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# Effects of simulated browsing on growth and leaf chemical properties in *Colophospermum mopane* saplings

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## Abstract

Browsing intensity influences a plant's response to herbivory. Plants face a trade-off between investment in the production of secondary compounds and investment in growth. To elucidate this trade-off, we simulated four browsing intensities (0%, 50%, 75% and 100%) on mopane saplings, *Colophospermum mopane* (J.Kirk ex Benth.) J.Léonard, in a greenhouse experiment. This showed that, with increasing defoliation intensity, plants change their investment strategy. At intermediate levels of defoliation (50%), mopane saplings increased the synthesis of condensed tannins, so that tannin concentrations followed a hump-shaped relation with defoliation intensity, with significantly higher tannin concentration at intermediate defoliation levels. When defoliated heavily (75% and 100%), tannin concentrations dropped, and plants were carbon stressed as indicated by a reduced growth rate of the stem diameter, and leaf production and mean individual leaf mass were reduced. This suggests that, at intermediate defoliation intensity, the strategy of the plants is towards induced chemical defences. With increasing defoliation, the relative costs of the secondary metabolite synthesis become too high, and therefore, the plants change their growing strategy. Hence, browsers should be able to benefit from earlier browsing by either adopting a low or a relatively high browsing pressure.

*Key words:* condensed tannins, induced defence, leaf mass, leaf production, trade-off

## Résumé

La réponse d'une plante à sa consommation dépend de l'intensité de ce phénomène. Les plantes sont confrontées à un compromis entre un investissement dans la production de composants secondaires et un investissement dans leur croissance. Pour élucider ce compromis, nous avons simulé quatre intensités de consommation (0%, 50%, 75% et 100%) sur des jeunes mopanes, *Colophospermum mopane* (J.Kirk ex Benth.) J.Léonard, lors d'expériences sous serre. Ceci a montré que, lorsque la défoliation s'intensifie, les plantes changent leur stratégie d'investissement. À des niveaux de défoliation intermédiaires (50%), les jeunes plants de mopanes augmentaient la synthèse de tanins condensés, de sorte que les concentrations en tanins suivaient une courbe en cloche (*hump-shaped*) selon l'intensité de la défoliation; elles étaient significativement plus élevées aux niveaux de défoliation intermédiaires. Lorsque les plantes sont fortement défoliées (75% et 100%), leurs concentrations en tanins chutent, et elles sont en stress carbone comme le montre le taux de croissance réduit du diamètre du tronc; la production de feuilles et la masse moyenne de feuilles par individu sont aussi réduites. Cela suggère que, quand l'intensité de défoliation est intermédiaire, la stratégie des plants va vers une défense chimique induite. Lorsque la défoliation augmente, le coût relatif de la synthèse du métabolite secondaire devient trop élevé et le plant change de stratégie de croissance. Donc,

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les herbivores qui les consomment devraient pouvoir bénéficier d'une consommation antérieure en adoptant une pression de consommation faible ou relativement élevée.

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## Introduction

Plants may respond to herbivory by changing tissue chemistry, growth rate or morphology (Belsky, 1986; Bryant *et al.*, 1992). These responses are considered to depend upon a number of factors, such as the browsing intensity, the soil nutrient status, and the moment of defoliation (Ouellet, Boutin & Heard, 1994; Smallie & O'Connor, 2000; Guillet & Bergstrom, 2006). Most dicotyledonous plants face a trade-off when defoliated (Rohner & Ward, 1997; Boege, 2005) as they can invest in rapid re-growth of relatively cheap photosynthetic tissue to compensate for the lost leaf biomass, or alternatively, they invest in more expensive leaves that contain a higher concentration of secondary metabolites to deter herbivores. The production of carbon-based secondary metabolites, such as condensed tannins, is relatively costly, because carbon used in the production of these metabolites is no longer available for growth and reproduction. This trade-off can be described by a cost benefit function, weighing the costs and benefits for the different strategies to cope with herbivory (Karban, Agrawal & Mangel, 1997; Stamp, 2003; Boege, 2005).

