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RESEARCH ARTICLE

Quantitative Variability of Cyanogenesis in *Cathariostachys madagascariensis*—the Main Food Plant of Bamboo Lemurs in Southeastern Madagascar

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Giant bamboo (*Cathariostachys madagascariensis*) is a major food plant for three sympatric species of bamboo-eating lemurs (*Hapalemur aureus*, *H. griseus*, and *Prolemur simus*) in the rain forests of southeastern Madagascar. This plant species is strongly cyanogenic. However, quantitative data on cyanide concentration in *C. madagascariensis* are scarce. Previous studies reported 15 mg cyanide per 100 g fresh shoot material (corresponding to approx. 57 μmol cyanide per gram dry weight). However, we found mean concentrations (±SE) ranging from 139.3 ± 19.32 in ground shoots to 217.7 ± 16.80 μmol cyanide per gram dry weight in branch shoots. Thus, cyanogenesis of *C. madagascariensis* was up to four times higher than reported before. In contrast to the strongly cyanogenic shoots no cyanide could be detected in differently aged leaves of *C. madagascariensis* confirming earlier studies. Within individual shoots fine-scaled analysis revealed a characteristic ontogenetic pattern of cyanide accumulation. Highest concentrations were found in youngest parts near the apical meristem, whereas concentrations decreased in older shoot parts. Beyond the general intra-individual variability of cyanogenic features analyses indicated site-specific variability of both, the ontogenetic pattern of cyanide concentration as well as the total amount of cyanide accumulated in shoots. Additionally, analyses of soluble proteins—one important nutritive measure affecting food plant quality—demonstrated a converse quantitative relation of protein concentrations in leaves to cyanide concentration in shoots at the site-specific level. We, thus, suggest integrative analyses on quantitative variation of cyanogenesis together with nutritive plant parameters in future studies. This approach would allow obtaining more detailed insights into spatial variability of giant bamboo's overall browse quality and its impact on lemur herbivores. Am. J. Primatol. 71:305–315, 2009.

Key words: giant bamboo; ontogenetic variability; cyanide; browse quality; Ranomafana National Park

INTRODUCTION

Giant bamboo (Poaceae: *Cathariostachys madagascariensis*) is endemic to Madagascar and represents a characteristic component of rainforests in the Ranomafana area [Dransfield, 1998]; In this area, giant bamboo is an important food plant to the three sympatric bamboo lemurs *Hapalemur aureus*, *H. griseus*, and *Prolemur simus* [Glander et al., 1989; Tan, 1999]. Observations in the Ranomafana National Park revealed selection of different plant parts by each of the three lemur species [Glander et al., 1989; Wright & Randriamanantena, 1989]. *H. aureus* and *H. griseus* rely heavily on *C. madagascariensis* that comprises 78 and 72% of their diets, respectively [Tan, 1999]. They select young leaf bases, the immature part of leaf-bearing branchlets, branch shoots (i.e., new sprouts from above-ground nodes in the canopy layer with a diameter of ~3 cm), and occasionally ground shoots (i.e., new sprouts from the subterraneous rhizomes with ~8 cm in diameter). Greater bamboo lemur (*P. simus*) lives almost entirely on *C. madagascariensis*, which accounts for more than 95% of its diet [Tan, 1999]. Thus, *P. simus* almost totally depends on the quality of one single plant species. Compared with the other two bamboo lemur species *P. simus* is less selective concerning foraging on different plant parts of giant bamboo and consumes leaves of different ages as well as ground and branch shoots and pith of mature culms. However, diet composition of these parts strongly depends on season [Tan, 1999].

In contrast to the wide-ranging distribution of *C. madagascariensis*, the present-day distribution of

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greater bamboo lemur is restricted to the south-central portion of the country's eastern rain forests at elevations of 200–1,100 m [Mittermeier et al., 1994]. According to the International Union for the Conservation of Nature (IUCN) this lemur species is one of the world's rarest mammals belonging to the 25 most endangered primates [Baillie et al., 2004; Mittermeier et al., 1992; Simons, 1997]. Historical records and subfossils confirmed a widespread occurrence of *P. simus* that covered wide areas of Madagascar [Godfrey & Vuillaume-Randriamanantena, 1986; Schwarz, 1931]. *P. simus* is threatened by environmental degradation (slash-and-burn agriculture, logging, as well as the extensive cutting of bamboo) and hunting [Simons, 1997]. Detailed information on the interaction between giant bamboo and its lemur herbivores is needed to better understand functional aspects of this highly specific relationship.

In addition to nutritional parameters, the suitability of food plants to herbivores is strongly affected by toxic plant features. Among such plant features cyanogenesis represents an outstanding characteristic of *C. madagascariensis* [Glander et al., 1989]. Cyanogenesis means the enzymatically accelerated release of hydrogen cyanide (HCN) from preformed inactive precursors in response to biotic or abiotic stresses [e.g. Møller & Seigler, 1999]. In intact tissues these precursors (generally cyanide-containing α-hydroxynitrile β-D-glucosides [for review see Seigler, 1998]) are stored in the central vacuole. In monocotyledonous plants, such as bamboo, specific β-D-glucosidases are located in chloroplasts and therefore are spatially separated from their substrates [Thayer & Conn, 1981]. After wounding, e.g. by herbivory or other disruptions of cellular integrity, β-D-glucosides are brought into contact with the cyanogenic glucosides. Hydrolysis of the *O*-glycosidic bond produces an unstable α-hydroxynitrile aglycone that is spontaneously or actively via α-hydroxynitrilase converted into HCN and a corresponding carbonyl compound, i.e., an aldehyde or ketone, depending on the structure of the cyanogenic glucoside [Møller & Seigler, 1999]. Toxicity of cyanide to vertebrates is mainly based on inhibition of the mitochondrial respiration pathway by blocking the cytochrome a/a3 dependent oxidase and on occupation of the oxygen-binding site in hemoglobin thus reducing oxygen transport capacities in blood [Solomonson, 1981].

