trade-off between growth (competitive ability) and differentiation processes (including secondary metabolism producing defences against herbivores). It predicts that any environmental factor that slows growth more than photosynthesis will increase the resources available for secondary metabolism. Similarly, the Carbon-Nutrient Balance hypothesis (Bryant, Chapin & Klein, 1983; Bryant *et al.*, 1991b; Lerdau

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& Coley, 2002) predicts that in situations of low nutrient but high light availability, plants will use the excess carbon to produce carbon-based secondary metabolites. Under opposite conditions (*i.e.* low light but high nutrient availability), plants are expected to increase production of nitrogen-based compounds. This hypothesis has, however, been under heavy debate (Hamilton *et al.*, 2001; Koricheva, 2002; Lerdau & Coley, 2002; Nitao, Zangerl & Berenbaum, 2002), and its application now seems to be restricted to secondary compounds produced in the shikimate pathway and present in large quantities, *i.e.* condensed tannins and lignin.

The benefit of defence is often difficult to measure directly as chemical defences, contrary to spines, are difficult to remove to measure reduction in fitness (Hamilton *et al.*, 2001). The benefit does, however, depend on the value of plant tissues (*i.e.* the reduction in fitness if plant parts are removed) and the probability of attack (Hamilton *et al.*, 2001). The value of plant tissues is addressed by the Resource Availability hypothesis (Coley, 1983; Coley, Bryant & Chapin, 1985), which has an evolutionary approach. It predicts that the optimal level of defence investment will increase as the potential growth rate of the plant, and its ability to compensate for herbivory, decreases. The hypothesis has been criticised for not accounting for plant resource competition (which also affects resource availability), and assuming that herbivory is more costly in resource poor environments without balancing this against the cost of defence (Loreau & de Mazancourt, 1999). Nevertheless, although differing in explanatory approaches, addressing only either the cost or benefit of defence, the Growth-Differentiation Balance and Resource Availability hypotheses make similar predictions about plant characteristics in environments with high or low resource availability (Coley, Bryant & Chapin, 1985; Herms & Mattson, 1992). Resource limited habitats are expected to favour plants with low growth rate, evergreen growth form, high levels of secondary metabolites and quantitative defences (sensu Feeny, 1976, e.g. phenols, tannins and fibre). In contrast, resource rich habitats are expected to favour plants with high growth rate, short leaf lifetimes, low levels of secondary metabolites and qualitative defences (sensu Feeny, 1976, e.g. alkaloids).

Although fast-growing plants are assumed to have less need for defences, rapid regrowth may not be enough to prevent mortality or growth retardation due to browsing in the juvenile phase. Bryant, Chapin and Klein (1983) therefore suggested that fast-growing plants may display a high degree of defence in their juvenile form. In agreement, preferences for mature over juvenile plants, or mature shoots higher in the canopy over juvenile basal shoots, in some cases linked to differences in levels of phenolic compounds, have been reported for several species of mammalian herbivores (Danell, Bergström & Dirke, 1983; Sinclair & Smith, 1984; Bryant *et al.*, 1985; Tahvanainen *et al.*, 1985; Danell *et al.*, 1987; Jachmann, 1989).

The Apparency hypothesis, predicts that plants with a high apparency in space (dominating vegetation) or in time (*e.g.* evergreens) should suffer a higher probability of attack and therefore exhibit more defences than less apparent plants (Feeny, 1976). These ideas have been incorporated into the Scarcity-Accessibility hypothesis (Grubb, 1992) which attempts to predict patterns of both structural (*i.e.*

spines) and chemical defences (although not distinguishing between the two). The variables considered are productivity, accessibility and proportion of landscape covered (*i.e.* apparency in space), architecture (*e.g.* scarcity of apical meristems), seasonal behaviour (*i.e.* apparency in time) and nutrient concentration relative to neighbours, and kinds of herbivores present. On a worldwide scale, Grubb (1992) found a horseshoe relationship between defences and resource availability with the maxima at the extremes. As an example, highly spinescent plants are found both in areas of low productivity due to water being scarce, as well as in moist tropical rainforests in the form of fast-growing gap-demanders (Grubb, 1992). Grubb (1992) also emphasized the role of the types of herbivores present during evolutionary time, and argued that as mammals in Southern Africa have high nutritional demands, only the most nutrient rich plants have evolved thorns. Several authors (e.g. Huntley, 1982; Campbell, 1986; Owen-Smith & Cooper, 1987) have reported spinescent species to be more typical of fertile than infertile soils in Southern Africa, which concurs with this idea.

Plant responses to browsing

Several factors determine the response of a plant to herbivory on the ecological time-scale, including plant intrinsic factors, *e.g.* growth rate (Bryant, Chapin & Klein, 1983), and abiotic factors, *e.g.* nutrient and water availability (van der Meijden, Wijn & Verkaar, 1988; Maschinski & Whitham, 1989). In addition, important biotic factors are competition, both between plant individuals (Hjältén, Danell & Ericson, 1993) and shoots on the same individual (du Toit *et al.*, 1990), and obviously intensity, frequency and timing of damage (Cissé, 1980; Canham, McAninch & Wood, 1994; Danell, Bergström & Edenius, 1994). On woody species, large herbivores mainly utilize shoots and leaves, which are removed through twig-biting, leaf-picking or leaf-stripping (Bergström, 1992), and plant responses are generally separated into the plants ability to compensate for tissue loss and the effect the damage has on structural or chemical defences.

Growth responses to twig-biting and leaf removal

Studies in temperate zones have shown that while most woody species are fairly tolerant to winter browsing, the extent of growth responses the following season varies between species (e.g. Krefting, Stenlund & Seemel, 1966; Wolff, 1978; Campa, Haufler & Beyer, 1992; Bilbrough & Richards, 1993; Canham, McAninch & Wood, 1994). Birches (*Betula* spp.) browsed during winter in boreal forests may increase shoot size, branching and leaf size on individual shoots during the following summer (Danell, Bergström & Edenius, 1994; Danell, Haukioja & Huss-Danell, 1997), although overall shoot biomass production may decrease (Danell, Bergström & Edenius, 1994). Lehtilä *et al.* (2000) reported that in terms of leaf biomass, apical parts compensate for bud loss by increasing shoot number, while basal parts, due to resource allocation constraints, are only able to increase leaf size. Similar growth responses are expected following dry season browsing in African savannas, although fewer such studies have been undertaken here. Late dry season clipping of *Combretum apiculatum* produced larger but fewer annual

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shoots and increased individual shoot biomass and leaf area during the following growth season (Bergström, Skarpe & Danell, 2000). Compensatory growth responses have also been reported for *Acacia nigrescens* and *A. tortilis*, in which lightly and heavily browsed trees did not differ in net annual shoot extension (du Toit *et al.*, 1990), and in *Acacia erubescens* following simulated twig-biting during the rainy season (Dangerfield & Modukanele, 1996). Increased growth following shoot browsing seems to be a reoccurring pattern in studies on African savanna trees, and growth in height may be especially important in this system with frequent fires (Dangerfield & Modukanele, 1996; Bond, 1997).