In the Southern African savannah, mopane woodland, *Colophospermum mopane* (J.Kirk ex Benth.) J.Léonard, is an important dry season refuge for many browsers, as mopane trees maintain leaves until late in the dry season (Dekker & Smit, 1996; Styles & Skinner, 1997; Ben-Shahar & MacDonald, 2002). Hence, high browsing pressures are reported in mopane woodlands. Mopane trees are known to produce high concentrations of secondary compounds, especially tannins, for defence against herbivory. However, there is limited knowledge at what browsing intensity mopane trees resort to increase the production of secondary compounds.

Browsing intensity plays an important role in determining whether plants will respond by vigorous regrowth of poorly defended tissue, or by producing slower growing, chemically defended leaves. At intermediate browsing intensities, some species have been found to invest in tannin-rich leaves, whilst the concentration of tannins in newly produced leaves dropped following browsing at higher intensity (Rohner & Ward, 1997; Peters & Const-

abel, 2002; Riipi *et al.*, 2005). Other studies reported no effect of defoliation on tannin concentration in leaves (Ferwerda *et al.*, 2005; Scogings, 2005) or reported increased leaf production with increasing browsing intensity (Guy, 1981; Ouellet *et al.*, 1994; Boege, 2005; Riipi *et al.*, 2005; Makhabu & Skarpe, 2006). For example, Makhabu & Skarpe (2006) found that lightly browsed mopane trees had fewer leaves than severely browsed trees. Similar trends were observed in experiments of simulated browsing of arctic vegetation, commonly browsed by caribou (Ouellet *et al.*, 1994).

The ability of plants to re-grow following browsing strongly depends on the amount of stored carbohydrates (Poorter & Kitajima, 2007). Repeated defoliation events are likely to deplete carbohydrate pools. Under such conditions, we can expect that plants preferentially invest in cheap leaves to rapidly regain a positive carbon balance, at the cost of an increased risk of leaf loss to browsing. The exact relationship between plant investment and defoliation is not clearly understood. In the present study, we therefore aimed to quantify the trade-off between investment in secondary metabolites or in growth of new plant tissue, under varying defoliation regimes. Because mopane is known to effectively deter herbivores with its high leaf tannin concentrations (Styles & Skinner, 1997), we focus our investigation of secondary compounds on tannins. We test the hypothesis that with an increase in browsing pressure the concentration of condensed tannins will increase up to a certain maximum level, but that it will ultimately decrease as browsing pressure is further increased, resulting in a hump-shaped relationship between browsing intensity and tannin concentration. Because of the high costs of producing carbon-based secondary compounds there will be a point at which the plant can no longer afford to invest in secondary compounds, because the plants have insufficient reserves to make the investment, and rapidly need to replace their photosynthetic tissue instead. Our second hypothesis is that leaf production, in terms of number of leaves, will increase with increasing browsing pressure as a 'risk spreading' strategy for plants under high browsing pressure.

## Methods

### *Plant material and growth conditions*

Mopane, *C. mopane* (Fabaceae, Caesalpinioideae) seeds, obtained from the Skukuza nursery, Kruger National Park,

South Africa, were germinated for a week at 20°C after peeling and soaking them for 24 h in distilled water. The germinated seeds were planted in plastic pots (diameter 15 cm, height 20 cm) filled with one part black soil and seven parts coarse river sand. The plants were grown for 2 years under ambient light and a 12 h : 12 h, 30°C : 25°C day–night regime in a greenhouse at Wageningen University, the Netherlands (51°9′N, 5°7′E), plants were maintained moist throughout and were watered twice per week; no additional nutrients were supplied, and no symptoms of mineral deficiencies were observed. At the beginning of the experiment ( $t = 0$  on March 1, 2005), the plants were on average 37 ( $\pm 10$ ) cm high (mean  $\pm$  SD;  $n = 160$ ), and had  $24 \pm 8$  green leaves per plant ( $n = 160$ ). The 160 saplings were randomly assigned to one of four treatments: control (0% defoliation), 50%, 75% and 100% defoliation. All plants were randomly repositioned within and between the four greenhouse benches on a biweekly basis.

