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## Induced chemical defences in *Colophospermum mopane* trees

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A field experiment was conducted in which Mopane (*Colophospermum mopane*) trees were subjected to three different canopy treatments: felling of trees at a mean height of 0.7m above ground level, felling at a height of 2m, or pruning of selected branches. These treatments were intended to simulate wood harvesting by local communities, pollarding by elephants, and browsing by herbivores, respectively. The foliar concentrations of total phenol, condensed tannin and protein-precipitating tannin were tracked for three consecutive years after treatments were effected. Coppicing, pollarding and pruning induced chemical defences in Mopane tree foliage. The order of magnitude of the response was positively related with impact severity: coppicing > pollarding > pruning > control. Differences between treatments remained the same for the duration of the study. The effects of canopy treatments on phenol and tannin concentrations were small compared to the seasonal and inter-seasonal fluctuations. The protein precipitation capacity of the secondary metabolites increased significantly with increasing impact. We concluded that secondary metabolite concentrations in Mopane foliage are dynamic and can be explained satisfactorily by season, year and treatment severity, with a high explanatory power ( $r^2 = 0.94\text{--}0.98$ ). Findings of this study have consequences for current harvesting, animal production and conservation practices.

**Keywords:** browsers, herbivory, phenol, protein precipitation, tannins

### Introduction

Plant secondary metabolites, those compounds that are not directly involved in plant primary metabolism, can have a significant negative effect on herbivory and consequently on animal distribution and productivity. Phenolic substances in plant tissues have the potential to reduce herbivory by lowering the nutritional value (Mattson 1980, Lundberg and Åström 1990) and through toxicity and by inhibiting digestion (Bryant *et al.* 1992, Coley 1988, Cooper and Owen-Smith 1985). Nevertheless, the construction of secondary metabolites is costly and plants face a trade-off; an investment either in secondary metabolites or in growth and reproduction. Switching photosynthetic resources towards growth and reproduction during periods of low herbivory, and muster 'defences' when browsed, might offer a strategy to optimise long-term fitness in unstable environments, such as those found in some African savannas (Stock *et al.* 1993). However, du Toit *et al.* (1990) found contrasting results, which confounds generalisations and calls for further investigation. Furthermore, information on the duration of heightened defences after induction as well as seasonal and inter-annual fluctuations in secondary metabolite concentration in an African context is scant.

In the southern African savanna, Mopane (*Colophospermum mopane*) ranges over an area of 571 500km<sup>2</sup> and commonly occurs in hot, low-lying areas (Coates Palgrave 1977, Walker 1980). Mopane tolerates clayey, poorly drained and alkaline soils and often dominates the woody biomass within its range (Ben-Shahar and Macdonald 2002, Timberlake 1995). In large parts of Mopane's distribution range the rainfall is low and erratic, rendering crop production uneconomical. Consequently, pastoralism, game ranching and wildlife conservation are the prominent forms of land use in these areas (Liengme 1981, 1983, Mashabane *et al.* 2001). Both wildlife and livestock make use of Mopane as a forage resource, which can be crucial for bridging the late dry season period when other deciduous species are leafless (Caughley 1976, Styles and Skinner 1997, Duffy *et al.* 1999, Smallie and O'Connor 2000, Smit 2001, Ben-Shahar and Macdonald 2002). Previous studies indicated that Mopane foliage has relatively high concentrations of condensed tannins, although these tend to diminish in older leaves, rendering it more acceptable to browsers during the dry season (Styles and Skinner 1997). We argue that at relatively high background levels, small induced increases of secondary metabolites can potentially have a large deterrent effect on herbivores that

apparently have to regulate the absolute intake of specific secondary metabolites. Induced defences in Mopane can have far-reaching consequences for browsers, especially if the effect is enduring. Van Hoven (1991), for instance, suggested that induced defences in browse species of fenced-in game ranches caused large-scale kudu mortalities during a regional drought. In addition, in communal areas, where foraging resources are often scarce, Mopane is widely harvested for fuel and construction purposes by local communities (Liengme 1981, 1983, Mashabane *et al.* 2001). Following wood harvesting, Mopane rapidly resprouts (coppice) resulting in increased browse availability at lower height strata (Smallie and O'Connor 2000). However, browse quality might decrease if wood harvesting induces chemical defences in regrowth. Similarly, in protected areas the pressure on woody resources intensifies as elephant populations increase in numbers and their range is restricted. Mopane, which occurs in many wildlife areas, forms one of the important forage species of elephants and other browsing ungulates (references in Styles and Skinner 2000). Forage quality changes due to elephant impact will consequently affect its subsequent use by a number of species.