A severe loss of leaf tissue in woody plants during the growing season may induce a fast response through refoliation. Nevertheless, refoliated leaves are usually smaller than mature, initially formed leaves (Faeth, 1992), and high defoliation intensities, early in the growth season or during previous growth seasons, may cause a reduction in final leaf biomass (Benjamin *et al.*, 1995; Bergström & Danell, 1995; Reichenbacker, Schultz & Hart, 1996). Several studies have reported a reduction in growth of shoots and height of plants after defoliation (Heichel & Turner, 1984; Marquis, 1992; Hjältén, Danell & Ericson, 1993; Bergström & Danell, 1995; Reichenbacker, Schultz & Hart, 1996), while others recorded increased branching and total shoot length (Torres, Gutierrez & Fuentes, 1980; Alados *et al.*, 1996).

Growth responses in plants following browsing must be put into the plant physiological context. Physiological ageing (senescence) is caused by internal nutrient stress, when shoot growth becomes limited by the supply of water and nutrients from the roots, and can be reversed by intensive pruning of shoots that reduces the number of growing points (Fontanier & Jonkers, 1976). In other words, a modified root:shoot ratio may lessen the competition for nutrients between shoots (McNaughton, 1983; Danell & Bergström, 1989; du Toit *et al.*, 1990). Loss of leaves obviously implies loss of photosynthetic tissue, as well as loss of nutrients and carbon stored in the leaves, and may potentially also affect the hormonal control system (Tuomi *et al.*, 1984; Haukioja & Honkanen, 1997). As an example, removal of terminal meristems can break the apical dominance and suppression of lateral meristems, altering the growth form of a plant (Aarssen, 1995). These physiological alterations can influence both the plants competitive ability, and future browsing.

Observations of increased growth of foliage on naturally grazed plants, compared to mechanically clipped plants (Reardon, Leinweber & Merill, 1974), has also lead to speculations that growth-promoting chemicals in ungulate saliva might mediate an increased regrowth of plants (McNaughton, 1985). Some studies have reported such growth responses in graminoids after application of bovine saliva (Reardon, Leinweber & Merrill, 1972) or thiamine which is found in saliva (Reardon, Leinweber & Merrill, 1972; Reardon, Leinweber & Merill, 1974; McNaughton, 1985). However, other studies have failed to show any effects of saliva (Johnston & Bailey, 1972; Reardon, Leinweber & Merill, 1974; Detling *et al.*, 1980). Until recently, the focus of these studies has been on grazers and graminoids. Grazers and browsers differ in their anatomical and physiological adaptations (Hofmann, 1989), and there are several morphological and

physiological differences between herbaceous and woody plants. Recently, Bergman (2002) showed that saliva from a browser, the moose (*Alces alces* L.), increased lateral branching in saplings of a woody species (*Salix caprea* L.) following simulated moose browsing.

Responses in defensive traits

Longer spines have been reported for trees in areas with, compared to those protected from, herbivores (Rohner & Ward, 1997; Young & Okello, 1998; Young, Stanton & Christian, 2003), and trees subjected to simulated browsing have responded with increased spine length (Young, Stanton & Christian, 2003) or biomass of spines (Gowda, 1997). Studies on *Acacia* species have also shown that juvenile trees, assumed more vulnerable to browsing, have longer and more closely spaced thorns, compared to mature trees (Brooks & Owen Smith, 1994; Rohner & Ward, 1997), and that thorns are longer on shoots within, compared to above, the reach of browsers (Young, 1987; Milewski, Young & Madden, 1991; Young & Okello, 1998). These observations support the idea that at least spine length may be an inducible defence.

The chemical composition of leaf tissues is also likely to be altered by removal of tissues. Newly refoliated leaves are younger, which in itself may imply a lower fibre content and higher digestibility compared to mature leaves. In addition, alterations in the plant's carbon/nutrient balance can induce differences in concentrations of nutrients (*e.g.* nitrogen) and secondary metabolites (*e.g.* condensed tannins) (Tuomi *et al.*, 1984; Fowler & Lawton, 1985; Haukioja & Honkanen, 1997), which in turn may affect future herbivory (Faeth, 1992; Alados *et al.*, 1996). However, chemical responses may differ between species of, for instance, different growth rates (Bryant *et al.*, 1991a; Stock, Le Roux & Van der Heyden, 1993).

Questions addressed

Many general theories explaining patterns in occurrence of plant defences, and other life-history strategies related to herbivory, originate from studies in boreal and northern temperate forests. Herbivory is, and has during a long evolutionary timescale been, an influential factor in African savannas. Nevertheless, fewer studies of plant defences and responses to herbivory have been undertaken here, and it is therefore interesting to test these general theories in this system. In addition, many species of large herbivores in African savannas, as well as livestock, browse to considerable extents on woody species. Even so, there are still gaps in our knowledge concerning specific interactions between browsers and woody species. More detailed studies of effects of browsing on woody species on the ecological timescale are therefore also relevant. In this thesis, I focus on patterns of defence against browsing in a wide range of woody species in semi-arid savannas, and on responses in woody species to different types of browsing.

More specifically, the follow questions were addressed:

1. How do the relative frequencies of herbivore-related traits of savanna trees, including thorns, chemical defences, leaf tardiness (evergreen vs. deciduous) and growth rates, change along a productivity gradient on a regional vs. landscape scale? (Paper I)

2. Are shoots on small, young tree individuals more defended than shoots on taller, mature individuals, and are shoots within browsing height more defended than shoots above browsing height on mature trees? (Paper II)

3. Is growth and morphology of annual shoots affected by dry season browsing, and if so, do responses differ between species and between shoots at different locations on the tree? (Paper III)

4. What are the short-term responses of a slow-growing species to simulated leaf browsing, in terms of shoot and height growth, leaf production and chemical composition of leaves? (Paper IV)

5. Can herbivore saliva affect growth responses of a woody species to browsing? (Paper V)

Study area

Climate and vegetation

All studies were undertaken in Botswana, Southern Africa. The region has a dry winter season, with warm days and cold nights including night frost, stretching from May to August, while September until the rainy season starts is dry and hot. The rainy season occurs during summer, usually from October to March, although rainfall is highly variable both within and between years, leading to longer dry seasons and even droughts. Paper I incorporated the rainfall gradient which stretches from *ca.* 200 mm per year in the SSW of Botswana, to *ca.* 650 mm per year in the NNE, over a distance of 1100 km (Fig. 1). Most of Botswana is covered by the savanna biome, defined as a tropical vegetation type co-dominated by woody plants and grasses (Scholes, 1997). The arid shrublands of the most southern part of the Kalahari, and the salt pans of the Madkgadikgadi depression (Fig. 1), are included as part of the Nama-karoo biome, which is separated from the savanna biome by aridity (Rutherford, 1997).