#### Sampling protocol

Leaves were handpicked seven times from 1 March to 11 November 2005, on average once every 43 days. Only leaves were sampled, and stems and twigs were not removed, similar to the effect of most browsers, like impala (*Aepyceros melampus* Lichtenstein) or kudu (*Tragelaphus strepsiceros* Pallas). To obtain sufficient leaf material for chemical analyses of the leaves after the final harvest and to sufficiently impact the seedlings on repeated defoliations, some preexisting leaves were harvested in addition to re-growth leaves. After each defoliation, two plants per treatment were excluded from further defoliations and left to recover. As a result, the number of repeats per treatment in the final analyses after seven sequential defoliations was reduced to 28. Data from the recovering plants are not presented in the present paper. Leaves were pooled per plant, oven dried at 70°C for 48 h, and weighed. In the control treatment the saplings were only defoliated at the end of the experiment and a subset of ten randomly chosen plants were used for chemical analyses.

#### Growth and leaf production

At the start and the end of the experiment the stem diameter was measured at 1 cm above the soil surface using digital callipers, and total plant height along the stems was measured. With these data relative diameter

growth rate (RGR<sub>d</sub>) and relative height growth rate (RGR<sub>h</sub>) were calculated following Beadle (1993) as:

$$\text{RGR}_d = \frac{\text{LN}(\text{diameter}_{\text{final}}) - \text{LN}(\text{diameter}_{\text{initial}})}{t_{\text{final}} - t_{\text{initial}}}$$
 and

$$\text{RGR}_h = \frac{\text{LN}(\text{height}_{\text{final}}) - \text{LN}(\text{height}_{\text{initial}})}{t_{\text{final}} - t_{\text{initial}}}$$

respectively, where diameter and height were measured in mm and time in weeks.

The number of leaves per plant was recorded before and after each defoliation, and the total leaf dry weight per plant was determined for the plants that were sampled. These data were used to calculate the average leaf weight for the different treatments.

#### Chemical analysis

Condensed tannins were extracted from dried and ground leaves following Hagerman (2002), using one pooled sample of the final harvest per plant. Relative concentrations of condensed tannins were determined using the improved acid butanol assay (Porter, Hrstich & Chan, 1986) standardized by purified Quebracho tannin (Asquith & Butler, 1985; Waterman & Mole, 1994). The reactivity of tannins to the acid butanol assay are species specific, hence, the obtained concentration is a relative measure of mopane tannin concentration. The results are therefore reported in terms of Quebracho tannin weight equivalents (Hagerman & Butler, 1989; Waterman & Mole, 1994).

#### Data analysis

Kolmogorov–Smirnov tests were used to test for the normality of residuals for all analyses. Where required, data were transformed to get a normal distribution (e.g. natural logarithm were applied to leaf weight). An ANOVA tests were used to test for differences between treatment means (number of leaves, relative growth rate, and mean leaf weight) with defoliation intensity and days since first defoliation as independent factors, followed by Tukey multiple comparison post hoc tests. For data that did not follow a normal distribution, a nonparametric Kruskal–Wallis test was applied to test for differences between treatments, followed by a conservative Scheffé’s multiple comparison tests to account for the nonnormal data distribution. All statistical analyses were carried out in SPSS 12.01 (SPSS, Inc., Chicago, IL, U.S.A.).