In this paper we report on the findings of a field experiment in which we treated Mopane trees as follows: (1) low stem cut that simulates wood harvesting (coppicing); (2) stem cut at 2m height that simulates severe elephant browsing (pollarding); and (3) pruning that simulates browsing by smaller herbivores. Dependent variables, which were followed for a three-year period, consisted of foliar total phenol content, condensed tannin and protein-precipitating tannin concentration. The latter was analysed as an indicator of the protein-binding capacity of the polyphenols. Zucker (1983) suggested that small structural changes in tannin molecules could drastically alter its protein precipitation capacity and subsequently its effect on herbivores. The objectives were to determine (1) if coppicing, pollarding and pruning induce increased foliar tannin levels relative to control trees, (2) for how long after treatments an induced effect is detectable, (3) if seasonal and year-to-year differences occur in secondary metabolite levels and (4) if the ratios between phenol concentrations and its protein precipitating capacity were affected by treatments.

### Study area

The study was conducted on the Messina Experimental Farm (MEF), situated 20km west of Musina in the Limpopo Province of South Africa (22°16'51"S, 29°51'54"E). According to the floristic classification of Acocks (1988) the MEF falls within the Mopani veld. The experimental site (1ha) is located in the *Kirkia acuminata* — *Enneapogon cenchroides* short closed woodland, as described by Dekker (1996). The woody layer is dominated by *C. mopane*, at an estimated density of 1 344 trees ha<sup>-1</sup>, in association with scattered *Boscia albitrunca*, *Combretum* spp., *Grewia* spp., and *Terminalia prunioides*, with a herbaceous layer of *Enneapogon cenchroides* and various forb species. In the landscape, the experimental site is situated on a foot-slope, in a relatively flat and homogenous area, with equal slope, exposure and aspect. Soil of the experimental plots is of the Oakleaf form (MacVicar *et al.* 1977).

The MEF is characterised by a hot wet summer and a mild dry winter. The mean temperature (1933–1984) is 22.3°C. The mean annual rainfall (1961–1990) for the MEF is 339mm (South African Weather Service 2001) with a unimodal peak during summer. The rainy season usually extends from September–May. Rainfall is highly variable (annual CV = 0.36; Dekker and van Rooyen 1995). The mean number of days with rainfall ≥1.0mm is the highest during January and February (7d).

Rainfall during the 2001–2002 leaf carriage period, December to end October, was lowest with a total of only 59mm (33.2% of corresponding long-term mean); the 2002–2003 leaf carriage period was wetter (256mm; 88.3% of the mean), and the 2003–2004 leaf carriage period the wettest (318mm; 9.7% above the mean).

## Procedures

### Pruning experiments

Stem cut and canopy treatments were carried out during February 2002 within a homogenous stand of similar-sized *C. mopane* trees. For coppicing, the main stems of 40 trees were felled at a mean height of 0.71m above ground level and all side branches below this height were removed from the main stem. This represents the height at which *C. mopane* trees are usually felled by rural inhabitants for timber and firewood. For pollarding, the top growth of 40 trees was removed at a mean height of 2m, simulating the severe effect of elephants (hedging, e.g. Smallie and O'Connor 2000). For the pruning treatment, seven healthy side branches of relatively equal diameter from each of 40 trees were selected from different sides of the tree and pruned by hand clean of leaves and twigs, leaving the main stems of selected branches intact. The latter was aimed at simulating browsing by large herbivores. The total amount of leaf biomass pruned was <10%. Forty trees were not treated and used as controls. A chainsaw was used to fell trees in the coppicing and pollarding treatments.