Based on aridity and soil fertility in the savanna biome, a general distinction is also made between broad-leaved (moist/infertile) and fine-leaved (arid/fertile) savannas (Huntley, 1982; Scholes & Walker, 1993). Most of Botswana consists of the fine-leaved savanna type on Kalahari sands, with the broad-leaved savanna type only stretching down into the northern most parts of the county (Fig. 1) (Huntley, 1982; Scholes, 1997). Fine-leaved woody species of arid savannas (*e.g.* Acacias and other genera of the Mimosideae subfamily) are typically deciduous, spinescent species (Huntley, 1982), of high palatability to browsers (Owen-Smith & Cooper, 1987). In contrast, broad-leaved species of the moist savanna (*e.g.*

genera of the subfamily Caesalpinioideae), are often tardily deciduous and not spinescent (Huntley, 1982), but less palatable due to high leaf concentrations of secondary metabolites, particularly tannins (Cooper & Owen-Smith, 1985; Owen-Smith & Cooper, 1987). Due to the seasonal periods of drought in the savannas, most trees are deciduous, with evergreens accounting for less than 5% of the leaf biomass (Scholes, 1997). Consumption by browsers amounts to 4.5 - 4.6% of leaf and shoot production (estimated as kg ha^{-1} yr^{-1}) in nutrient-rich and intermediate savannas in Southern Africa with no or low densities of elephants, and up to 7.0% in nutrient-poor savannas with large elephant populations (Owen Smith & Danckwerts 1997).

Study sites

Field work entailing natural browsing by large herbivores was conducted in Chobe National Park (17° 45´ - 19° 25´S, 23° 50´ - 25° 10´E) in northern Botswana (Papers I and II), and in Mokolodi Nature Reserve, 15 km south west of Gaborone (24° 45´S, 25° 48´E), in south eastern Botswana (Papers I, II and IV) (Fig. 1). The Chobe site was situated in the northernmost part of Chobe National Park. The site stretched *ca.* 50 km east-west along the Chobe River and *ca.* 10 km south of the river. Mean annual rainfall is 650 mm and the area is predominately of broad-leaved savanna type, although different vegetation zones can be distinguished running parallell to the river (Skarpe pers. com.). The river is flanked by treeless floodplains or steep slopes, with a narrow, fragmented strip of riverine forest forming a boarder towards *Capparis* shrubland on raised alluvial flats. On Kalahari sands, and transition zones between alluvial soils and sand, *Combretum* shrublands take over, followed by mixed woodlands further up on the sand ridge, and *Baikea* woodlands highest up and furthest away from the river. A large part of the shrublands used to be woodlands in the mid-1900's. These woodlands were probably established at low densities of large herbivores due to rinderpest and ivory hunting, before the recovery of elephant and ungulate populations. The Chobe area has been a national park since 1968 and today holds high densities of large herbivores, resulting in high browsing pressure, especially during the dry season when antelopes, buffalo and elephants concentrate near the river. Fires used to be frequent, but are now controlled and greatly reduced.

The Mokolodi area was a 3000 ha fenced nature reserve (it has since been extended) in which several species of native large herbivores, including four elephants and eight species of browsing ungulates, have been reintroduced. It was traditionally grazed by local livestock, and later served as a cattle ranch, entailing heavy grazing, until 1986. The game fence was erected in 1985, electrified in 1992 and the nature reserve officially opened in 1994. Mean annual rainfall is 540 mm and the area is predominately of fine-leaved savanna type, on shallow to very shallow soils, from sand to clay loams. The vegetation types vary with the topography, with *Combretum* shrublands on hilltops and upper slopes, *Acacia* shrublands on lower slopes, and *Spirostachys* woodland in bottom land along drainage lines (Bråten, 1997). Density of large herbivores, and thus browsing pressure, is intermediate and animal mobility is restricted by fencing. Fires have been prevented since the reserve was established in 1994.

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Controlled studies of responses to simulated browsing were conducted on fenced experimental fields at Veld Products Research (VPR), Gabane, 20 km west of Gaborone (24º 39´S, 25º 47´E, altitude 1230m) (Papers III and V) and Botala Farm (BF), Notwane, 20 km south of Gaborone (24º 47´S, 25º 50´E, altitude 1020m) (Paper III).
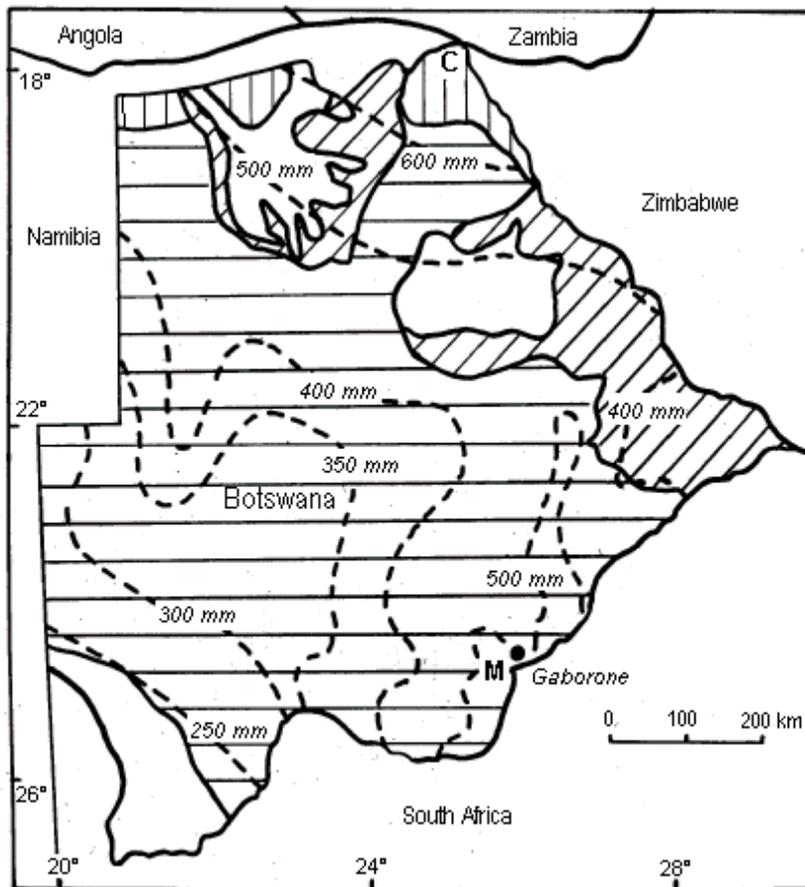
Fig. 1. *Map of Botswana showing rainfall isohyets (mm) from Pike (1971), and the location of the two main study sites; Chobe (C) and Mokolodi (M). Areas filled with parallell lines mark the distribution of general savanna types; fine-leaved (arid/fertile) savanna (horizontal lines), broad-leaved (moist/infertile) savanna (vertical lines) and Mopane woodland (diagonal lines), from Scholes (1997). Blank areas within Botswana on the map are the Okavango Delta (N Botswana), the Madkgadikgadi Depression (salt pans, NE Botswana) and arid shrublands in the Kalahari (S Botswana).*

