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Seasonal variation in phenolics in several savanna tree species in Botswana

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SUMMARY

Total phenolics (50% methanol) in leaves, flowers, pods and seeds of 10 tree species, *Acacia burkei, A. erubescens, A. fleckii, A. karroo, A. mellifera, A. nilotica, A. robusta, A. tortilis, Dichrostachys cinerea* and *Terminalia sericea* were investigated over one year. In all species the concentration of phenolic substances was high in young leaves, in immature fruits and seeds and low in mature leaves and pods. The decrease in phenolic concentration during the year occurred in waves, which may be related to climatic factors. Monomeric phenolics made up a high percentage of water-soluble phenolics, which was significantly correlated with total phenolics. There was a general tendency for a positive correlation between high concentrations of nitrogen, phosphorus and potassium and high concentrations of phenolics. Results are discussed in relation to herbivory and the hypothesis of a carbon-nutrient balance.

Key-words: Acacia spp., nitrogen, phenolics, phosphorus, pods, potassium.

INTRODUCTION

Phenolic compounds, particularly polyphenolics such as tannins, are widely distributed in tree foliage and play an important role in ecosystem processes (Kuiters 1990). In the living plant they are involved in lignin synthesis, but ecological emphasis is given to their function as a chemical defense against foraging animals (Crawley 1983; Cooper & Owen-Smith 1985). Young leaves often contain high concentrations of nitrogen, phosphorus and potassium (Tolsma *et al.* 1987; Ernst 1990) and are attractive to herbivores. They seem to be less protected from foraging animals than mature leaves (Feeny 1970) in which phenolic substances are present in higher amounts (Lowman & Box 1983, Mole *et al.* 1988). These observations have been formalized in the carbon-nutrient balance hypothesis (Bryant *et al.* 1983). It postulates that ‘the decline in growth with nutrient stress is generally larger than the decline in photosynthesis so that carbohydrates and carbon-based secondary metabolites such as phenols accumulate’. In contrast to the C/N balance hypothesis, it has been found that leaves of birch (*Betula pendula*) and spruce (*Picea abies*) have high levels of phenolics in spring which strongly decrease during the summer (Dittrich & Kandler 1971; Palo *et al.* 1985).

In savanna ecosystems large herbivores not only depend on the grass layer, but some of them, for example giraffe (Pellew 1984) and impalas (Cooper & Owen-Smith 1985), prefer to browse shrubs and trees. Together with introduced cattle they consume large amounts of seeds and pods, often rich in tannins (Ernst & Tolsma 1989; Wrangham & Waterman 1981). In many studies, leaf chemistry is treated as an invariable factor throughout the season, although strong changes in leaf components throughout the season are well documented not only for nutrients (Ernst 1990) but also for phenolic compounds (Feeny 1970; Dement & Mooney 1974).

In the savanna of Botswana, *Acacia* species are the dominant tree species. Here a comparison was made of the seasonal change in leaf phenolics at the species level, to differentiate between environmental impact and interspecific physiology. To avoid effects of browsing on phenolic contents (Danell *et al.* 1955; Du Toit *et al.* 1990), a non-grazed savanna was studied. The results will be compared with the concentrations of mineral nutrients in the leaves (Tolsma *et al.* 1987) and with reports about phenolics in a South African savanna (Cooper & Owen-smith 1985; Owen-Smith & Cooper 1987; Cooper *et al.* 1988; Du Toit *et al.* 1990).

MATERIALS AND METHODS

The study area was a non-grazed tree savanna at Gaborone with predominant *Acacia erubescens* and *A. tortilis* (Tolsma *et al.* 1987). Leaves, flowers, fruits and seeds (if present) were sampled at 2–4 week intervals from October 1982 to September 1983 from at least three individual plants of each of the following species: *Acacia burkei* Benth., *A. erubescens* Welw. ex Oliv., *A. fleckii* Shinz, *A. karroo* Hayne, *A. mellifera* (Vahl) Benth., *A. nilotica* (L.) Willd. ex Del., *A. robusta* Burch., *A. tortilis* (Forsk.) Hayne, *Dichrostachys cinerea* (L.) Wight & Arn., and *Terminalia sericea* Burch. The sampling procedure was the same as described for the analysis of nutrients in these savanna plants (Tolsma *et al.* 1987).