### Leaf sampling

Leaves, including petioles, were randomly collected at intervals of 55d from experimental trees. The first samples were collected immediately before the treatments were administered. Seven leaves were randomly collected from the canopies of experimental trees per sampling event, transported in plastic bags to the laboratory in a cooler box where they were dried in plant presses at room temperature (Hagerman 2002, Mueller-Harvey 2001). The dried leaves from each treatment were thoroughly mixed, and a pooled sample of 10g per treatment per sample cycle (55d) was sent to the Botany Department, University of Cape Town, for the determination of total phenolic (TP), condensed tannin (CT) and protein-precipitating tannin (PPT) concentration as described by Hagerman (2002).

### Statistical analyses

We used General Linear Models (GLMs, SPSS 2000) to analyse whether independent factors could explain variation in the dependent variables (TP, CT and PPT). Independent factors included in the models were type of

treatment, leaf year (December to end of November) and sampling month (February, April, June, August, October, December). Seasons used in the model were dry seasons (May to end of October) and wet seasons (November to end of April). The data were arcsine transformed to normalise the data prior to analysis. The normal distribution was confirmed by non-significant ( $p > 0.05$ ) Kolmogorov-Smirnov tests. The atypical October 2002 data points were treated as outliers to conform to the assumption of data normality and equal variances (Levene's test) (SPSS 2000). Tukey's test ( $p < 0.05$ ) was used as a *post hoc* test. Only significant ( $p < 0.05$ ) terms were retained in final models.

Additionally, a non-parametric Friedman test (SPSS 2000) for related samples (same sampling date) was used to test for treatment effects on the foliar TP, PPT and CT concentrations. This approach rigorously tested for treatment effects on foliar secondary metabolite concentrations over the study period.

The temporal pattern in the magnitude of the secondary metabolite treatment response was analysed using best-fit regression curve estimations (SPSS 2000). The deviation of TP, CT and PPT from control concentrations per sampling event was used as dependent variables, and the time (months) since the start of the treatments (June 2002,  $t = 0$ ) as an independent variable.

## Results

### Treatment effects

GLMs, including year, month of sampling, and canopy treatment as independent variables, explained 94–98% of the variation in TP, CT and PPT (Table 1). Concentrations of TP, CT and PPT tended to increase with increasing severity of harvesting (Figure 1), with the largest significant differences between control and coppiced trees for all compounds (Tukey,  $p < 0.05$ ). The treatment effect was robust for all secondary compounds measured and was confirmed by a non-parametric Friedman test ( $\chi^2 > 19$ ,  $n = 15$ ,  $p < 0.001$  for all three analyses). The concentrations in TP, CT and PPT between treatments were all significantly different and consistently followed the following rank order: coppiced > pollarded > pruned > control (Figure 1).

TP, CT and PPT were significantly affected by the month and leaf year in which samples were collected and by the canopy treatments administered (Table 1). The mean values per leaf carriage period for TP, CT and PPT gradually increased over the three consecutive seasons and seemed positively correlated with the corresponding precipitation received (Table 2). Of the possible interactions, only the month  $\times$  leaf year terms were significant. *Post hoc* tests revealed that the three years were all significantly different (Tukey,  $p < 0.05$ ), except for TP in the second and third years. The general pattern in TP, CT and PPT over the seasonal cycle was as follows: high during the early growing season (October and December), lower levels during the late wet season (February and April) and decreasing to their lowest levels during the dry season (June and August) (Table 3).

Significant differences occurred between the different dry (May to end of October) and wet (November to end of April) seasons in TP ( $F_{4,55} = 77.634$ ,  $p < 0.001$ ), CT ( $F_{4,55} = 53.98$ ,  $p < 0.001$ ) and PPT ( $F_{4,55} = 26.598$ ,  $p < 0.001$ ). The mean TP concentrations of the 2002 dry season were significantly lower (Tukey,  $p < 0.05$ ) than any other dry or wet season, which were not significantly different from each other (Table 2). The mean CT for the 2003 dry season, although higher than the 2002 dry season, was significantly lower than either the 2004 dry season or 2002–2003 wet season, but not different from the 2003–2004 wet season. The 2003 dry season PPT concentration also differed significantly from other seasons. A cluster consisting of both the 2002–2003 and 2003–2004 wet seasons, and the 2004 dry season had significantly higher PPT concentrations than the 2002 and 2003 dry season values, but were not significantly different from each other (Tukey,  $p < 0.05$ ).