Methods

Methods used included both surveys of plant traits relevant to browsers (including chemical analyses of leaf material) over a range of woody species (Papers I and II), and controlled experiments to study plant responses to simulated browsing (Papers III, IV and V).

Surveys of plant traits

The relative frequency of woody species with different plant traits related to herbivory (including thorns, chemical defences, evergreen growth forms and growth rates) along productivity gradients were studied at two different scales (Paper I). On the regional scale, with the rainfall gradient across all of Botswana as a productivity gradient, we used information on traits of 357 tree and shrub species reported in Botswana in the literature, together with data from vegetation plots at three different rainfall levels (Christina Skarpe, unpublished data). On the landscape scale, we used data from vegetation plots in different vegetation types, following a gradient in soil water and nutrient availability, in Chobe (Christina Skarpe, unpublished data) and in Mokolodi (Bråten, 1997; Skarpe *et al.*, 2000). In addition, we recorded spinescence and performed chemical analyses on leaf material from 51 species in Chobe and 30 in Mokolodi. We also had data on contents of P, Mg, Na, K and Ca in twigs and leaves from 14 species in Chobe (Wilson Marokane & Christina Skarpe, unpublished data).

Defensive traits in savanna trees, in relation to tree size and shoot exposure to browsing, were studied, at the peak of the rain season, on nine species in Chobe and five species in Mokolodi (Paper II). We chose species that are common in the area and grow to at least 6 m in height, that differed in leaf fall patterns, growth rates, use by browsers, and presence of structural defences (spinescent vs. non-spinescent species) (Table 1). We sampled individual trees in sets of one mature individual (>6 m high) and the nearest young (<3 m high) individual, selecting ten sets of each species at the site. From these sets of trees we randomly sampled three types of shoots, which were cut 20 cm from the shoot apex: 1) shoots within browsing height (0-2 m above ground) on young trees, 2) shoots within browsing height (0-2 m above ground) on mature trees, and 3) shoots above browsing height (over 5 m $\pm$ 1 dm above ground) on mature trees. The number and length of thorns on shoots were recorded on spinescent species. Leaves from collected shoots were saved for chemical analyses.

Simulated browsing

Many large herbivores rely on woody species especially during the dry season, and as most species are deciduous, twig-biting is the most common mode of feeding during this time. Morphological responses to simulated dry season browsing (twig-biting) were studied on five woody species planted at VPR and BF

(Paper III). All species were common in the area, and selected to represent species with different growth rates, use by browsers, and presence of structural defences (spinescent vs. non-spinescent species) (Table 1). Treatments consisted of clipping 25, 50, 75 and 100% of the shoots on tree individuals, at a diameter of ≤ 4 mm (except one species cut at ≤ 6 mm), and cutting trees at ground level. Control trees were left untouched. At the end of the following growth season, data were collected on leaf length, shoot length, base diameter, number of branches, number of pairs of thorns and dry weight of three annual shoots per tree, at different height levels (bottom, middle and top third of the tree).

During the growth (wet) season, browsers may feed by leaf-biting or leaf stripping. This mode of feeding is more difficult to monitor than twig-biting, and has thus been less studied. Responses to simulated leaf browsing were studied on *Combretum apiculatum* (Table 1) at Mokolodi Nature Reserve (Paper IV). This species is common in the area and known to be browsed, at least during the dry season, although it has been less studied than, *e.g.*, Acacia species. Treatments, performed at the final stages of shoot growth, consisted of removing leaves (including shoot apices) from every second leaf node (50% defoliation) or all leaf nodes (100% defoliation) on the trees. Control trees were left untouched. Additional trees were individually fenced in, to prevent browsing by ungulates, and either subjected to 100% defoliation or used as fenced controls. Both prior to treatment and after the growth season (just before leaf fall), data were collected on tree height and length of top shoot and, on five marked annual shoots per tree, total shoot length, number of lateral shoots produced, and number of leaves present and missing (*i.e.* scars). Furthermore, we counted the number of leaves per tree after the growth season, and recorded initial leaf area (in a leaf-area meter), leaf area missing due to insect herbivory (measured with mm-paper), and leaf dry weight. For comparison, we also recorded insect herbivory on leaves picked at time of treatment. Leaf samples were saved for chemical analyses.

Controlled studies of simulated browsing always risk being criticized for not simulated natural browsing accurately enough, neglecting factors such as the patchy distribution of natural damage or the potential effects of saliva. Effects of saliva on a woody species was studied during on *Combretum apiculatum* (Table 1) planted at VPR (Paper V). Three trial groups were used: 1) control, 2) clipping without saliva and 3) clipping with saliva. As any effects of saliva are more likely when shoot are actively growing, in contrast to when they are dormant, the trial was performed when the rainy season had started and shoot growth was intense. Clipping treatments, on three shoots per tree, consisted of pulling off the shoot top with pliers, simulating the damage caused by browsing goats. Saliva was collected on site, from female tswana goats (*Capra hircus* L.), in a silicone tube which the goat chewed on, and immediately applied to the clipped shoot. Prior to treatments and at the end of the growth season, length, diameter, number of leaves and number of nodes were recorded, on trial shoots and on new lateral shoots initiated on trial shoots after the time of treatment. Dry weight of shoots and tree height were also recorded.