## Results

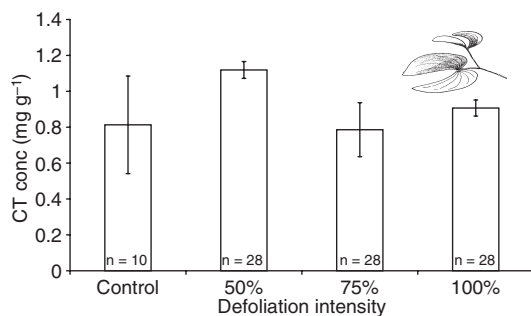
### The effect of defoliation intensity on chemical defence

The defoliation intensity significantly influenced the condensed tannin concentration of the mopane leaves (Fig. 1; Kruskal–Wallis,  $\chi^2 = 23.66$ ,  $df = 3$ ,  $P < 0.001$ ), with intermediate level of defoliation (50%) yielding the regrowth with the highest concentration of condensed tannins (Scheffé's test,  $P < 0.05$ ). The tannin concentration of the 75% and 100% defoliation were not significantly different from the control treatment (Fig. 1).

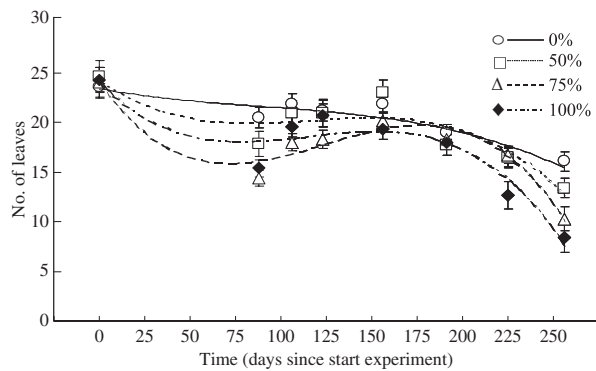
### The effect of defoliation intensity on leaf production and growth

The mean numbers of leaves per sapling just before each defoliation (i.e., total regrowth for 100% treatment, or regrowth plus old leaves for lower intensity treatments) did not differ significantly between treatments (Fig. 2). The mean number of leaves per sapling before each defoliation decreased over time for all treatments ( $F_{1,134} = 29.14$ ,  $P < 0.001$ ). The mean number of leaves per sapling remained highest for the control plants almost continuously. In early summer, the plants from the 100% defoliation treatment produced a larger numbers of leaves than those of the 50% treatment, but at the end of the experiment, the 100% defoliation plants had the smallest numbers of leaves of all treatments (Fig. 2).

Table 1 shows the  $RGR_d$  and  $RGR_h$  for the different treatments. Due to large plant-to-plant variation in growth rates, no significant differences were found in  $RGR_h$ , while the  $RGR_d$  in the higher defoliation treatments (75% and 100%) were significantly lower than those of control



**Fig 1** Mean ( $\pm 95\%$  confidence intervals) condensed tannin concentration (CT,  $mg\ g^{-1}$ ) of mopane sapling leaves at different defoliation intensities. Bars indicated with a different letter are significantly different (Scheffé's test,  $P < 0.05$ )



**Fig 2** The mean number of mopane leaves per sapling  $\pm 1$  SE, as counted prior to the successive defoliations, for control plants (0%) and the plants of the 50%, 75%, and 100% defoliation treatments, where  $t = 0$  stands for March 1st 2005. Third order polynomial trend lines were fit through the data to visualize the changes over time ( $R^2 > 0.8$  for all treatments)

plants (One-way ANOVA;  $P < 0.05$  in Tukey post hoc tests).

### Mean dry weight per leaf

There was a significant effect of treatment on leaf mean dry weight ( $F_{2,134} = 4.41$ ,  $P < 0.05$ ). The mean leaf weight in the 50% defoliation treatment was higher than at 100% defoliation (Tukey test,  $P < 0.05$ ). There was a decrease in leaf dry weight over time for all treatments ( $F_{1,134} = 113.6$ ,  $R^2_{adj} = 0.46$ ,  $P < 0.001$ ; Fig. 3), due to the increased number of immature leaves in the samples.