One major aspect complicating functional analyses of cyanogenesis in natural systems is the high quantitative variability of cyanogenic plant features among populations or sites [e.g. Buhrmester et al., 2000; Gleadow & Woodrow, 2000; Goodger et al., 2002]. Therefore, detailed information on variability of cyanogenic plant traits is essential for analyzing the role of plant cyanogenesis in herbivore–plant interactions [Ballhorn et al., 2005, 2007, 2008]. In addition to defensive traits such as cyanogenesis, suitability of host plants to herbivores is strongly affected by nutritive plant parameters, e.g. concentrations of protein and digestible sugars [e.g. Belovsky & Schmitz, 1994; Cooper et al., 1988; Fowler, 1983; Robbins, 1993]. Thus, we quantitatively analyzed both cyanogenesis and soluble protein concentration in individual bamboo plants at the intraspecific and ontogenetic level. All analyses were conducted with fresh material immediately after collection from natural sites. Naturally occurring variability of biochemical plant features might be used for studying functional associations between cyanogenic giant bamboo and bamboo lemurs.

METHODS

Study Site and Plant Species

This study was conducted in the Ranomafana area in southeastern-central Madagascar near the Talatakely area (~S21°15′, E47°25′) in January 2008. The area is composed of submontane rain forests with elevations ranging from 900 to 1,100 m [Wright, 1995, 1997]. Annual temperature averages 21°C [Wright, 1995] with the lowest temperatures (4–6°C) occurring from June to August and the highest (28–30°C) from November to January. The total precipitation averages 2,300–4,000 mm per year. Monthly rainfall in this area is highest from December to March (400 mm) and lowest from May to October (90 mm). Samples were mainly collected outside the protected areas of Ranomafana National Park (permit N° 020/08/MEEFT/SG/DGEF/DSAP/SSE as obtained by the MINISTERE DE L ENVIRONMENT, DES EAUX ET FORETS ET DU TOURISME). The majority of samples were collected at two sites. Site A was located near the Centre ValBio, whereas site B was further down the road to the village Ranomafana (for detailed GPS information of each plant individual see Table I). We included three individual mature culms carrying leaves and branch shoots per site, whereas three ground shoots were included from site A and two from site B. We localized a third site of *C. madagascariensis* on the road from Ranomafana to Kianjavato (site C), where we collected one additional sample representing a ground shoot, which had just started to develop leaf-bearing branchlets. This ontogenetic stage was not found at the other sites at time of analyses in January 2008. In total we analyzed 81 samples from stems, including 25 samples derived from five ground shoots and 56 samples taken from 31 branch shoots. Altogether, we included 192 leaf samples from six plant individuals and 42 leaf-bearing branchlets.

Plant Material

We quantitatively analyzed cyanogenic potential (HCNp; the total amount of cyanide that can be

TABLE I. Collection Data of *Cathariostachys madagascariensis* Individuals Included in This Study

Site and plant individual	GPS S	GPS E	Elevation (m)	Date
A_1	21°14.991	47°25.176	1,121	Jan-21-2008
A_2	21°14.923	47°25.148	1,110	Jan-23-2008
A_3	21°14.943	47°25.178	1,121	Jan-23-2008
A_4	21°14.995	47°25.193	1,101	Jan-23-2008
B_1	21°15.148	47°26.014	938	Jan-22-2008
B_2	21°15.157	47°26.028	936	Jan-24-2008
B_3	21°15.150	47°26.021	940	Jan-24-2008
C_1	21°19.990	47°39.601	519	Jan-25-2008

All plant samples were collected by Daniel. J. Ballhorn, Stefanie Kautz, Fanny P. Rakotoarivelo, and Georges Razafindrakoto.

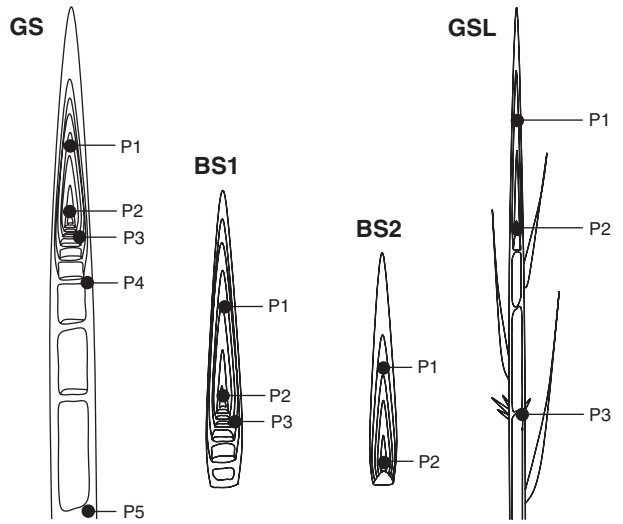

Fig. 1. Sampling of shoot material. Ground shoot (GS), branch shoot >20 cm (BS1), branch shoot <20 cm (BS2), and ground shoot developing leaves (GSL) of *C. madagascariensis*. We sampled at five (GS), three (BS1), and two (BS2) positions thus including material from young infolded leaves (P1), meristematic leaf bases (P2), and young pith (P3–P5). Three samples were taken from the GSL. Positions of samples are indicated by black dots.