Table 1. General characteristics of woody species included in Papers II, III, IV and V. Scientific names and structural defence (i.e. spines, thorns or prickles) according to (Coates Palgrave, 2000). Leaf fall pattern (deciduous or evergreen) and growth rate (slow or fast) compiled from Miller (1952a,b), Palmer & Pitman (1972 & 1973), Timberlake (1980), Grignon & Johnsen (1986), Noad & Birnie (1992), Roodt (1992), van Wyk (1992), van Wyk & van Wyk (1997) and Coates Palgrave (2000). Palatability to mammalian herbivores from [1]Coates Palgrave (2000), [2]van Wyk (1992), [3]Palmer & Pitman (1972 & 1973), [4]Walker (1980) and [3]Berry (undated).

Species (Family/subfamily)	Leaf fall	Structural defence*	Growth rate	Palatability to mammalian herbivores	Paper
Acacia nigrescens (Mimosoideae)	dec	prickles	slow	Eaten but not selected[4]	II
Acacia tortilis (Mimosoideae)	dec	spines	slow	Palatable[1,2,4,5]	II, III
Baikiaea plurijuga (Caesalpinioideae)	dec	no	?	No info.	II
Boscia albitrunca (Capparaceae)	ever	no	slow	Palatable[1,4,5]	II
Capparis tomentosa (Capparaceae)	ever	spines	?	Unpalatable to very palatable[1,4]	II
Colophospermum mopane (Caesalpinioideae)	dec	no	slow	Eaten but not selected to palatable[1,2,4,5]	III
Combretum apiculatum (Combretaceae)	dec	no	slow	Eaten but not selected to palatable[4]	II, IV, V
Croton gratissimus (Euphorbiaceae)	dec	no	?	Unpalatable to palatable[1,5]	II
Croton megalobotrys (Euphorbiaceae)	dec	no	fast	No info.	II
Erythrophleum africanum (Caesalpinioideae)	?	no	?	Eaten but not selected[1,4]	II
Grewia flava (Tiliaceae)	dec	no	?	Unpalatable to very palatable[1,2,4]	III
Markhamia acuminata (Bignoniaceae)	dec	no	slow	No info.	II
Pappea capensis (Sapindaceae)	dec	no	slow	Palatable[1,4]	II
Peltophorum africanum (Caesalpinioideae)	dec	no	fast	Unpalatable[2,8]	II, III
Spirostachys africana (Euphorbiaceae)	dec	no	slow	Palatable[5]	II
Ziziphus mucronata (Rhamnaceae)	dec	spines	fast	Unpalatable to palatable[1,2,4,5]	III

* A. nigrescens: pairs of small hooked prickles below the nodes; A. tortilis: pairs of sharply hooked or long and straight stipular spines; C. tomentosa: pairs of sharply hooked stipular spines; Z. mucronata: pairs of one short straight and one short curved stipular spine (Coates Palgrave, 2000).

Chemical analyses

Acid-detergent fibre (ADF) content in leaf material was determined using detergent analysis (Goering & Van Soest, 1970; Mould & Robbins, 1981). Total nitrogen (N) content was analysed in an elemental analyser (Perkin Elmer 2400 CHN) (Papers I, II and IV). Both ADF and N concentrations were expressed as percentage of dry weight of leaf sample. Total phenolics were assayed by the Folin-Ciocalteu method (Waterman & Mole, 1994), with tannic acid as a standard (Papers I, II and IV). Concentrations of condensed tannins were determined with the Proanthocyanidin (butanol/HCl) assay (Waterman & Mole, 1994; Dalzell & Kerven, 1998), with quebracho tannin as a standard (Paper IV). The potential biological tannin activity in the samples was quantified through protein precipitation, using the radial diffusion assay (Hagerman, 1987), with tannic acid as a standard (Papers I and II). Levels of total phenolics, condensed tannins and tannin activity were expressed as mg tannic acid, or quebracho tannin, per g dry weight of the leaf sample.

Results and Discussion

Defensive traits along productivity gradients

Following the predictions of the Growth-Differentiation Balance (Herms & Mattson, 1992) and Resource Availability (Coley, Bryant & Chapin, 1985) hypotheses (Paper I), we expected to find an increasing frequency of fast-growing species, a decreasing frequency of evergreen species, a decrease in levels of C-based defences, and an increase in levels of nutrients in species, with increasing resource availability (Table 2). The Scarcity-Accessibility hypothesis (Grubb, 1992) predicts a horseshoe relationship between defences and resource availability with the maxima at the extremes. These defences may be structural (*i.e.* spines) or chemical. However, we anticipated that the horse-shoe relationship could, at least in part, be explained by differences in spatial scales. On a large spatial scale, we expected defences to be negatively correlated to resource availability (as predicted by Resource Availability hypothesis), but on a smaller scale, scarce gaps or edges of nutritious growth will be sought out by selective herbivores, causing a stronger selection for defences in these plants. This fine scale variation, with increased defence in resource-rich patches, could be found anywhere within the resource gradient on a larger scale, but would be concealed by patterns on the larger scale. Consequently, we predicted that, with increasing resource availability, the frequency of spinescent plants would decrease on the regional scale, but increase on the smaller landscape scale (Table 2).

Our study (Paper I) showed that, with increasing resource availability on a regional scale (the rainfall gradient across Botswana), the frequency of fast-growing species increased, while the frequency of spinescent species and of evergreen species decreased, as expected (Table 2; Paper I: Fig. 1 & 2). With increasing resource availability on the smaller landscape scale in Chobe, the frequency of spinescent species and fast-growing species increased (Table 2;

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Paper I: Fig. 3, Table 4). However, in Mokolodi the frequency of fast-growing species decreased with increasing resource availability, while there was no difference in the frequency of spinescent species (Table 2; Paper I: Fig. 4, Table 5). In addition, the frequency of evergreen species increased along the gradients at both sites (Table 2; Paper I: Fig. 3 & 4, Table 4 & 5). As predicted, mean contents of C-based defences (phenolics, tannin activity and ADF) in woody species generally decreased with increasing resource availability in Chobe (Table 2, Paper I: Fig. 5, Table 4). In Mokolodi the pattern was less clear, with no difference in phenolics, an increase in tannin activity and a decrease in ADF with increasing resource availability (Table 2, Paper I: Fig. 6, Table 5). The mean contents of N increased with increasing resource availability in Mokolodi but not in Chobe, although other nutrients (P, Mg, K, Ca and Na) increased along the gradient in Chobe (Table 2, Paper I: Fig. 7 & 8, Table 4 & 5). Most evergreens are unpalatable, and generally associated with resource poor environments and quantitative defences, although exceptions occur which are heavily browsed (Scholes, 1997). The increase in evergreen species, and decrease in C-based defences, with increasing resource availability on the landscape scale, may indicate that these species have qualitative (*e.g.* N-based) instead of quantitative defences.