Plant material was air-dried and milled before extraction of 0·1 g with 25 ml distilled water for 20 h and the supernatant was collected after centrifugation at 3500 rpm. After filtration (Schleicher & Schüll paper) water-soluble phenols (monomeric and polyphenols) were determined by using the Folin–Ciocalteau reagent (Box 1983). Tannic acid was used as a standard and the concentration of water-soluble phenolics was expressed as milligrams TAE (tannic acid equivalents) per gram dry matter (Kuiters & Denneman 1987). Although the Folin–Ciocalteau reagent is reduced by amino acids and proteins in addition to phenolic compounds, the reduction by these compounds was found to be negligible compared to the reduction by phenolics after precipitation with trichloracetic acid (TCA), as was the case in Australian tree foliage (Lowman & Box 1983). Phenolic compounds were also extracted by boiling (80°C) 1 g air-dried plant material in 50% v/v aqueous methanol for 15 min, as described by Lowman & Box (1983). This fraction will hereafter be denominated as 'total phenolics', although phenolics bound to cell wall substances are not released by this extractant. However, the important fractions of phenolic glycosides, hydrolyzable and condensed tannins are extracted. Development-dependent differences of phenolic content were tested by linear regression (Sokal & Rohlf 1981).

Recently Bernays *et al.* (1989) have suggested that naturally occurring variation in tannins may be accompanied by an associated variation in simple phenolics. For determination of monomeric phenolics in leaf-water extracts, separations were performed on a FPLC (Pharmacia GP 250), equipped with a 50 μl injector loop and an ultraviolet detector at 280 nm (Kuiters 1987). Although wavelengths from 235 to 280 nm are used (Roston &

Table 1. Relative absorbance of 21 phenolic compounds with the Folin–Ciocalteau reagent, compared to tannic acid (100). Each value is the mean (±1 SD) of three measurements

Phenolic compound	Relative absorbance
Cinnamic acid	0±0
Benzoic acid	0±0
p-Anisic acid	1±0·1
p-Hydroxybenzaldehyde	4±0·3
Gentisic acid	30±2·4
Salicylic acid	38±2·4
m-Coumaric acid	57±8·5
p-Hydroxybenzoic acid	65±3·4
Syring aldehyde	69±1·7
Vanillin	74±2·4
o-Coumaric acid	74±12·5
Ferulic acid	77±5·9
Syringic acid	81±3·9
p-Coumaric acid	85±18·9
Caffeic acid	86±3·7
Tannic acid	100±3·2
Catechol	109±28·1
Gallic acid	121±14·5
(+)-Catechin	123±11·5
Vanillic acid	139±9·4
Protocatechuic acid	156±21·6

Kissinger 1982), ultraviolet absorption at 280 nm is preferred, because there is less interference with aliphatic compounds (Kögel 1983). Peak height, peak area and retention times were obtained with an integrator. The chromatographic column was a Pep RPC HR 5/5 C2/C18 column (Pharmacia). Standards of 18 different chemically purified phenolic compounds were used as reference material (Kuiters & Sarink 1986). Gradient elution was carried out at a flow rate of 1 ml min^{-1}. Fifty microlitres were chromatographed isocratically for 2 min with eluent A (93% distilled water, 5% methanol and 2% acetic acid) and then eluted with a linear gradient to 45% B (25% eluent A and 75% acetonitrile) for 18 min. Acetic acid was added to eluent A to decrease ionization of the acids. The column was re-equilibrated for 5 min by 100% eluent B, followed by 5 min with 100% eluent A. For a correct judgement of the various phenolic compounds in the water-soluble phenol fraction, the reduction of the Folin–Ciocalteau reagent was determined for 21 phenolics. Each compound was dissolved in 2% aqueous methanol (75 µg ml^{-1}). The absorbance values were expressed in milligrams tannic acid per litre. The standard curve was linear up to at least 25 µg ml^{-1}. Dihydroxy-substituted phenolics had a stronger reductive ability than mono-hydroxy compounds. Cinnamic, benzoic and *p*-anisic acid formed no coloured complexes with the phenol reagent.

