

## Leaf traits – BCI (draft 1 December 2007)

This document describes the rationale, methods and calculations used for BCI leaf trait determinations. BCI leaf collections were also used for paleobotanical and, for lianas only, barcoding studies. This document also describes how leaves are preserved for DNA barcoding and scanned for paleobotanical studies.

### Rationale

The CTFS plant traits working group selected six leaf traits to be measured across CTFS sites. Table 1 presents these six traits, their rationale, and sample sizes recommended by Cornelissen et al. (2003). The final working group report is Appendix A at the end of this document. Five additional traits were measured on BCI. These include leaf lamina thickness ( $\mu\text{m}$ ), leaf fractal toughness ( $\text{J m}^{-2}$ ), carbon concentration, and nitrogen and carbon isotopic composition. Leaf thickness should be measured at all CTFS sites.

Table 1. Leaf functional traits. ‘+’ marks denote well established associations with environmental gradients in climate or disturbance regime, competitive ability, and defense against herbivores and pathogens. Recommended sample sizes are numbers of individuals and numbers of leaves per individual from Cornelissen et al. (2003).

Trait (units)	Literature association of trait with				Sample Size	
	Response to		Com- petitive ability	Defense	Indi- viduals	Items
	Climate	Distur- bance				
Size ( $\text{mm}^2$ )	+		+	+	10	2
Specific leaf area ( $\text{m}^2 \text{kg}^{-1}$ )	+		+	+	5-10	2
Leaf thickness ( $\mu\text{m}$ )						
N concentration ( $\text{mg g}^{-1}$ )	+	+	+	+	5-10	2
P concentration ( $\text{mg g}^{-1}$ )	+	+	+	+	5-10	2
Dry matter content ( $\text{mg g}^{-1}$ )				+	5-10	2
Fiber content ( $\text{mg g}^{-1}$ )				+	5-10	2

### Methods

Methods follow the recommendations of Cornelissen et al. (2003) unless otherwise stated.

#### Methods Leaf Collection

We collected leaves between August and December 2007 from the six largest and the six smallest individuals of each free-standing tree species in the BCI 50-ha plot. We only collected from the six largest individuals for shrubs. We used the 2005 census to identify these individuals and chose six individuals at random when several individuals were tied in size. We collected leaves from every individual for tree species with  $\leq 12$  individuals and shrub species with  $\leq 6$  individuals.

We collected entire leaves including petioles. We did not collect the attached twigs, as recommended by Cornelissen et al. (2003), because it was impossible to collect twigs for most canopy trees and lianas and to minimize damage to the smallest individuals and to lianas (petioles emerge from the main stem of most lianas).

We collected three or more leaves from each individual; however, we never collected more than 10% of the leaves from an individual. Thus, we collected zero, one and two leaves for individuals with 1-9, 10-19, and 20-29 leaves, respectively.

We collected leaves between 0900 and 1400 hours. We immediately placed leaves in “Ziplock” bags with a slightly moistened piece of paper towel and placed sealed “Ziplock” bags in a cooler with ice. Many leaves were processed the afternoon they were collected. Others were stored overnight in a cold room at 3° C and processed the next morning. A few leaves were collected on Friday and processed the following Monday. Morphological traits were unaffected by storage time (Appendix B). Leaves for DNA bar coding were treated differently (see Methods Bar Coding).

Leaf traits vary with light availability, and it is crucially important to collect leaves under standard light conditions. Cornelissen et al. (2003) recommend collecting leaves exposed to full sun whenever possible and leaves from the top of fully shaded individuals (most juveniles as well as adults of shrubs and understory treelets). We followed this recommendation. We collected leaves by hand for the shortest individuals, with a pole cutter for individuals up to 12 m tall, and with a shotgun for taller individuals. We used shotgun shells with non-toxic steel shot.

We also recorded a five-point, qualitative crown exposure (CE) index to estimate light availability for the crown of each individual. Dawkins and Field (1978) developed the CE index. Table 2 describes the CE index.

Table 2. The crown exposure index (adapted from Clark and Clark (1992)).