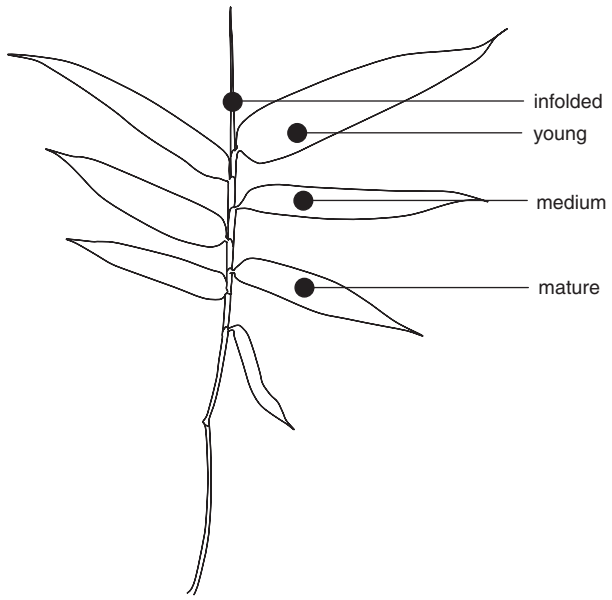

Fig. 2. Sampling of leaf material. Within leaf-bearing branchlets of *C. madagascariensis* different ontogenetic leaf stages were analyzed for HCNp and protein representing still infolded leaves (youngest), young unfolded leaves, medium aged leaves, and mature leaves. Positions of samples are indicated by black dots.

released from a given tissue [Loyd & Gray, 1970]) and concentration of soluble proteins of ground shoots, branch shoots and leaves of giant bamboo—*volohosy* in the local dialect of Malagache—(Poaceae: Bambusoideae: *C. madagascariensis* (A. Camus) S. Dransf.). All of these parts are consumed by bamboo lemurs [Tan, 1999]. Five samples each were taken from individual ground shoots at the following positions: 10 cm in acripetal direction of the meristem (young infolded leaves), on level with the meristem (young leaf bases) as well as three, six, and nine nodes in basipetal direction from the meristem (pith) (Fig. 1, GS). At time of analyses ground shoots (GS) were 1.4 ± 0.25 m tall. Branch shoots developing at mature culms inserted 7–15 m from the ground and were 10–35 cm at time of analysis. They were grouped according to size, either 20–35 cm (BS1) or <20 cm (BS2) in length. Of the larger branch shoots three samples were taken: one 10 cm acripetal of the meristem on top of the shoot (position 1), one 1 cm above the meristem (position 2), and one three nodes down in basipetal direction (position 3) (Fig. 1, BS1). Of branch shoots <20 cm two samples were taken, one 10 cm acripetal of the meristem (position 1) and one 1 cm above the meristem (position 2) (Fig. 1, BS2). We included three samples at positions 1–3 (Fig. 1, GSL) of the ground shoot (site C) that had just started to develop leaves. For analyses of green leaf material eight leaf-bearing branchlets were collected per mature shoot and from each branchlet four leaflets of different ontogenetic stages were analyzed (Fig. 2). Leaf-bearing branchlets were gathered every third node beginning 2 m down the top of a mature culm.

Voucher specimens are deposited in the herbarium of the University of Duisburg-Essen, Germany (DB_0090–0136). *C. madagascariensis* specimens

sampled at natural sites were authenticated by Georges Razafindrakoto, Centre ValBio, Ranomafana National Park, Madagascar.

Quantification of HCNp

The cyanogenic potential (HCNp) was measured by extraction of cyanogenic precursors from plant material. To avoid a premature release of HCN by degradation, sampling of plant material was conducted fast and by avoiding any injury of shoot or leaf material during collection and transport. Collected leaves were transported in Ziplock Bags® (Toppits, Minden, Germany) supplemented with moist filter paper to minimize effects of wilting. Immediately before analysis shoots were cut lengthwise and defined segments of shoot material (Fig.1) were removed at defined positions at the stem using a cork borer (9 mm in diameter). Each sample was cut lengthwise with a razor blade, while one part was used for analysis and the other dried (75°C, until constancy of weight) and weighed to the nearest 0.001 g. Plant material was ground for extraction of cyanogenic precursors with a mortar and pestle kept on ice and under adding ice-cold 0.067 mol^{-1} disodium hydrogen phosphate (2 ml $g\,fwt^{-1}$) and sterilized sea sand (Sigma Aldrich, Deisenhofen, Germany). The homogenized samples were filtered using 5 ml PE syringes (B. Braun AG, Melsungen, Germany) supplemented with cotton and the filtrate was used immediately for analyses.

For enzymatic degradation of the cyanogenic precursors, exogenous β-glucosidase from almonds (Fluka Chemie AG, Buchs, Switzerland) in phosphate–citrate buffer (McIlvaine buffer), pH 5.6, was added to the respective sample in an amount that corresponded to 20 nkat. Activity of β-glucosidase was determined by using *p*-nitrophenyl-β-D-glucopyranoside (Merck KGaA, Darmstadt, Germany) as artificial chromogenic substrate. Thunberg vessels were used as reaction flasks and stopped by a glass stopper with a side bulb of about 1 ml volume. The closed headspace prevented the released gaseous HCN from leaking. The mixture for incubation consisted of 0.05 ml filtered sample, 0.45 ml 0.067 mol l^{-1} aqueous sodium dihydrogen phosphate solution, 0.10 ml β-glucosidase solution, and 0.60 ml 0.2 mol l^{-1} NaOH in the side bulb of the stopper. This mixture was incubated in a water bath for 25 min at 30°C. We inverted the vessel and brought the NaOH solution from the side bulb into contact with the incubation mixture. This stopped the enzymatic reaction and sodium salt of HCN was formed.

One aliquot (0.025 ml of shoot extract and 0.250 ml of leaf extract, respectively) from the stopped incubation mixture was used for spectrophotometric measurement of cyanide. The sample was neutralized by adding the respective amount of 0.1 mol l^{-1} HCl (0.025 or 0.250 ml) and made up to a total volume of 5 ml by adding deionized water. The concentration of the chromogenic product was measured after 5 min of incubation. The concentration of cyanide was measured spectrophotometrically at 585 nm (Genesys 20, Thermo Spectronic, Madison, WI) by use of the Spectroquant® cyanide test (Merck). 1 mol of formed polymethine dye corresponds to 1 mol cyanide.