Rainfall can only be used as a general resource gradient up to a certain limit (roughly 650 mm per year), beyond which soil leaching decreases nutrient availability (Huntley, 1982). The northernmost part of Botswana lies in this transition zone (Fig. 1), and our two study sites (Chobe and Mokolodi) can thus be used to compare plant traits in broad-leaved (moist/infertile) and fine-leaved (arid/fertile) savannas (Fig. 1). Woody species typical for fine-leaved savannas have been characterized as deciduous, spinescent (Huntley, 1982; Scholes, 1997), and of high palatability to browsers (Owen-Smith & Cooper, 1987). Species typical for broad-leaved savannas have been reported as less deciduous, non-spinescent (Huntley, 1982; Scholes, 1997), and containing high leaf concentrations of C-based defences (Cooper & Owen-Smith, 1985; Owen-Smith & Cooper, 1987). However, we found higher levels of C-based defences (tannin activity, ADF and a trend in phenolics), and lower levels of N, in species in Mokolodi (fine-leaved savanna), compared to in Chobe (broad-leaved savanna), and no significant differences in the proportion of spinescent or evergreen species (Table 3; Paper I: Table 3).

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Table 2. *Differences in plant traits with increasing resource gradients at different scales in Botswana, measured as relative frequency (% spinescent, fast-growing and evergreen), or mean (levels of total phenolics, tannin activity, fibre, C/N, N, P, Mg, K, Ca and Na) of tree or shrub species. Predicted increase (+) or decrease (−) of traits with increasing productivity, based on the Scarcity-Accessibility hypothesis (Grubb, 1992) (spinescence), or the Growth Differentiation Balance (Herms and Mattson, 1992) and Resource Availability hypotheses (Coley, Bryant & Chapin, 1985) (all other traits). Observed patterns shown for a regional scale (only three traits), covering the rainfall gradient across Botswana (357 species), versus a landscape scale, covering shorter productivity gradients at two study sites; four vegetation types in Chobe (51 species, except P, Mg, K, Ca & Na which is based on 14 species) and three vegetation types in Mokolodi (30 species). Lack of significant differences (p > 0.05) are shown as zero (0). Data on the regional scale did not allow for statistical tests and where only evaluated graphically*

Plant traits	Regional		Landscape		
	Predicted	Observed	Predicted	Observed	
		Botswana		Chobe	Mokolodi
Spinescent	−	−	+	+	0
Fast-growing	+	+	+	+	−
Evergreen	−	−	−	+	+
Total phenolics			−	−	0
Tannin activity			−	−	+
Fibre (ADF)			−	−	−
C/N			−	−	−
N			+	0	+
P,Mg,K,Ca,Na			+	+	no data

Table 3. *Differences in plant traits between fine-leaved and broad-leaved savannas, measured as higher (+) or lower (−) frequency of spinescent and evergreen woody species, and mean levels of total phenolics, tannin activity, ADF, C/N and N in woody species. Predicted differences based on Huntley (1982), Owen-Smith & Cooper (1987), Scholes & Walker (1993) and Scholes (1997). Observed differences from a comparison of woody species in two sites in Botswana, representing a fine-leaved (Mokolodi, 30 species) and a broad-leaved savanna (Chobe, 51 species). Close to significant trends (p < 0.10) are shown in parenthesis, and lack of significant differences as zero (0).*

Plant traits	Fine-leaved compared to broad-leaved savannas	
	Predicted	Observed
Spinescent	+	0
Evergreen	−	0
Total phenolics	−	(+)
Tannin activity	−	+
ADF	−	+
C/N	−	+
N	+	−

Defensive traits in trees and shoots with different exposure to browsers

We only found weak support for our hypothesis that shoots on small, young trees are more defended than shoots at the same height on high, mature individuals (Paper II: Table 2, Fig. 1). Consistent with our predictions, we found an overall higher concentration of total phenolics in shoots on young compared to mature trees. However, at the species level, this pattern was only significant, or marginally significant, in three of the 13 species studied. Moreover, young trees did not generally have higher tannin activity, ADF concentration or more and longer spines than mature trees. In contrast, four species had higher concentrations of N in shoots on young trees, indicating potentially higher palatability. We found no evidence indicating that shoots within browsing height are more defended than shoots above the reach of browsers, on mature trees (Paper II: Table 2, Fig. 2). Instead, shoots within browsing height were actually less chemically defended (total phenolics and tannin activity) than shoots above browsing height in six of 13 species. Two species had less ADF in lower shoots, and no differences were found in N content or spinescence for any species.

Apart from browsing pressure, other variables, such as growth conditions and life-history traits of plants, are assumed to influence investment in defensive traits, and have been incorporated into general theories of plant defence (e.g. the carbon-nutrient balance hypothesis: Bryant, Chapin & Klein, 1983; the resource availability hypothesis: Coley, Bryant & Chapin, 1985; the growth-differentiation balance hypothesis: Herms & Mattson, 1992). Our study covered 13 species, differing in final height, growth rate, presence of thorns, and natural browsing pressure (Table 1; Paper II: Table 3). Nevertheless, we were unable to use any of these characteristics to distinguish the species which showed significant differences in chemical composition (phenolics, tannin activity or N) between different shoot types in this study.

The browsing pressure by large herbivores, amounting to 10-14% of shoots browsed in the more heavily browsed species in Chobe (Christina Skarpe, unpublished data) and Mokolodi (Bråten, 1997; Skarpe et al., 2000), should be strong enough to instigate an adaptation in spatial allocation of defences. Such patterns have also previously been found in Acacia species, where longer thorns are produced within, compared to above, the reach of browsers (Young, 1987; Milewski, Young & Madden, 1991; Young & Okello, 1998). Alternatively, the restricted browsing height of ungulates could be counterbalanced by herbivory on higher shoots by other herbivores. However, we found this unlikely as arboreal mammals (*e.g.* primates and rodents) probably rely more on fruits, seeds and other food sources (Apps, 2000), and damage by insects (amounting to 5-10% leaf area removal on the most attacked species) was greater on lower, compared to higher, shoots in our study (Tuulikki Rooke, unpublished data).

An alternative explanation for our results is that other factors, such as plant physiology, may constrain plant allocation to certain defensive traits. Several studies have, for example, shown that concentrations of phenolic compounds in plants increase under high light conditions (Larsson *et al.*, 1986; Mole, Ross &

Waterman, 1988; Lavola, 1998; Lavola *et al.*, 2000; Tegelberg & Julkunen-Tiitto, 2001). Exposure to sunlight may therefore explain increased levels of such compounds in shoots above browsing height, compared to lower down, and on low-growing trees, compared to shoots at the same height on larger, self-shading trees. However, other factors must also be involved as only certain species showed this pattern.