### PPT:CT ratio

The PPT:CT ratios were significantly different between treatments ( $F_{3,42} = 2.955$ ,  $p < 0.001$ ). Leaves from control plants and pruned plants had the lowest PPT concentrations relative to CT, whilst leaves from coppiced trees had the highest PPT:CT ratio (LSD test,  $p < 0.05$ ). The two other ratios (PPT:TP and CT:TP) did not show any significant changes in relation to treatment.

**Table 1:** Summary statistics of the GLMs, testing the effects of leaf year and month of sampling, and canopy treatment (independent variables) on the concentrations of arcsine-transformed total phenolics (TP), protein-precipitating tannin (PPT) and condensed tannin (CT) in *Colophospermum mopane* leaves

| Independent variables | Dependent variables |        |         |        |         |        |
|-----------------------|---------------------|--------|---------|--------|---------|--------|
|                       | TP                  |        | PPT     |        | CT      |        |
|                       | F value             | p      | F value | p      | F value | p      |
| Month                 | 98.2                | <0.001 | 41.0    | <0.001 | 71.0    | <0.001 |
| Year                  | 297.0               | <0.001 | 72.0    | <0.001 | 235.9   | <0.001 |
| Treatment             | 11.8                | <0.001 | 11.8    | <0.001 | 13.9    | <0.001 |
| Month*year            | 96.0                | <0.001 | 45.0    | <0.001 | 107.2   | <0.001 |
| Model statistics      |                     |        |         |        |         |        |
| df                    | 17.42               |        | 17.42   |        | 17.42   |        |
| F value               | 115.46              |        | 40.96   |        | 107.4   |        |
| p                     | <0.001              |        | <0.001  |        | <0.001  |        |
| r <sup>2</sup>        | 0.98                |        | 0.94    |        | 0.98    |        |

**Table 2:** Mean concentration of total phenols (TP), condensed tannins (CT) and protein-precipitating tannins (PPT) per leaf year (December–October) in relation to the rainfall measured in the corresponding period

| Leaf year  | Rainfall (mm) | Concentration (mg g <sup>-1</sup> )* |                    |                    |
|------------|---------------|--------------------------------------|--------------------|--------------------|
|            |               | TP                                   | CT                 | PPT                |
| 2001–2002  | 59.0          | 39.5 <sup>a</sup>                    | 44.3 <sup>a</sup>  | 48.4 <sup>a</sup>  |
| 2002–2003  | 256.0         | 77.9 <sup>b</sup>                    | 88.0 <sup>b</sup>  | 83.5 <sup>b</sup>  |
| 2003–2004  | 318.2         | 78.3 <sup>b</sup>                    | 96.2 <sup>c</sup>  | 114.7 <sup>c</sup> |
| Dry 2002   |               | 39.5 <sup>a</sup>                    | 44.3 <sup>a</sup>  | 48.5 <sup>a</sup>  |
| Wet 2002/3 |               | 87.5 <sup>b</sup>                    | 109.1 <sup>c</sup> | 110.8 <sup>b</sup> |
| Dry 2003   |               | 69.1 <sup>b</sup>                    | 66.9 <sup>b</sup>  | 56.3 <sup>a</sup>  |
| Wet 2002/3 |               | 73.2 <sup>b</sup>                    | 87.1 <sup>b</sup>  | 116.6 <sup>b</sup> |
| Dry 2004   |               | 82.5 <sup>b</sup>                    | 105.4 <sup>c</sup> | 112.8 <sup>b</sup> |

\* Values with the same superscripts in columns are not significantly ( $p > 0.05$ ) different