Mineral nutrients were analysed after digestion with strong acids ($HNO_3/HClO_4$, 7:1, v/v) by column chromatography (nitrogen), spectrophotometry (phosphorus) and atomic absorption spectrometry for all other elements (for details see Tolsma *et al.* 1987).

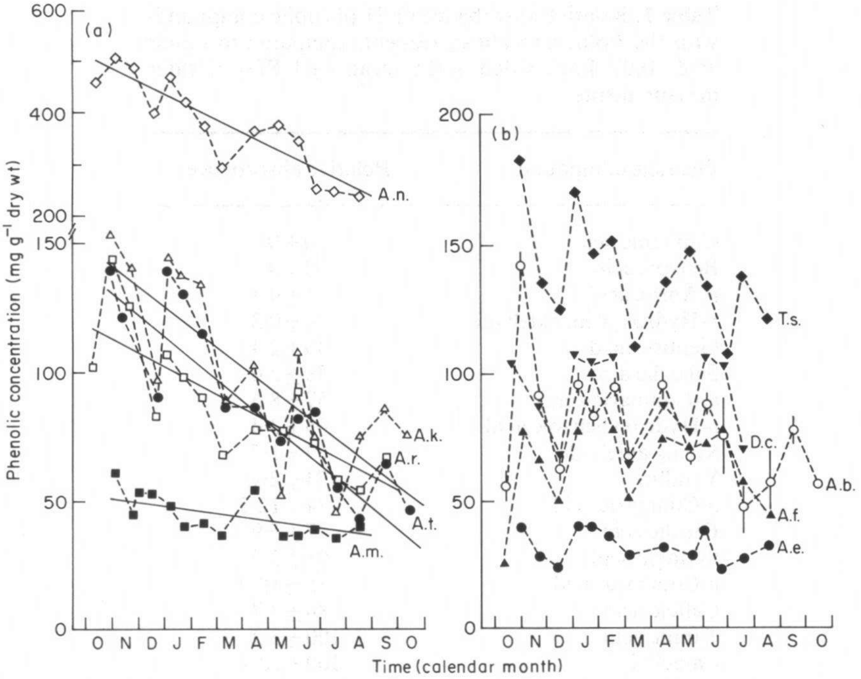

Fig. 1. Seasonal changes in the concentration of total phenolic compounds in the leaves of the savanna tree species from October 1982 to September 1983 in a savanna at Gaborone. (a) Species with a significant ($P < 0{\cdot}05$) decrease in the phenolic concentration of the leaves with leaf senescence: (△) *A. karroo*, (■) *A. mellifera*, (◇) *A. nilotica*, (□), *A. robusta*, (●), *A. tortilis*. (b) Species with a tendency for a decreasing concentration of phenolics with leaf senescence: (○) *Acacia burkei*, (●), *A. erubescens*, (▲) *A. fleckii*, (▼) *Dichrostachys cinerea*, and (◆) *Terminalia sericea*. Each value is the mean of the leaves of at least three individual trees. For *A. burkei* and *A. erubescens* the standard deviation is given as an indication of variation between trees.