Plant responses to browsing

Simulated dry season twig-biting

Simulated dry-season twig-biting was performed on woody species differing in growth rate, occurrence of spines and use by mammalian herbivores (Table 1). Nevertheless, all five species revealed a general pattern of compensatory growth responses during the following growth season (Paper III: Fig. 1). According to the Resource Availability hypothesis, fast-growing species, adapted to high-resource environments, should be able to respond to herbivory by rapid regrowth, while slow-growing species are forced to invest in defence (Coley, 1983; Coley, Bryant & Chapin, 1985). No such patterns were, however, obvious in this study, where the strongest growth responses appeared in one slow- and two fast-growing species. The differences in strength of responses among species in this study could not either be related to natural browsing pressure.

Growth responses, in annual shoots, consisted of increased shoot length, shoot diameter, number of lateral shoots and, consequently, increased shoot biomass with increased clipping intensity (Paper III: Table 2, 3 & 4, Fig. 1). These responses may be a result of the reduction of competing growing points (McNaughton, 1983; Danell & Bergström, 1989; du Toit *et al.*, 1990) and disrupted apical dominance (Aarssen, 1995), and could be an effect of competition for light (Hjältén, Danell & Ericson, 1993; Aarssen, 1995; Järemo, Nilsson & Tuomi, 1996). However, they may still be adaptive responses to physical damage, including herivory. Growth responses were stronger in shoots with higher location in the tree (Paper III: Table 2, 3 & 4, Fig. 2), which may reflect the pressure on trees to attain a certain height to avoid browsing, reach reproductive size and increase chances of surviving a fire (Dangerfield & Modukanele, 1996; Bond, 1997).

Acacia tortilis, the only species that did not increase individual shoot biomass with increased clipping intensity, showed the strongest response in basal shoots to the stump treatment (Paper III: Fig. 1). It is possible that this species also responded to severe clipping by producing basal, instead of axillary, shoots. In savanna systems with frequent fires, the ability to sprout from the base of the stem, if the canopy is burnt, is important for juvenile survival in many species (Bond, 1997; Bond & Midgley, 2001). Trees over 2 m in height are, however, more resistant to fire (Scholes, 1997), and sprouting ability may decline or even be lost completely when trees grow taller (Bond, 1997; Bond & Midgley, 2001). Sprouting after damage also requires stored reserves, the cost of which is traded off against normal growth or reproduction (Bellingham & Sparrow, 2000; Bond & Midgley, 2001). In other words, sprouting is expected to be more common in

younger trees of short height, and in trees with slow growth or maturation rates. Interestingly, all five species studied showed a strong response to the stump treatment by sprouting (Paper III: Fig. 1), irrespectively of growth rate or stage of maturation.

In both spinescent species studied, there was a trend towards a decrease in the number of spines per unit shoot length with increasing clipping intensity (Paper III: Table 5, Fig. 3). This may be an effect of an increase in internode length in annual shoots, resulting from the compensatory growth responses following clipping. Based on the same idea, but with opposite responses in terms of growth, other researchers have previously reported an increase in spine density on browsed shoots, as an effect of reduced growth rates and internode length (Milewski, Young & Madden, 1991; Dangerfield, Perkins & Kaunda, 1996).

Simulated growth season leaf browsing

Removal of leaves and shoot apices on *Combretum apiculatum*, entailing a loss of resources and breaking apical dominance (Aarssen, 1995), reduced growth in tree height and increased shoot mortality (Paper IV; Table 3). Surviving shoots on defoliated trees increased production of lateral shoots, and there was no difference in total length of annual shoots between defoliated and control trees at the end of the growth season (Paper IV; Table 3). Similar responses in growth have previously been reported for other woody species (e.g. Torres, Gutierrez & Fuentes, 1980; Bergström & Danell, 1995; Alados *et al.*, 1996; Reichenbacker, Schultz & Hart, 1996), although reductions in shoot growth may still occur in subsequent growth seasons (Hjältén, Danell & Ericson, 1993), or after repeated defoliations (Heichel & Turner, 1984). Significant refoliation only occurred after 100% defoliation and, as previously shown in other species (Benjamin *et al.*, 1995; Bergström & Danell, 1995; Reichenbacker, Schultz & Hart, 1996), the smaller size of refoliated leaves resulted in lower final leaf biomass on defoliated compared to control trees (Paper IV; Table 3, Fig. 1). Total leaf biomass production (including experimentally removed leaves) was, however, equal for all treatment groups (Paper IV; Fig. 1), indicating an exact compensation (sensu Belsky, 1986).

Growth rate and timing of damage are known to affect chemical responses in plants. Bryant *et al.* (1991a) found increased levels of N and decreased levels of total phenolics and condensed tannins in slow-growing species one year after severe defoliation, but the opposite responses in fast-growing species. Danell, Bergström and Edenius (1994) found increased levels of N in birches (*Betula pendula*) one year after late summer browsing, but decreased levels of N one year after early summer browsing or repeated late summer leaf stripping. In accordance, we found that refoliated leaves on the slow-growing *C. apiculatum,* subjected to late season leaf removal, contained more N, and tended to have less ADF, total phenolics and condensed tannins (Paper IV; Fig. 2). Despite the fact that refoliated leaves were potentially more palatable to herbivores, we found no evidence of increased levels of ungulate or insect herbivory on defoliated trees (Paper IV; Fig. 3 & 4). We may have had too small sample sizes to detect ungulate herbivory, although defoliated trees may also have produced insufficient biomass

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to attract ungulates. The smaller proportion of insect damaged refoliated leaves compared to mature leaves is probably a reflection of the differences in temporal availability of leaves.

The effects of saliva

Application of goat saliva to clipped shoots of *Combretum apiculatum* significantly enhanced total shoot growth (tripled in length) and leaf production (2.7 times more leaves), compared to shoots clipped without saliva (Paper V; Fig. 1). Nevertheless, this increase in growth could not compensate the decrease in total shoot length, production of leaves, diameter, and overall shoot biomass production that resulted from clipping alone (Paper V; Fig. 1).

Although relatively small, the growth responses induced by saliva in this study are significant and could be important at higher browsing pressures. Further support is provided by Bergman (2002), who reported increased lateral branching in *Salix caprea* saplings in response to application of moose saliva during simulated browsing. Previous studies have suggested that growth responses in plants may be mediated by animal hormones (Geuns, 1978; Dyer, 1980), thiamine (Bonner & Greene, 1939; Reardon, Leinweber & Merrill, 1972; Reardon, Leinweber & Merill, 1974; McNaughton, 1985) or salts (Detling *et al.*, 1981) found in the saliva. However, a critical question is whether or not sufficient amounts of these compounds can be transferred via saliva to have an affect on plants. A more simple explanation could be that saliva may act as a protective coating, against desiccation or even microbial infections, on damaged shoots. This could be especially important in arid climates.