**Table 3:** Bimonthly concentration of total phenols (TP), condensed tannins (CT) and protein-precipitating tannins (PPT) in *Colophospermum mopane* foliage

| Month    | N  | Concentration (mg g <sup>-1</sup> )* |                    |                     |
|----------|----|--------------------------------------|--------------------|---------------------|
|          |    | TP                                   | CT                 | PPT                 |
| February | 8  | 74.7 <sup>b</sup>                    | 98.0 <sup>c</sup>  | 90.0 <sup>d</sup>   |
| April    | 8  | 69.6 <sup>b</sup>                    | 83.9 <sup>b</sup>  | 87.5 <sup>c</sup>   |
| June     | 12 | 51.2 <sup>a</sup>                    | 62.4 <sup>a</sup>  | 68.7 <sup>ab</sup>  |
| August   | 12 | 48.4 <sup>a</sup>                    | 53.1 <sup>a</sup>  | 58.8 <sup>a</sup>   |
| October  | 12 | 91.5 <sup>c</sup>                    | 101.0 <sup>c</sup> | 111.6 <sup>bc</sup> |
| December | 8  | 96.7 <sup>c</sup>                    | 112.4 <sup>d</sup> | 142.0 <sup>e</sup>  |

\* Values with the same superscripts in columns are not significantly ( $p > 0.05$ ) different

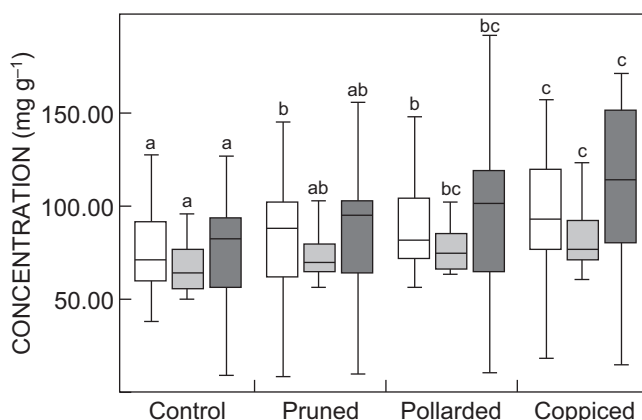
### TP, CT and PPT deviation from controls over study period

The correlation between the deviation of treatment TP concentration from control concentrations in mg g<sup>-1</sup> over time since the start of the canopy treatments, was described by a linear function ( $y = ax + b$ ,  $a = 0.48$ ,  $b = -0.83$ ;  $F_{43} = 7.97$ ,  $r^2 = 0.16$ ,  $p < 0.01$ ), indicating a linear increase in TP concentrations over time. The condensed tannin concentrations in treatment foliage in relation to control concentrations, also increased over time but was best described by a logarithmic function ( $y = alnx + b$ ,  $a = 10.0$ ,  $b = -15.2$ ,  $F_{43} = 7.80$ ,  $r^2 = 0.15$ ,  $p < 0.01$ ). The deviation of treated PPT from control concentrations did not vary significantly ( $p > 0.05$ ) over the study period.

## Discussion

### Treatment effects

Coppicing, pollarding, or pruning induced an increase of secondary metabolite levels in Mopane foliage. Higher concentrations of TP, CT and PPT were found in the foliage of treated plants relative to control trees in our experiment. In addition, we demonstrated a positive correlation between the severity of treatments and the magnitude of the response. Mopane accordingly invested heavily in secondary metabolites where the whole canopy was removed (coppice treatment), with positive but diminishing effects in



**Figure 1:** Boxplot showing the leaf concentrations (mg g<sup>-1</sup>) of condensed tannin (open bars), total phenol (light grey), and protein-precipitating tannin (dark grey) in control, pruned, pollarded, and coppiced *Colophospermum mopane* trees. The boxes cover the inter-quartile ranges (25–75%) around the median, the error bars indicate maximum and minimum values. Similar letters above bars indicate insignificant differences ( $p > 0.05$ )

the less severe treatments (pollard > pruned > control). Phenols, notably condensed tannins, have been implicated as deterrents protecting plant tissue against herbivory (Cooper and Owen-Smith 1985, Coley 1988, Bryant *et al.* 1992, Stamp 2003). Our results, showing increased levels of secondary metabolites, therefore support the 'induced defence' theory that browsed plants increase herbivore-detering substances in subsequent regrowth. Results of this study contrast with the recent findings of Ferwerda (2005), who found no significant induced condensed tannin response in Mopane foliage following defoliation. The conditions under which the respective experiments were carried out, however, differed and could explain the disparate results; controlled climatic conditions with saplings in a three-month study (Ferwerda 2005) versus field conditions using trees studied over three years (this study).