RESULTS

The mean concentration of total phenolics in the leaves of shrubs and trees varied by a factor of 11, from 33 mg g^{-1} dry wt in *Acacia erubescens* to 374 mg g^{-1} in *A nilotica* (Fig. 1). With regard to the seasonal variation, leaves of all species contained the highest amounts of phenolics at the start of the growing season in October with the exception of very young leaves of *A. burkei*, *A. fleckii*, *A. nilotica* and *A. robusta*. In leaves of five *Acacia* species, i.e. *A. karroo*, *A. mellifera*, *A. nilotica*, *A. robusta* and *A. tortilis* the concentration of total phenolics decreased significantly to July (Fig. 1a), when leaves became senescent. In the other five species, i.e. *Acacia burkei*, *A. erubescens*, *A. fleckii*, *Dichrostachys cinerea* and *Terminalia sericea*, there was a trend towards lower phenolic concentrations with leaf ageing (Fig. 1b). This decrease in phenolics, however, occurred in several waves and was most pronounced in species with intermediate levels of phenolics. In all species, there was a sharp decrease in the phenolic concentration after heavy rains in December, with a new increase to almost the October-level in January, followed by a further sharp decrease in March, and once more a pronounced increase in April. Species with green leaves throughout the winter (July, August) and up to early spring (September), i.e. *Acacia burkei*, *A. karroo*, *A. robusta*, and *A. tortilis* showed an increase in phenolic concentration in old leaves during August and September, followed by a decrease in October just before leaf shedding.

Table 2. Mean concentration (± 1 SD) of phenolics and the linear relation between total (y) and water-extractable phenolics (x) in leaves of savanna tree species at Gaborone, Botswana. The mean is based on at least 13 samples. Mean concentrations with different letters indicate a significant difference at least at $P<0{\cdot}05$

Tree species	Mean concentration of phenolics (mg g^{-1} dry wt)	Correlation function ($y=a+bx$)		Correlation coefficient
		a	b	$r_{1,2}$
Acacia burkei	$77{\cdot}4\pm23{\cdot}3^{a}$	31·8	1·32	0·60
A. erubescens	$33{\cdot}2\pm5{\cdot}7^{b}$	2·7	1·53	0·78
A. fleckii	$65{\cdot}5\pm18{\cdot}6^{a}$	14·1	1·41	0·89
A. karroo	$101{\cdot}4\pm35{\cdot}6^{c}$	25·2	2·09	0·90
A. mellifera	$43{\cdot}2\pm6{\cdot}4^{d}$	6·7	1·36	0·80
A. nilotica	$373{\cdot}7\pm93{\cdot}2^{e}$	202·1	0·79	0·83
A. robusta	$87{\cdot}9\pm24{\cdot}2^{ac}$	45·2	1·40	0·67
A. tortilis	$90{\cdot}9\pm31{\cdot}4^{ac}$	14·0	2·26	0·90
Dichrostachys cinerea	$92{\cdot}0\pm16{\cdot}3^{ac}$	42·1	1·32	0·95
Terminalia sericea	$137{\cdot}9\pm22{\cdot}1^{f}$	75·5	0·86	0·85

Water-soluble phenolics contributed from 33·5 (*Acacia robusta*) to 62·7% (*Acacia mellifera*) to the total concentration of phenolic compounds. A test of the correlation between water-soluble and the total phenolic concentration (Table 2) showed a very high positive correlation for nearly all investigated woody plant species ($r>0{\cdot}78$). Exceptions were *A. burkei* and *A. robusta* with a correlation coefficient of 0·60 and 0·67, respectively.