Value of crown exposure index	Definition
5	Crown completely exposed (emergent trees)
4	Full overhead light (> 90% of the vertical projection of the crown exposed to direct light when the sun is directly overhead; canopy trees and shorter trees located in canopy gaps)
3	Some overhead light (10-90% of the vertical projection of the crown exposed to direct light when the sun is directly overhead; crown partially overtopped by a taller tree)
2	Lateral light (< 10% of the vertical projection of the crown exposed to direct light when the sun is directly overhead; crown receives direct light from a canopy gap located to one side of the crown)
1	No direct light

## Methods Leaf Morphology

We measured leaf morphological traits for up to three leaves for each individual before 10 November and for up to two leaves for each individual after 10 November 2007. Leaf sample size was reduced on 10 November because collections were going too slowly and morphological measurements were the rate limiting step. Sample size was occasionally smaller for small individuals (we never collected more than 10% of the leaves on a plant) and for the tallest individuals because we did not fire a second shot if the shotgun brought down just one or two leaves.

Leaf morphological traits were usually measured in the order presented below. The traits most sensitive to desiccation in the air conditioned lab were measured first. Each measured leaf was also lettered with a “Sharpie” magic marker so that fresh measurements could be associated with dry masses several days later. Appendix C is the data form used for all morphological measurements except fractal toughness.

Cornelissen et al. (2003) recommend rehydrating leaves before determining fresh mass and area. We conducted trial rehydrations for several species and found that our fresh leaves were fully hydrated (Appendix D). We therefore did not rehydrate leaves.

*Leaf fresh mass (g)* – We measured leaf fresh mass immediately upon removing leaves from the cooler and “Ziplock” bag using electronic balances accurate to 0.1 mg. Leaf fresh mass included the petiole for simple leaves and the petiole, rachis and petiolules for compound leaves. Figure 1 defines these terms for a compound leaf.

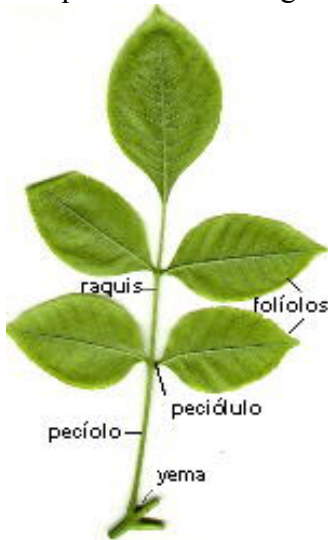


Figure 1. A compound or pinnate leaf with major parts defined. Image taken from [http://www.biologia.edu.ar/botanica/tema2/tema2\\_2dicot.htm](http://www.biologia.edu.ar/botanica/tema2/tema2_2dicot.htm)

*Leaf size (mm<sup>2</sup>)* – We measured leaf area using a LI-3100C leaf area meter (Licor, Lincoln, Nebraska). We removed leaflets from the petiole and rachis for compound (pinnate) and doubly compound (bipinnate) leaves to ensure that leaflets did not overlap as they passed through the leaf area meter.

Cornelissen et al. (2003) recommend that leaf size should exclude the petiole, rachis and petiolules (lamina only) while the leaf area used to calculate specific leaf area (SLA) should include the petiole, rachis and petiolules as well as the lamina. To obtain both measures of leaf area, we first recorded the area of the entire leaf including petiole, rachis, petiolules and lamina using the leaf area meter. We then recorded the area of petioles, rachises and petiolules. We used the leaf area meter for winged petioles and rachises. We measured length (L) and diameter (D) at the midpoint to estimate the area ( $L \times D$ ) of unwinged petioles, rachises and petiolules. We measured one representative petiolule for each leaf. Leaf size was estimated as the area of the entire leaf minus the summed areas of petioles, rachises and petiolules.

*Fresh mass of leaf disc (g)* – A leaf punch with a diameter of 2 cm was then used to collect a disc of leaf lamina. This disc excluded major veins unless the leaf was very small. The fresh mass was determined immediately.

*Leaf thickness ( $\mu\text{m}$ )* – We determined leaf thickness with a micrometer screw gauge (X company, city, country) accurate to 0.01 mm. This measurement was made in the broadest part of the leaf avoiding major veins for virtually all leaves. The micrometer contacted the leaf over a circle of X mm diameter.

*Leaf fractal toughness ( $\text{J m}^{-2}$ )* – The first two leaves measured for each individual were then returned to the ice chest in sealed “Ziplock” bags. A piece of leaf (1.5 cm length and 0.6 to 0.7 cm width) was removed and mounted in a portable mechanical toughness tester that controls the force and directs the fracture of a leaf using cobalt-steel scissors (Darvell et al. 1996). We fractured leaves with a single transverse cut perpendicular to the midrib. When the midrib was too thick, we made a fracture perpendicular to a secondary vein. We used the cut.vi application of Lab View 6.1 to obtain the leaf fracture measurements.