The demonstrated link between simulated utilisation and increased secondary metabolite levels has consequences for Mopane browsers. A higher secondary metabolite concentration in utilised plants translates to regrowth of a lower nutritional value. In systems where Mopane constitutes an important forage resource, this finding might therefore have negative repercussions for animal performance. It remains to be resolved whether an improved height accessibility, foliage density and seasonal availability of Mopane regrowth can offset the decreased forage quality in utilised plants. A further consequence of decreased Mopane palatability can be that alternative forage species are proportionally more heavily impacted by browsers avoiding high secondary metabolite loads in Mopane. However, the decreased forage quality in utilised plants might be offset by the improved height accessibility, foliage density and seasonal availability of Mopane regrowth, especially for bulk-feeders that can tolerate low quality browse (Clauss *et al.* 2003).



### **Continuous versus a single severe pruning event**

Continuous removal of regrowth (monthly or annually), as opposed to a single treatment event, might cause opposite responses to what was found in this study. This has been demonstrated for *Acacia nigrescens* trees, where severe, continuous pruning caused decreased foliar condensed tannin levels and increased foliar nitrogen levels (du Toit *et al.* 1990). The increased nutritional value of the browse of these trees attracted further browsing in a self-sustaining feeding loop. Elephants, which make extensive use of *C. mopane* as a food source (Duffy *et al.* 1999, Styles and Skinner 2000), are known to heavily and repetitively utilise certain *C. mopane* stands while avoid feeding on adjacent patches (Ben-Shahar and Macdonald 2002), and previously utilised Mopane trees were browsed more frequently than trees showing no previous impact (Smallie and O'Connor 2000). Smallie and O'Connor (2000) attributed the increased utilisation of previously browsed Mopane to enhanced branch availability at preferred browsing heights and earlier leaf flushing in the new season, and speculated that quality increases might also be responsible for upholding this attraction. It seems possible that regularly browsed *C. mopane* can also be caught in a similar feeding loop of continuous browsing, which indicates increased quality under a continuous browsing regime rather than induced defences associated with a once-off or infrequent browsing event. The alternative is that induced defences do take place but that certain herbivore species, such as elephants, are insensitive to high phenol concentrations (Styles and Skinner 2000, Lagendijk *et al.* 2005).

### **Protein precipitation in relation to condensed tannin concentration**

The increase in PPT:CT ratio with increasing severity of treatments suggests that the protein-precipitating capacity of the CT molecules was influenced by the treatments. If true, this means that a compound effect takes place; damage to Mopane canopies induces higher phenol concentrations, which also have a superior protein-precipitation capacity. As increased condensed tannin concentrations reduce the acceptability of forage for large mammals mainly by immobilising (precipitating) protein in the rumen of animals such as greater kudu and giraffe (Cooper and Owen-Smith 1985), this finding predicts a significantly lower intake of severely impacted Mopane canopies due to a relatively larger increase in PPT. However, some studies report that browsers tend to return to previously browsed patches (Smallie and O'Connor 2000, Makhabu and Skarpe 2006). A plant's responses to browsing are complex and we need to understand changes in relative abundance of secondary compounds in relation to changes in bulk density and nutrient composition (Fornara and du Toit 2007) in order to fully understand the tree-browser interaction.

### **Low defences in first year following treatments**

The near absence of phenolic substances in *C. mopane* foliage during the wet season of 2001–2002 was probably caused by the culmination of several factors affecting growth versus photosynthetic rates. Firstly, an extensive outbreak of *Imbrasia belina* larvae occurred in December

2001, which defoliated all *C. mopane* trees at the experimental site and surrounding area. The timing of the defoliation, at the beginning of the growing season, might have exacerbated the defoliation effect on plant chemicals. Removal of young new-season growth, as opposed to mature parts, has been reported to result in decreased chemical defences (Bryant *et al.* 1992). Young growth is produced from stored carbohydrate reserves, which subsequently results in the post-defoliation allocation of carbohydrates towards growth rather than defences. Secondly, severe drought conditions prevailed during the latter part of the 2001–2002 rainy season. The dynamics of woody plant phenolics as influenced by water limitations are poorly understood (Bryant *et al.* 1992). According to the growth-differentiation hypothesis (Bryant *et al.* 1992, Stamp 2003), moderate water stress will affect growth processes more than photosynthetic rates, with a subsequent rise in carbon-based secondary production. Severe water stress, however, suppresses photosynthesis more than growth, resulting in reduced chemical defences (Bryant *et al.* 1992). Hence, the defoliation by *I. belina* larvae occurred during a vulnerable stage in the seasonal carbohydrate status of *C. mopane*, which was exacerbated by the dry conditions. The treatment effects were found even in the presence of the *I. belina* larvae in December 2001, indicating that the treatment effects were larger than those generated by the larvae outbreak.