Quantification of Soluble Proteins

Shoot and leaf samples were analyzed for concentration of soluble proteins according to Bradford [1976]. Bradford reagent (Biorad Laboratories, Munich, Germany) was diluted 1:5 with water and 20 μl of each sample were added to 1 ml of diluted Bradford solution. Bovine serum albumin (BSA; Fluka Chemie AG, Buchs, Switzerland) at different dilutions was used as standard. After 5 min of incubation, concentration of protein was spectrophotometrically measured at 595 nm. We used the same individual plant extracts for protein measurements that were used for HCNp analyses. Thus, both parameters could be quantitatively related to the same individual sample. Quantitative effects of potential interference of plant phenolic compounds with plant protein during analyses were investigated in preliminary experiments in which individual samples were cut lengthwise while one subsample was analyzed with and the other without addition of polyvinylpolypyrrolidone (PVPP; Sigma-Aldrich, Buchs, Switzerland) before extraction ($N = 25$ shoot samples and $N = 33$ leaf samples). Protein concentration under addition of PVPP was never higher than in samples not treated with PVPP (data not shown).

All experiments presented here complied with the current laws of Germany and adhered to the legal requirements of the government of Madagascar.

Statistical Analyses

Data obtained from shoots were statistically analyzed using general linear model (GLM) for analysis of variance after a multivariate design with HCN per dry weight, protein per dry weight, and HCN per protein as variables and ''Type of Shoot,'' ''Plant Individual,'' and ''Position (Type of Shoot∗Individual)'' as fixed factors. The term ''Position (Type of Shoot∗Individual)'' is nested, because each measure for position of sample comes from only one combination of the type of shoot (ground shoot or branch shoot) and individual plant. We analyzed a total of 81 shoot samples. These were assigned to two different ''Types of Shoot'' ($N = 30$ samples per ground shoots and $N = 51$ samples per branch shoots), to seven different ''Plant Individuals'' ($N = 5$ samples per three ground shoots, and $N = 15$–18 samples of the four ground shoots that

additionally carried branch shoots), and to five different ''Positions'' in ground shoots, to three different ''Positions'' in branch shoots >20 cm, and to two different ''Positions'' in branch shoots <20 cm. Data obtained from leaves were statistically analyzed using the GLM for analysis of variance after a univariate design with ''Leaf Age'' and ''Position of Branchlet'' as fixed factors and ''Plant Individual'' as random factor. We analyzed a total of 192 leaf samples. These were assigned to four different ''Leaf Ages'' ($N = 48$ samples per leaf age), to eight different ''Positions of Branchlets'' ($N = 28$ samples per position), and to six different ''Plant Individuals'' ($N = 32$ samples per plant individual). All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) 16.0 (SPSS for Windows, SPSS, Chicago, IL).

RESULTS

Cyanide Concentration

Ontogenetic variability of cyanogenic potential (HCNp)

Analyses of different parts of *C. madagascariensis* revealed high ontogenetic variability of cyanide concentration (HCNp). We found mean ($\pm$SE) cyanide concentrations of 139.30 ± 19.32 for ground shoots ($N = 5$) and 217.67 ± 16.80 μmol HCN per gram dry weight for branch shoots ($N = 31$). The difference in HCNp between ground shoots and branch shoots was not significant [according to multivariate GLM: $F = 2.581$, df = 1, $P = 0.116$; Table II]. However, within shoots substantial ontogenetic variability of HCNp occurred (Fig. 3). The factor ''Position (Type of Shoot∗Individual)'' had a significant effect on HCNp (according to multivariate GLM: $F = 1.942$, df = 32, $P = 0.023$; Table II).

Variability of HCNp in ground shoots

Ground shoots of site A had increasing HCNp levels from young, still infolded leaves at the top of the stem (position 1), meristematic leaf bases (position 2) to highest concentrations in young pith (position 3), whereas pith of older stem segments (positions 4 and 5) showed a slight decrease in HCNp (Fig. 3). In contrast, shoots from site B exhibited highest HCNp in meristematic leaf bases (position 2) followed by a basipetal decrease in HCNp in young pith (positions 3–5). The total amount of accumulated cyanide was substantially lower in ground shoots at site A compared with site B. Shoots collected at site B contained 60% more cyanide per gram dry weight than shoots from site A (site A: 109.81 ± 17.88; site B: 183.53 ± 37.13 μmol HCN $g\,dwt^{-1}$; mean $\pm$ SE). However, the differences between sites were not significant (according to one-way ANOVA: $F = 1.999$, df = 1, $P = 0.169$), because position effects could not be included in statistical evaluations. The effect of ''Plant Individual'' on HCNp was highly significant (according to multivariate GLM: $F = 3.708$, df = 6, $P = 0.005$; Table II).

Variability of HCNp in branch shoots

For both sites, branch shoots possessed the same ontogenetic pattern of HCNp as the respective

TABLE II. Effects of ''Type of Shoot,'' ''Individual,'' and ''Position'' on Plant Traits

Source	Dependent variable	SS	df	*F*	*P*
Model	HCN dwt^{-1}	3,859,782.260	40	10.632	0.001
	Protein dwt^{-1}	232,196.477	40	7.330	0.001
	HCN $protein^{-1}$	1,378.187	40	26.277	0.001
Type of shoot	HCN dwt^{-1}	23,426.987	1	2.581	0.116
	Protein dwt^{-1}	1,667.568	1	2.106	0.154
	HCN $protein^{-1}$	0.312	1	0.238	0.628
Plant individual	HCN dwt^{-1}	92,161.745	6	3.708	0.005
	Protein dwt^{-1}	4,421.839	6	1.692	0.147
	HCN $protein^{-1}$	29.171	6	3.708	0.005
Position (Type of Shoot∗Individual)	HCN dwt^{-1}	563,936.684	32	1.942	0.023
	Protein dwt^{-1}	17,290.694	32	0.682	0.867
	HCN $protein^{-1}$	120.210	32	2.865	0.001
Error	HCN dwt^{-1}	372,122.644	41		
	Protein dwt^{-1}	32,468.326	41		
	HCN $protein^{-1}$	53.759	41		
Total	HCN dwt^{-1}	4,231,904.904	81		
	Protein dwt^{-1}	264,664.803	81		
	HCN $protein^{-1}$	1,431.946	81		