As far as compounds could be identified, water-soluble phenolics consist of a high percentage of monomeric phenolic acids (Table 3). Gallic acid contributed between 11·7% (*Acacia fleckii*) and 26·7% (*A. erubescens*), ferulic acid contributed between 10·4% (*A. karroo*) and 52·9% (*A. erubescens*). Only in *A. burkei* was protocatechuic acid involved for more than 15% in water-soluble phenolics; in all other species this phenolic acid remained below 10%. Coumaric acid was restricted to three species, *p*-coumaric acid to *A. fleckii* and *A. tortilis*, *o*-coumaric was found in *A. karroo* and *A. tortilis*, in the latter species the value was 20·4%. *p*-Hydroxybenzoic acid and vanillic acid were present in all species, but they contributed less than 10 and 5%, respectively, to the water-soluble phenolics. Seasonal changes (data not presented) were not accompanied by a change in some precursors of lignin synthesis, i.e. cinnamic acid, *p*-coumaric acid and ferulic acid. Their concentrations, based on dry weight, remained very constant. With an increasing concentration of water-soluble phenolics, the contribution of monomeric phenolics to this fraction decreased significantly ($P<0{\cdot}05$), independent of the *Acacia* species investigated.

The total number of phenolics in flowers was in the same range as the mean concentration in leaves of the various species (Table 4). The phenolic concentration of the fruits showed the same species-specific tendency as that of leaves, being lowest in *A. erubescens* (17 mg g^{-1} dry wt) and highest in *A. nilotica* (387 mg g^{-1}). In contrast to the fruits, the phenolic concentrations of seeds varied only by a factor of three between the species and were unrelated to the concentration in fruits.

To test the hypothesis of an increase in carbon-based defence substances at very low nutrient availability in trees (Tuomi *et al.* 1988), the relation between the concentration of total phenolics and leaf nutrients was analysed. As shown for leaves of *A. nilotica* (Fig. 2),

Table 3. Contribution of various monophenolic acids in leaves of *Acacia* species to the concentration of water-soluble phenolics. The values are corrected for the difference in extinction of the specific monophenolic acid in comparison to the used tannic acid standard. The percentage contribution of cinnamic acid is given in brackets because cinnamic acid does not react with Folin–Ciotalteau reagent. Values are based on two sampling dates. The highest value of each species is underlined

Monophenolic acid (% of water soluble phenolics)	*Acacia*							
	burkei	*erubescens*	*fleckii*	*karroo*	*mellifera*	*nilotica*	*robusta*	*tortilis*
Chlorogenic	—	—	—	4·5±0·1	8·6±1·8	5·4±0·9	3·8±0·7	3·3±0·3
Cinnamic	(6·4±1·7)	(8·8±4·4)	(9·3±1·0)	(7·9±2·6)	(4·3±2·3)	(8·7±6·2)	—	(14·1±8·9)
p-Coumaric	—	—	12·5±3·9	—	—	—	—	8·2±2·1
Ferulic	14·6±5·8	52·9±14·1	40·7±8·0	10·4±0·9	28·8±6·4	15·4±1·1	14·7±0·7	13·0±1·7
Gallic	14·8±2·3	26·7±7·9	11·7±2·0	18·0±4·8	18·5±4·3	19·5±9·5	12·9±6·2	16·2±2·2
p-Hydroxybenzoic	4·0±0·2	9·4±1·8	6·9±1·2	4·7±0·3	7·4±0·7	8·8±4·1	5·5±0·5	3·7±0·7
Protocatechuic	15·6±5·2	6·0±1·3	2·9±0·5	3·1±0·1	3·2±0·6	1·5±0·4	2·5±0·4	2·6±0·5
o-Coumaric	—	—	—	12·0±1·5	—	—	—	20·4±4·3
Vanillic	2·1±0·2	4·3±0·8	2·1±0·5	2·1±0·1	3·7±0·6	1·3±0·2	3·2±0·5	1·7±0·5

Table 4. Total phenolic concentration (mg g^{-1}): in flowers, fruits, seeds of shrubs and trees in an *Acacia tortilis* savanna at Gaborone (Botswana). n.d. = Not determined