### **Duration of increased defences**

No significant interaction between leaf season and treatment was found (Table 1), suggesting that the effect of treatment on the dependent variables did not change significantly over the study period. However, the treatment deviation (difference between treatment and control values) for TP and CT concentrations, a more sensitive measure, increased significantly over the 28 month period after treatments were implemented. In the case of PPT the relationship was not significant, indicating that the increased PPT concentrations of treated Mopane remained constantly above control concentrations for the full duration of the study period, although a slight increasing trend was noticeable. Long-lasting induced effects have also been reported for a temperate forest, where induced secondary metabolite concentrations remained significantly higher than controls five years after a single logging treatment (Forkner and Marquis 2004). The prolonged effect that harvesting induced in *C. mopane* in our experiment has implications for the utilisation of this species by browsers. The data suggests that a single, severe event of damage to *C. mopane* canopies, which can be expected through wood harvesting or impact by large herbivores such as elephants, will increase concentrations of deterrent substances for extended periods, even into subsequent growth seasons. This may affect subsequent herbivory as browsers may be deterred by an increased secondary compound load.

### **Secondary metabolites and seasonal effects**

Osier *et al.* (2000) reported that for *Populus tremuloides* concentrations of phytochemicals were highly correlated between two consecutive years, suggesting that the phyto-

chemical profiles of aspen are predictable from year to year. This finding contrasts with our observations of *Colopospermum mopane* foliar secondary metabolite concentrations, which showed significant inter-annual variation, which might be an artefact of the variable rainfall received in these parts. Rutherford (1984) reported that leaf production by woody plants in a nutrient-poor savanna was influenced by the preceding season's rainfall due to shoot extension occurring prior to the onset of the rainy season. Unlike *C. mopane* trees that occur in Namibia, shoot extension of *C. mopane* trees in the study area normally occurs after the onset of the rainy season. Therefore, differences in the concentrations of condensed tannins and total phenolics in young mopane leaves are expected to respond to rainfall events during the onset of the growing season. Hence, leaves collected during the early growing season of 2002–2003 and 2003–2004 had the highest tannin levels relative to other months, similar to the findings of Ernst *et al.* (1991) and Vaithyanathan and Singh (1989).

In conclusion, coppicing, pollarding and pruning induced chemical defences in Mopane tree foliage. The magnitude of the response in TP, CT and PPT concentrations was positively related to the proportion of the canopy removed: coppicing > pollarding > pruning > control. These effects can be enduring and our data suggest a period of at least three years. The effects induced by canopy treatments on phenol and tannin concentrations, although significant, were small compared to the seasonal and inter-seasonal fluctuations thereof. These fluctuations were apparently in response to water availability and a defoliation event by *I. belina* larvae. The correlation between the severity of canopy treatments and the increase in PPT:CT ratio suggests that the protein-precipitating efficiency of CT molecules increased with increasing impact. Severe canopy damage induced increased phenol concentrations, mainly CT, which has a relatively higher protein-precipitating capacity. Consequently, forage value will be significantly lowered. This study indicated that secondary metabolite concentrations in Mopane foliage are dynamic and controlled by interactive factors. Findings of this study have implications for current harvesting, animal production and conservation practises. The finding that induced chemical defences remain high for years afterwards challenges the perception that coppicing and pollarding facilitate smaller browsers by improving browse conditions. Furthermore, the seasonal and inter-seasonal fluctuations in secondary compounds mean that browsers have to constantly adjust their diet to cope with potentially harmful substances. Alternatively, dropping Mopane from the diet can increase the impact on other browse species.

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