Results were obtained using the general linear model (GLM) for analysis of variance after a multivariate design with HCN per dry weight, protein per dry weight, and HCN per protein as variables. The term ''Position (Type of Shoot∗Individual)'' is nested, because each measure for position of sample comes from only one combination of the type of shoot (ground shoot or branch shoot) and individual plant. All terms were set as fixed factors.

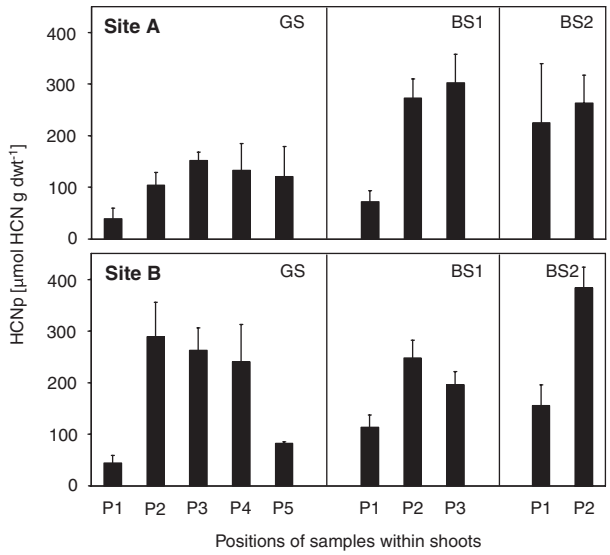

Fig. 3. HCNp of shoots. Two sites (A and B) were included in the analysis. Of each site, ground shoots (GS), branch shoots >20 cm (BS1), and branch shoots <20 cm (BS2) were included. Within ground shoots, samples were taken at five positions (P1–P5), within branch shoots >20 cm, at three positions (P1–P3), and within branch shoots <20 cm, samples were taken at two positions (P1 and P2). Values are means ± SE.

ground shoots (Fig. 3). At site A, branch shoots of more than 20 cm (Fig. 3) revealed lowest cyanide concentrations in infolded leaves at top of the shoot (position 1), intermediate concentration in meristematic leaf bases (position 2), and highest concentrations in young pith (position 3). At site B, highest HCNp was again found in meristematic leaf bases (position 2) whereas both young leaves at the top as well as young pith showed lower HCN concentrations. In branch shoots <20 cm, pith was not developed yet, thus only positions 1 and 2 could be considered for analyses. At both sites, meristematic leaf bases (position 2) had higher HCNp compared with infolded leaves at position 1. In contrast to ground shoots, HCNp in branch shoots of both types (< and >20 cm) indicated no clear site-specific differences.

HCNp in leaves and leaf-developing shoots

The single shoot developing leaf-bearing branchlets collected at site C exhibited lower HCNp in positions 1–3 than ground and branch shoots in positions 1–3 (Fig. 4). In contrast to all shoots, leaves of different ontogenetic stages contained no cyanide at all.

Concentration of Soluble Proteins

Protein in ground shoots

Protein concentration in ground shoots at site A and B corresponded to the observed pattern of HCNp. We found highest concentrations of proteins at position 3 (site A) and at position 2 (site B). In

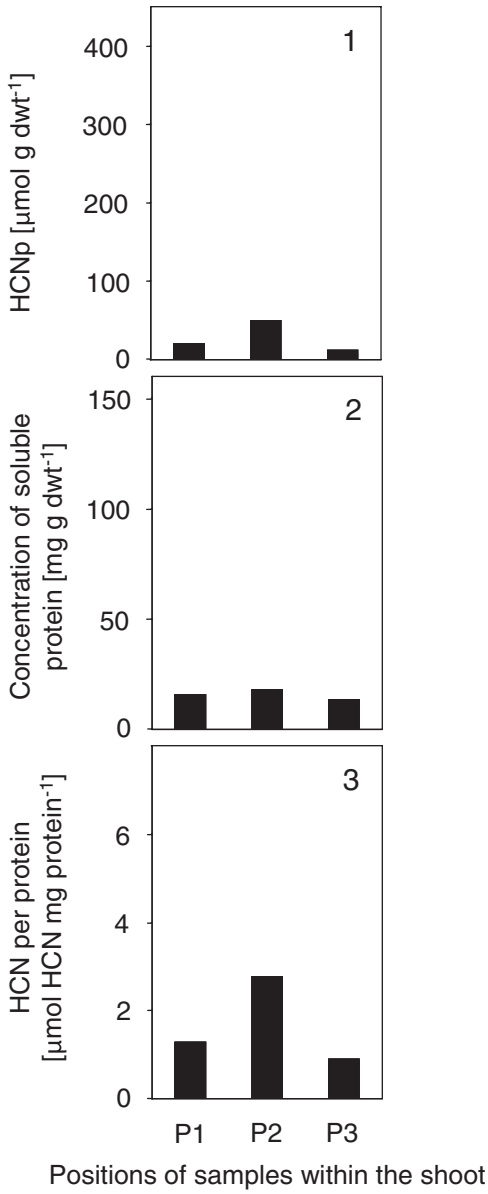

Fig. 4. HCNp in ground shoot developing leaves. A single shoot (GSL, site C) was analyzed for HCNp (1), soluble proteins (2), and HCN per protein (3). Values represent single measurements.

shoots collected at site A concentration of soluble proteins was almost homogeneous among positions 2–5 (Fig. 5). Protein concentration in shoots did not differ significantly between sites (according to one-way ANOVA: $F = 0.362$, df = 1, $P = 0.549$).