## Discussion

Simulated browsing of mopane saplings resulted in altered leaf tannin concentrations; a significant increase in con-

**Table 1** Mean values ( $\pm 1$  SE) of relative height growth rate ( $RGR_h$ ) and relative diameter growth rate ( $RGR_d$ ) are given for plants in three different defoliation treatments and control plants. Values followed by different letters are significantly different from one another (one-way ANOVA, with Tukey *post hoc* tests).

Treatment	$RGR_h$ ( $mm\ mm^{-1}\ week^{-2}$ )	$RGR_d$ ( $mm\ mm^{-1}\ week^{-2}$ )
Control	$0.0013^a \pm 0.0004$	$0.0019^a \pm 0.0002$
50% defoliation	$0.0002^a \pm 0.0004$	$0.0011^{ab} \pm 0.0003$
75% defoliation	$0.0010^a \pm 0.0006$	$0.00004^b \pm 0.0005$
100% defoliation	$0.0010^a \pm 0.0004$	$0.0005^b \pm 0.0002$

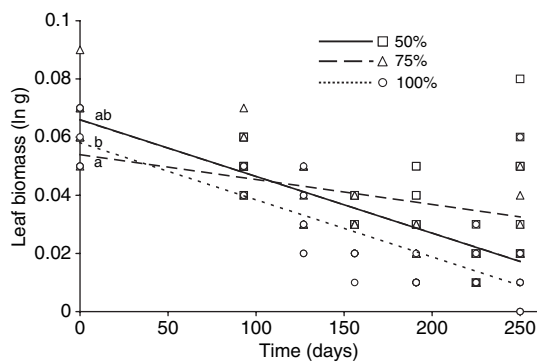


Fig 3 Mean mopane leaf dry weight at 50%, 75% and 100% defoliation over time with fitted linear regression lines showing the decrease in leaf weight over time per treatment. Letters are used to discriminate significant different treatments, i.e., a difference in rate of decrease over time (Tukey test  $P < 0.05$ )

densified tannin concentration was found at intermediate defoliation intensity, while greater defoliation intensities (repeated defoliation of 75% and 100%) neither increased nor decreased the levels of tannins in the leaves (Fig. 1). Other studies with defoliation intensities  $< 50\%$  also reported an increase of foliar condensed tannin concentration following physical damage (*Populus tremuloides* Michx: Peters & Constabel, 2002; *Betula pubescens* subsp. *czerepanovii* (N.I. Orlova) Hämet-Ahti: Riipi *et al.*, 2005). Previous studies using very intense defoliation treatments with Mopane saplings – 100% (Ferwerda *et al.*, 2005) and *Acacia karroo* Hayne – 87% (Scogings, 2005) also reported no significant changes in leaf tannins, similar to our results when the 100% defoliation treatment is compared with the control. Our results corroborate with these studies, and support our hypothesis that beyond a certain threshold level of defoliation/physical damage, plants do not further increase the investment in anti-herbivore defence. However, the subtlety of the observed responses indicates that a wide range of defoliation intensities should be included to elucidate the exact relationship between defoliation intensity and secondary metabolite concentrations.

#### Allocation to defence is mediated by resources and storage

The increased synthesis of condensed tannins after repeated defoliation is dependent on the plant's carbohydrate reserves and on factors such as soil nutrient status and water availability (Coley, 1986; Bryant *et al.*, 1992; Richburg, 2005). We have stated that production of tannins is expensive for plants, but it is important to realise