Plant species	Flowers	Fruits	Seeds
Acacia burkei	n.d.	71·4±12·8	36·0±11·1
A. erubescens			
Young	n.d.	40·9±4·2	n.d.
Mature	33·4±1·8	17·3±5·0	32·1±6·9
A. fleckii	82·9	35·4±9·3	14·2±4·1
A. karroo	94·3±22·7	n.d.	n.d.
A. mellifera	54·4±2·9	n.d.	n.d.
A. nilotica	403·1	387·3±79·2	37·9±6·9
A. tortilis			
Young		58·9±9·4	192·2±12·6
Mature	69·7±4·8	36·1±1·1	43·5±8·7
Dichrostachys cinerea	96·9±24·9	93·8±32·9	20·3±5·3
Terminalia sericea	n.d.	25·9±2·2	36·8±4·5

the species with the highest phenolic concentration, the overall decline of phenolics in waves was paralleled by a decrease in nitrogen, phosphorous and potassium, whereas the calcium concentration increased with leaf senescence. The correlation coefficient between nutrient and phenolic concentrations, however, was not consistent for all investigated *Acacia* species (Table 5). Although nitrogen, phosphorus and potassium were positively correlated with phenolic concentration, this correlation was only significant ($P<0·05$) for N, P, K in *A. mellifera* and for K in *A. robusta*. The Ca concentration in leaves was negatively associated with a total phenolic concentration, but only in *A. mellifera* this correlation was statistically significant ($P<0·05$). The Mg concentration of leaves was positively correlated with the phenolic concentration in *A. burkei, A. fleckii* and *A. robusta*, and negatively correlated in *A. erubescens, A. karroo, A. nilotica* and *A. tortilis*. There was no correlation in *A. mellifera*.

DISCUSSION

The total phenolic concentration in leaves of the woody savanna species investigated, fell within the range reported for rain-forest plants in Africa (Mole, Ross *et al.* 1988), Australia (Lowman & Box 1983) and Central America (Janzen & Waterman 1984), with the exception of the high phenolic concentration in *Acacia nilotica*, which is a well-known source of commercial tannins (Peak 1952). In contrast to the situation in the leaves of rain-forest trees in Central America (Janzen & Waterman 1984), there was a pronounced decrease in total and water-soluble phenolics with leaf senescence in the investigated savanna tree species, as was also found for *Brachystegia* and *Julbernardia* in the miombo woodlands of Malawi (Jächmann 1989). There is no indication, to date, which abiotic (climatic) factor is involved in the wave pattern of the decrease in total and water-soluble phenolics. The strong decrease in December can be correlated with a period of very cloudy weather and heavy rains. It is known from rain-forest trees that low light intensity diminishes the production of polyphenolics and simple phenolics (Mole *et al.* 1988). This coincidence in December, however, does not hold for the decrease in March and July,