Protein in branch shoots

Protein concentrations in branch shoots tended to be higher compared with ground shoots (Fig. 5). At both sites, we found highest concentrations of protein in meristematic leaf bases (position 2) of branch shoots >20 cm. Young leaves at the top possessed lowest and young pith intermediate concentrations. Branch shoots <20 cm revealed the highest protein

concentrations among all shoots at site A. These branch shoots exhibited highest concentration of proteins (100.4 ± 55.3 mg protein g dwt^{-1}) in young infolded leaves (position 1). At site B, highest protein concentrations were observed for meristematic leaf bases (position 2) corresponding to ontogenetic patterns observed for branch shoots >20 cm and ground shoots. However, the factors "Type of Shoot" and "Position (Type of Shoot∗Individual)" had no significant effect on the concentration of soluble proteins in shoots (according to multivariate GLM: $F = 2.106$, df = 1, $P = 0.154$ and $F = 0.628$, df = 32, $P = 0.867$; Table II), whereas "Plant Individual" had a significant effect on protein content in shoot material (according to multivariate GLM: $F = 1.692$, df = 6, $P = 0.005$; Table II).

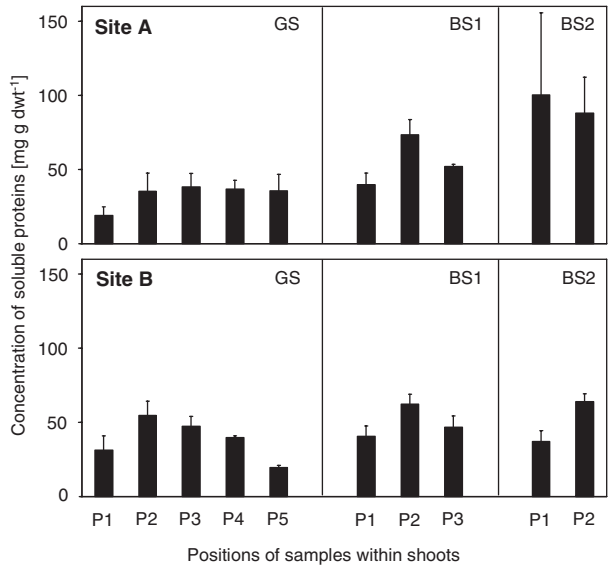

Fig. 5. Concentration of soluble proteins in shoots. Two sites (A and B) were included in the analysis. Of each site, ground shoots (GS), branch shoots >20 cm (BS1), and branch shoots <20 cm (BS2) were included. Within ground shoots, samples were taken at five positions (P1–P5), within branch shoots >20 cm, at three positions (P1–P3), and within branch shoots <20 cm, samples were taken at two positions (P1 and P2). Values are means ± SE.

Protein in leaves

Leaves of branchlets from different mature shoots showed differences in leaf protein concentration depending on ontogenetic stage (except "Plant 1" from site A; Fig. 6). According to univariate GLM with "Leaf Age" as factor, the differences in content of soluble proteins between the four defined stages were highly significant ($F = 13.441$, df = 3, $P < 0.001$; Table III). Within leaf-bearing branchlets we found a gradient of protein concentration from unfolded leaves (lowest protein concentration) to mature leaves (Fig. 6). However, the "Position of Branchlets" on the main shoot, did not have any significant effect on the concentration of proteins (according to univariate GLM: $F = 0.886$, df = 7, $P = 0.519$; Table III). Between "Plant Individuals," differences in protein content were highly significant (according to univariate GLM: $F = 8.972$, df = 4, $P < 0.001$; Table III). Protein concentrations in leaves of site

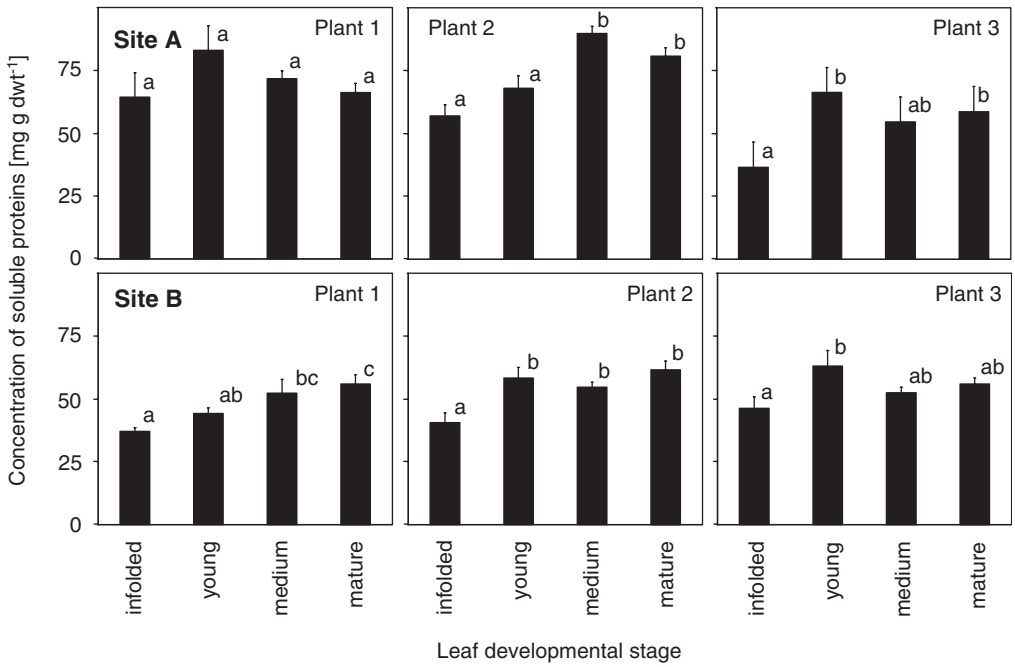

Fig. 6. Concentration of soluble proteins in leaves. Six individual plants from two sites (three individuals per site A and B) were analyzed for concentration of soluble proteins in leaves of different developmental stages. Of each ontogenetic leave stage, eight samples (each one from eight leaf-bearing branchlets per individual plant) were included. Values are means ± SE. Significant differences between leaf stages were calculated by a post hoc test (LSD; $P < 0.05$) after one-way ANOVA and are indicated by different letters at the columns.