that when plant growth is limited by factors other than light availability, such as water, the excess photosynthates thus produced may make tannins relatively inexpensive. When nutrients appear nonlimiting, as in the current experiment, plants face a trade-off whether to use carbohydrate reserves for rapid growth or synthesis of secondary compounds (Coley, 1986; Bryant *et al.*, 1992; Boege, 2005). We conclude that the defoliation intensity influences the allocation of carbon to these different ways of coping with or preventing further browsing. The significant reduction in  $RGR_d$  suggests that repeated severe defoliation caused carbon stress, and when experiencing such carbon stress the mopane plants no longer invested in increased levels of condensed tannins (Fig. 1). This is because the synthesis of tannins is relatively costly, and nutrients and energy used will not be available to compensate for lost photosynthetic tissue (Coley, 1986). When sufficient leaves are available for photosynthetic carbohydrate production, i.e. at low carbon stress (50% defoliation or less, when stem diameter growth was not reduced compared to control plants), mopane saplings invested relatively more in the synthesis of tannins than in compensation for leaf loss.

Defoliation causes carbon stress and also reduces nutrient competition by growth points, i.e., apical dominance (Rooke & Bergström, 2007) triggering the growth of new buds and leaves (Bryant *et al.*, 1992). The emergence of many buds and new leaves increases the carbohydrate demand and hence limits the production of carbon-based secondary metabolites (Danell, Huss-Danell & Bergström, 1985). Such a response was not found in the present study (Fig. 2). We simulated browsing by picking leaves, not twigs, and as a result defoliation did not result in the loss of the hormonally controlled apical dominance, and we did not find an increase in auxiliary shoots (data not shown).

This study suggests that Mopane saplings do not continue to invest in tannin production at repeated defoliation intensities over 50% (Fig. 1). Hence, heavy browsing can improve the palatability of the available forage for browsing herbivores, and indeed various studies reported a decrease of tannin concentration under severe browsing (Coley, 1986; Du Toit, Bryant & Frisby, 1990; Boege, 2005; Hikosaka *et al.*, 2005) and an increase in leaf nitrogen content (Du Toit *et al.*, 1990). Together with the positive effects of browsing on browse quality and availability, this might explain the mechanisms behind browser re-visitation of previous browsed trees or patches, so-called browsing lawns; dense vegetation of trees and shrubs that are

continuously browsed by browsers (Fornara & Du Toit, 2007). This plant–herbivore interactions is characterized by positive feedback mechanisms that are triggered and maintained by browsers, so that these trees and shrubs continuously invest in growth of poorly defended leaves (Fornara & Du Toit, 2007). The defoliation by handpicking of leaves, as carried out in our study, is assumed to mimic browsing of large herbivores. However, it is unclear to what extent the impact of elephant and other browsers differ in triggering the responses in mopane. Elephants also take leaves, twigs and branches, which are almost not taken by browsers such as impala or kudu. It would be useful to investigate whether there is a difference in the responses of mopane under these different defoliation strategies.

Defoliation intensity is an important factor that is able to influence the plant's investments in either chemical defences or leaf tissue production. Evidence presented in this paper shows that condensed tannin concentration followed a hump-shaped relationship with an increasing intensity at which plants were repeatedly defoliated. In contrast to our expectations, leaf production did not increase with increasing defoliation intensities. Hence, browsing intensity might trigger the plant's trade-off between investing in chemical defences and leaf production *per se*, the carbon stress caused by repeated defoliation may be too severe to actually compensate, let alone over-compensate leaf production. Browsing herbivores are able to influence palatability and browsable biomass. We expect that the best strategy for herbivores foraging on mopane is either a very low or a very high browsing pressure. The investment in plant secondary compounds depends on the soil nutrient status, and is larger on nutrient poor soils (Du Toit *et al.*, 1990; Bryant *et al.*, 1992; Guillet & Bergstrom, 2006). Hence, we hypothesise that it is less likely to find browsing lawns under nutrient rich conditions, especially of herbivore species that are sensitive to high secondary compound concentrations. This is in contrast to grazing lawns that are expected to form especially under conditions with a high soil nutrient availability (Ruess & McNaughton, 1984; Cromsigt, 2006).

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