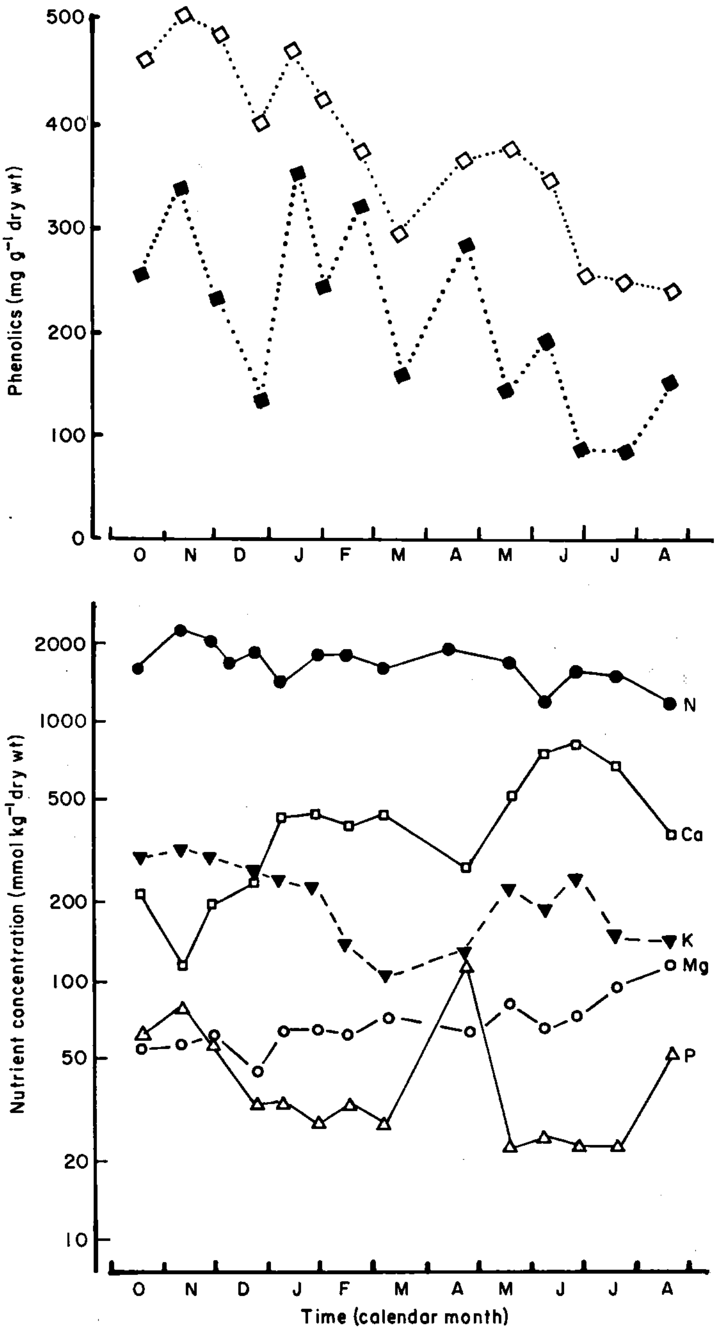

Fig. 2. Seasonal changes in the concentration of total phenolic compounds (◇) and mineral nutrients (◆) in the leaves of the *A. nilotica* from October 1982 to August 1983 in a savanna at Gaborone, Botswana. The data of mineral nutrients are based on Tolsma *et al.* (1987).

Table 5. Correlation coefficients of the concentration of mineral nutrients with the concentration of total phenolics in leaves of *Acacia* species in a savanna in Botswana during a growing season

Acacia species	N	P	K	Ca	Mg
A. burkei	+0·20	+0·14	+0·20	−0·08	+0·45
A. erubescens	+0·12	+0·03	+0·18	−0·44	−0·45
A. fleckii	+0·32	+0·44	+0·38	−0·40	+0·53
A. karroo	+0·56	+0·53	+0·53	−0·46	−0·28
A. mellifera	+0·74*	+0·72*	+0·67*	−0·68*	+0·06
A. nilotica	+0·41	+0·06	+0·14	−0·54	−0·43
A. robusta	+0·21	+0·58	+0·80*	−0·46	+0·39
A. tortilis	+0·46	+0·22	+0·28	−0·65	−0·27

* = significant difference at $P < 0.05$.

when light intensity is very high. It has been argued (Mole *et al.* 1988) that an accumulation of phenolics may be related to a hampered translocation from the site of formation. The high content of water-soluble phenolics of young pods and seeds, as found for *A. tortilis*, may be interpreted as a storage of precursors for cell wall formation.