TABLE III. Effects of Leaf Developmental Stage on Protein Per Dry Weight in Leaves

Source	SS	df	*F*	*P*
Leaf age				
Hypothesis	3,160.686	3	13.441	<0.001
Error	235.148	155		
Position of branchlet				
Hypothesis	208.435	7	0.886	0.519
Error	235.148	155		
Plant individual				
Hypothesis	2,109.791	4	8.972	<0.001
Error	235.148	155		

Results were obtained using the general linear model (GLM) for analysis of variance after a univariate design with ''Leaf Age'' and ''Position of Branchlet'' as fixed factors and ''Plant Individual'' as random factor.

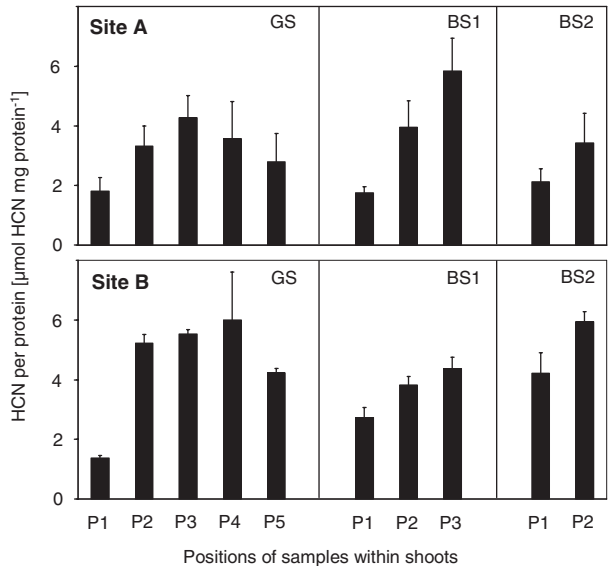

Fig. 7. Concentration of HCN per protein in shoots. Two sites (A and B) were included in the analysis. Of each site, ground shouts (GS), branch shoots >20 cm (BS1), and branch shoots <20 cm (BS2) were included. Within ground shoots, samples were taken at five positions (P1–P5), within branch shoots >20 cm, at three positions (P1–P3), and within branch shoots <20 cm, samples were taken at two positions (P1 and P2). Values are means ± SE.

A and B revealed clear site-specific differences. Leaves of site A showed significantly higher concentration of protein than leaves of site B (according one-way ANOVA: $F = 32.137$, df = 1, $P < 0.001$).

HCN per Protein

The quantitative relation of HCN per protein in ground and branch shoots (Fig. 7) resembled the observed ontogenetic pattern of HCNp (Fig. 3). The factor ''Position (Type of Shoot∗Individual)'' of a respective sample had a highly significant effect on the concentration of HCN per protein (according to multivariate GLM: $F = 2.865$, df = 32, $P = 0.001$; Table II). The factor ''Type of Shoot'' had no significant effect on the relation of HCN per protein (according to multivariate GLM: $F = 0.238$, df = 1, $P = 0.628$; Table II), thus no significant differences occurred in HCN per protein between ground and branch shoots. The effect of ''Plant Individual'' on HCN per protein was significant (according to multivariate GLM: $F = 3.708$, df = 6, $P = 0.005$; Table II). Comparing HCN per protein of all shoots (ground and branch shoots) revealed significantly higher values for site B (according to one-way ANOVA: $F = 5.901$, df = 1, $P = 0.017$).

DISCUSSION

Since the late 1980s researchers have investigated home range size, group composition, and behavior of the three bamboo lemur species sympatrically occurring in the Ranomafana area [e.g. Meier et al., 1987; Wright & Randriamanantena, 1989]. Although considerable research has been conducted on diet selection and foraging behavior as well [e.g. Overdorff et al., 1997; Tan, 1999] only one previous study had been carried out addressing the impact of bamboo's cyanide concentration on the three bamboo eating lemurs [Glander et al., 1989]. This study by Glander and co-workers provided important first results on plant biochemical parameters, however, was complicated by methodical difficulties of quantitative HCN analyses under field conditions. In the present study, we employed a highly precise method by spectrophotometrically quantifying cyanide in fresh plant material. This allows for detection of minimal quantitative differences in HCN concentration in different samples. Referring the amount of cyanide to the dry weight of samples rather than to the wet weight further eliminates variation owing to differing water content of plant material. As cyanogenesis is known to strongly depend on plant ontogeny and plant organ, we carried out small-scaled analyses of different parts of *C. madagascariensis*. These high-resolution analyses of plant features contribute to filling the gap of understanding the role of food plant biochemistry on lemur herbivores in natural systems.

In addition to toxic compounds, nutritive plant components crucially affect quality of browse [e.g. Awmack & Leather, 2002; Ball et al., 2000; Mattson, 1980] and the quantitative relationship of toxins to nutrients is a crucial measure of plants' overall food quality. Therefore, we conducted a simultaneous quantitative analysis of the variability of cyanogenesis and soluble protein concentration as one detrimental and one beneficial plant feature. We differentiated among different ontogenetic stages and plant organs.