Conclusions

On a regional scale, a rainfall and productivity gradient across Botswana, our data on changes in frequency of spinescent, evergreen vs. fast-growing species support general predictions made by the Scarcity-Accessibility (SA) (Grubb, 1992), Resource Availability (RA) (Coley, Bryant & Chapin, 1985) and Growth-Differentiation Balance (GDB) (Herms & Mattson, 1992) hypotheses (Paper I). On the shorter landscape scale, changes in plant traits along the gradient were more consistent with the above hypotheses in Chobe than in Mokolodi (Paper I). Spinescence decreased with increasing resource availability on the regional scale, but increased on the shorter landscape scale in Chobe. This pattern agrees with our interpretation of the predictions by the SA hypothesis (Grubb, 1992) on different spatial scales. Along resource gradients on the landscape scale we furthermore found support for the RA and GDB hypotheses in data on frequency of fast-growing species, C-based defences and nutrients (but not N) in Chobe, and N in Mokolodi. However, C-based defences and frequency of fast-growing species in Mokolodi did not follow the predictions. In addition, the increasing frequency of evergreen species with increasing resource availability on the landscape scale, the opposite pattern to that found on the regional scale, disagrees with the hypotheses.

This emphasizes the importance of defining the spatial scales considered. We also found higher levels of C-based defences in the fine leaved, compared to the broad-leaved savanna (Paper I), which is the opposite to generalisations that have previously been suggested for plant traits in the two contrasting savanna types. An explanation to this could be the historical difference in browsing pressure between the two sites.

We found some evidence of increased levels of defence (phenolics) in young trees, compared to mature trees, but contrary to our predictions, shoots above, compared to within, browsing height on mature trees had more phenolics and tannins (Paper II). This pattern may be explained by exposure of shoots to sunlight, and supports the proposal that these substances have not evolved as a direct response to herbivory, although they may still function as such to a certain degree. Future studies, on the spatial effects of browsing pressure on individual trees, should include other defensive compounds, such as N-based metabolites. Alternatively, cafeteria tests with herbivores could be employed, although such studies cannot identify which plant properties actually function as deterrents.

Although previous studies on *Acacias* have reported evidence suggesting that spines may be an inducible defence, we found little difference in spinescence between shoots on young vs. mature individuals, or within vs. above browsing height in three studied spinescent species (Paper II). In addition, clipping resulted in increased shoot length and a lower density of spines on annual shoots the following growth season (Paper III). Density of spines (modified stipules) and thorns (modified branches arising from the axils of leaves) are constrained by shoot morphology, which may imply a trade-off between growth and defence. However, this does not apply to prickles, which are small sharp outgrowths from the cortex and epidermis (Raven, Evert & Eichhorn, 1992), and not necessarily connected to nodes.

The species used in studies of simulated browsing (Paper III & IV) showed a high tolerance to twig-biting during the dry season (five species) or severe late growth season defoliation (one species). Twig-biting (Paper III) induced compensatory growth responses in annual shoots during the following growth season, especially in shoots located higher up in the tree. All five species also responded to cutting at ground level by sprouting, indicating a high tolerance to fire. Defoliation induced foliage re-growth in the single species studied (Paper IV), although total leaf production during the season did not increase, implying a lower leaf biomass in defoliated trees, compared to controls, at the end of the season. Overall, the studied species seem tolerant to herbivory in the short-term, but resource losses, and the potentially higher palatability to herbivores shown after defoliation (Paper IV), may still produce negative effects during later growing seasons. The application of saliva (Paper V) had a small, but significant, effect on shoot growth following clipping, which could provide a slight competitive advantage at higher browsing pressures. Further studies are needed to determine the cause of this response, whether mediated by compounds in the saliva or simply by providing a protective coating.

We found no evidence for a connection between patterns in allocation to defensive traits between shoot types (on young vs. mature trees, or within vs.

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above browsing height) and other species characteristics, *e.g.* leaf fall patterns, growth rate, presence of spines or natural browsing pressure (Paper II). Likewise, strength of responses to clipping (growth of axillary shoots) and stumping (growth of basal shoots) was independent of growth rate, stage of maturation or natural browsing pressure (Paper III). This stresses the importance of looking at several species, as general rules of plant defence based on studies of single species may not hold when comparing several species.

In response to my dismay over lack of expected results, a wise professor once said "*Our objective is to describe nature, not prove theories*". Most general theories on plant defences against browsing have been developed in northern temperate and boreal forests, from studies on single species or single plant-herbivore combinations. While the largest herbivores that once roamed these regions have gone extinct, several mega-herbivores, as well as other large mammalian herbivores, still exist on the African savannas and pressure from herbivory is assumed to be high. As Darwin (1889, p. 63) remarked: *"With regard to the number of large quadrupeds, there certainly exists no quarter of the globe which will bear comparison with Southern Africa."* In addition, the savanna ecosystems are considered older than the northern temperate and boreal systems, due to smaller temperature fluctuations during the last ice ages. This may have lead to a belief that patterns in plant defence found in boreal forests should be even more pronounced in African savannas. Nevertheless, this may not be entirely true. Boreal systems are limited, above all, in nitrogen (Tamm, 1991), which has been named a key factor in determining plant defences. In savanna systems, however, nitrogen is not as limiting, and models built on C/N-balance of plants may be less relevant, and other factors, such as fire and water availability, may be more important.

Future perspectives

Hypothesis predicting the occurrence of plant defences will only attain generality when they can apply to different spatial scales and the variety of resources limiting plant growth (*e.g.* light, nutrients or water). At the least, these models should be clearly defined with respect to their limitations. Chemical defences must also be weighed against structural defences of different ontogenetical origin (i.e. spines, thorns and prickles). Herbivory is never the sole factor exerting pressure on a plant. Growth responses following browsing may be an adaptive response to a combination of factors, including herbivory, competition for resources, tolerance to fire *etc*. Likewise, the production of certain chemical compounds, such as tannins, may have a number of advantages, including deterrence of herbivores and protection from abiotic stresses or microbial attacks. One does not necessarily exclude the other. To evaluate the significance of what we believe are defensive traits, I propose studies including actual cafeteria tests on plants, preferably considering potential effects of different traits on different types of herbivores. Such studies could also give an insight into interactions between different types of herbivores, mediated by food resources. Finally, I stress the importance of studying a wider range of plant species, and plant-herbivore combinations, to instigate the development of novel research angles.

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