The decrease in total phenolics is paralleled by the decrease in the nutrients nitrogen, phosphorus and potassium (Tolsma *et al.* 1987), and this does not support the hypothesis of a plant carbon/nutrient balance. In contrast to the postulated increase in carbon-based defence substances at low nutrient availability (Bryant *et al.* 1983; Tuomi *et al.* 1988), as far as availability can be measured by the concentration in the leaves, the present study demonstrates a relationship between nutrient concentration and phenol concentrations, but not opposite to each other. Therefore, it does not support the idea of a 'response to herbivores over physiological time scales' (Bryant *et al.* 1983, p. 366). The hypothesis may fit for a small group of plants but it is not reliable at the genus level, when data for *Betula nana* are compared with those of *Betula pendula* (Palo *et al.* 1985). One of the shortcomings of the carbon-nutrient balance hypothesis is the lack of consideration of the investigations that do not fit, e.g. all species with a significant decrease in phenolics with leaf senescence or without a seasonal pattern (Macanley & Fox 1980). The high nitrogen concentration in leaves of *Acacia* species, due to the symbiosis with nitrogen-fixing bacteria, may have a special attraction for large herbivores, if the mineral nutrition hypothesis is correct (McNaughton 1990). In each case, a single sampling in the wet season (Du Toit *et al.* 1990) is insufficient for correct judgement to be made on the dynamics of nutrients and phenolics in leaves and their impact on foraging animals. The predominant inhibitory effect of only one phenolic compound on ruminant digestibility of birch (Sunnerheim *et al.* 1988), and the presence of cyanogenic glucosides in Australian (Maslin *et al.* 1988) and African *Acacia* species (P. Kakes, personal communication), are indications of a specific defence rather than an overall involvement of particular tannins, as demonstrated for white spruce (Sinclair *et al.* 1988). Due to the study being restricted to 'non-grazed' savanna, the impact of browsing on leaf chemistry was excluded (Danell *et al.* 1985; Du Toit *et al.* 1985) in order that the physiological aspect could be differentiated from the herbivorous involvement, however, an interpretation of the impact of herbivores on a stimulation of phenolic synthesis was not possible.

The observed decrease in phenolics with senescence of the leaves, however, may be interpreted as an increased use of carbon for growth at a time when the attractiveness of a formerly high nitrogen/phosphorus foodstuff to ruminants has been lowered by plant internal retranslocation processes (Tolsma *et al.* 1987) and by the development of physical defences, e.g. thorns (Cooper & Owen-Smith 1986; Young 1987; Du Toit *et al.* 1990). Browsing by ruminants is said to be strongly deterred by condensed tannins in woody plants in a South African savanna in the late summer (Cooper & Owen-Smith 1985), but further research has not established a consistently significant correlation between deterrents and nutrients (Cooper *et al.* 1988). The lack of an effect of high tannin concentration may be related to differences in the structure of tannins (Zucker 1983) and/or caused by the presence of tannin-binding proteins in saliva of wild ungulates and their absence in domestic ungulates (Austin *et al.* 1989). Cattle will accept leaves with a high phenolic concentration as soon as the leaf chemistry is changed by insect-derived exudates. As observed for *Colophospermum mopane*, cattle favour leaves covered by the sweet excretes of the mopane psyllid *Arytaina mopane* (Ernst & Sekhwela 1987). As Lindroth (1989) has pointed out, herbivores may adapt their feeding habits to obtain the best variety of nutrients instead of selecting one foraging method.

At this point it should be mentioned that fruit-eating ungulates have a preference for ripe but tannin-rich pods of *A. nilotica* and *A. tortilis* (Peak 1952; Lamprey *et al.* 1974) instead of tannin-poor pods of *A. mellifera* and *A. erubescens*. In addition, larvae of seed-eating bruchid beetles were undeterred by high tannin concentrations of the pods and seed coat, as indicated by a degree of infestation of *Acacia* seeds (Ernst *et al.* 1989, 1990). The increased knowledge of the various types of simple- and polyphenols, their seasonal fluctuation, and the high adaptation of wild ungulates to tannin-containing plant extracts, may require caution in the interpretation of these compounds as plant defence mechanisms. The difference in tannin content of *Acacia nigrescens* leaves at a heavily grazed site (low tannin) and a control site (high tannin), and a lack of a difference in *A. tortilis* at the same site (Du Toit *et al.* 1990), does not only indicate a species-specific reaction but demonstrates that severe browsing can increase palatability.

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