In shoots, concentrations of all parameters investigated, i.e., cyanide per dry weight, protein per dry weight, and cyanide per protein, varied among ontogenetic stages of the respective parts. Cyanide and protein concentrations correlated and

the maximum amounts of both parameters were found in the ontogenetically youngest shoot parts (i.e., position 2) (Figs. 3–5, 7). This finding provides a causal explanation why bamboo lemurs select the highest cyanogenic parts when they are seasonally available [Tan, 1999]. The young parts are both at the same time the most toxic and the most attractive parts—i.e., the most desirable protein source to herbivores. Nevertheless, compared with average concentration of extractable proteins in forest tree leaves in the Ranomafana area (~6.4% of dry weight) as investigated by Ganzhorn [1992] protein concentration in shoots and leaves of bamboo is lower (ground shoots: $3.5 \pm 0.3\%$; branch shoots: $5.5 \pm 0.4\%$; leaves: $5.9 \pm 1.3\%$). Thus, at least for shoots—which represent an important seasonal food source—findings suggest that bamboo lemurs might be limited in protein availability.

In general, quantitative analyses of plant soluble protein using Bradford methodology are complicated by interference of plant phenolic compounds with proteins during preparation of samples [Mattoo et al., 1987]. However, as we conducted comparative preliminary experiments with effects of phenole-binding agents (PVPP) on quantitative analyses of soluble protein in different organs of giant bamboo—and we found no differences in plant protein concentration with and without addition of PVPP—our results obtained by the Bradford assay were reliable [e.g. Faeth, 1985; Jones et al., 1989]. Furthermore, these preliminary findings indicate low concentrations of phenolics in bamboo.

The giant bamboo/bamboo lemur system is highly suitable to study quantitative and functional association of host plant quality and herbivore fitness under natural conditions. In contrast to most other tropical herbivore–plant interactions this system has an extremely low diversity at the producers' and most of all the consumers' level. Utilization of *C. madagascariensis* as food source appears to be restricted to a few specialist herbivores, such as bamboo lemurs, which are able to tolerate cyanide in high amounts. For *H. aureus*, Glander et al. [1989] calculated a daily intake of 1,850 μmol cyanide kg^{-1}, which corresponds to 12 times the lethal dose of cyanide based on a averaged lethal dose of mouse (137.04 μmol kg^{-1}), dog (148.15 μmol kg^{-1}), cat (74.07 μmol kg^{-1}), rat (370.37 μmol kg^{-1}), and sheep (74.07 μmol kg^{-1}) [Christensen & Fairchild, 1976; Harborne, 1982; Montgomery, 1969]. Thus, *H. aureus* and the other bamboo lemurs (most of all *P. simus*) are considered to be strongly adapted to high cyanide concentrations in *C. madagascariensis*. Sensitive spectrophotometric analyses used here revealed almost the four-fold amount of accumulated cyanide in plant tissues as reported by the earlier study of Glander et al. [1989] indicating an even more extreme adaptation of bamboo lemurs to highly cyanogenic food.

The specialist-herbivore paradigm predicts that specialist herbivores are less affected by toxic compounds of their host plants than generalists [Van Dam et al., 2000; Van Der Meijden, 1996]. However, this does not mean that specialists are not negatively affected by plant toxins at all. Our recent studies demonstrated reduced fitness of the specialist Mexican bean beetle (*Epilachna varivestis*) on high cyanogenic lima bean (*Phaseolus lunatus*) compared with lower cyanogenic host plant genotypes [Ballhorn et al., 2007]. Although these results were obtained using an insect–plant experimental system, results might be transferred to vertebrate herbivores, because special physiological adaptations (e.g. enhanced synthesis of enzymes involved in detoxification of cyanide) generally create costs [e.g. Iason, 2005]. The major mechanism of cyanide detoxification in mammals is the conversion of cyanide to thiocyanate by activity of rhodanase [e.g. Nahrstedt, 1985]. This enzymatic reaction requires presence of *S*-containing amino acids, such as methionine or cysteine [Montgomery, 1969]. Glander et al. [1989] argued that based on nutritional data on Asian bamboo species used as browse by giant panda [Schaller et al., 1985] the concentration of methionine and cysteine in *C. madagascariensis* has to be considered low. However, whether low concentration of *S*-containing amino acids as observed in Asian bamboos hold true for *C. madagascariensis* and other Malagasy bamboos remains elusive.

For giant bamboo we found that ontogeny is an important factor determining biochemical characteristics (Figs. 3–7). Although subject to intraspecific variability, within sites the same ontogenetic patterns of HCN and protein concentration that were found in ground shoots could be observed in branch shoots (Figs. 3, 5, and 7). Analysis on the site-specific level (comparing site A and B), revealed strong differences in the relation of cyanide per protein accumulated in ground shoots (Fig. 7) as well of protein concentration in leaves (Fig. 6). We found significantly higher cyanide concentration per protein in shoots at site B, whereas protein concentration in leaves at the same site were significantly lower compared with site A. As both plant parts are consumed by bamboo lemurs [Tan, 1999], our results suggest spatial patterns of browse quality of *C. madagascariensis*. However, these findings on site-specific differences in food plant quality of giant bamboo are considered preliminary owing to small sample sizes and only comparing two different sites.

Although speculative at this time, in addition to effects of environmental degradation, limited distribution of highly specialized bamboo lemurs, *H. aureus* and most of all *P. simus* might depend on such patterns of biochemical characteristics among bamboo populations. Whether variability of cyanogenic features is based on bamboo genetics or on phenotypic plasticity and whether quantitative

variability can be correlated to site-specific ambient conditions, such as water and nutrient availability, will be analyzed in further studies using larger sample sizes. This approach will help to understand the nutritive demands of bamboo lemur species and is promising in providing information for successful conservation programs.

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