*Dry masses (g)* – We dried all leaves for 72-96 h at 60°C and then recorded the dry mass of the lamina (including the leaf disc and the piece used to determine fractal toughness), petiole, rachis and petiolules separately with balances accurate to 0.1 mg.

Before drying, we placed all parts of a single leaf (lamina, leaf disc, petiole, rachis and petiolules) together in one paper bag and labeled the bag with the tag number of the tree and the letter of the leaf. We then placed the 1, 2 or 3 bags for an individual plant inside a paper bag labeled with the tag number of the plant. Finally, we discarded the petiole, rachis and petiolules of any extra leaves and placed them in the outer bag. The bag was then placed in a convection oven. Multiple tests were performed to determine that mass was indeed constant after 72 h of drying.

There were at least three exceptional species whose leaf morphologies defied the above protocol. This section will be written when we are done. We must still solve the problem posed by *Acacia melanoceras* and *Enterolobium schomburgkii*.

Liana leaflet modified to become tendril. We recorded tendril length, diameter and fresh and dry weights separately. How many species? Just one? X

The palmately compound leaf of this neotropical liana, *Anemopaegma orbiculatum* (Family Bignoniaceae), has one of the leaflets modified as a tendril.

<http://www.botgard.ucla.edu/html/botanytextbooks/generalbotany/typesofshoots/tendril/a1178tx.html>



We took fresh and dry mass of the modified leaflet separately from the other leaflets.

Leaf traits were not determined for *Acacia melanoceras* and *Enterolobium schomburgkii* because their leaflets were too small and leaflets close after being collected.

## Methods Leaf Nutrients

Leaf nutrient concentrations were determined for leaf lamina (with all veins) only following the recommendation of Cornelissen et al. (2003). We discarded all petioles, rachises and petiolules after dry masses were determined. Lamina were then ground to a powder and stored. Ben Turner determined ... X

## Methods Leaf Scanning

Beginning on Sept. 26, 2007, leaves were also scanned to obtain outlines for morphometric analysis. After collection but before measurement of Cornelissen et al. (2003) traits, leaves were placed abaxial side down on a flat-bed scanner and scanned to grey-scale tagged image format (.tif) digital images. Each image also included a 1 by 5 cm. scale bar as an area control to be used during image processing. Resolution of initial images was 600 dpi, but this was reduced to 300 dpi after Oct. 18. Leaves that were too large for the scanner bed were cut and scanned as multiple images for subsequent manual reattachment. In the case of compound leaves, only a single leaflet and petiolule was scanned.

## Methods Leaf DNA Collection

We collected liana leaves only for DNA bar coding. We treated these leaves exactly as we treated all other leaves until they reached the laboratory (see *Methods: Leaf Collection*). We placed leaves for DNA analyses in sealed “Ziplock” together with dry-rite as soon as they reached the laboratory. The dry-rite was checked after 24, X and X hours and replaced as necessary. The leaves were judged to be dry when the dry-rite no longer had to be replaced. We then placed individual bags into larger “Ziplock” bags also with dry-rite so that each sample was double bagged with dry-rite in each bag.

## Calculations

volume  $[L \times \Pi \times (D/2)^2]$

## Literature Cited

Clark, D. A., and D. B. Clark. 1992. Life history diversity of canopy and emergent trees in a neotropical rain forest. *Ecological Monographs* 62:315-344.

Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, ter Steege H, Morgan HD, van der Heijden MGA, Pausas J, Poorter H (2003) Handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* 51, 335-380 ([http://www.igbp.kva.se/cgi-bin/php/sciencehistory.show.php?section\\_id=11&article\\_id=328](http://www.igbp.kva.se/cgi-bin/php/sciencehistory.show.php?section_id=11&article_id=328))

Darvell, B. W., P. K. D. Lee, T. D. B. Yuen, and P. W. Lucas. 1996. A portable fracture toughness tester for biological materials. *Measurement Science & Technology* 7:954-962.

Dawkins HC, Field, DRB. 1978. A long-term surveillance system for British woodland vegetation. Department of Forestry, Oxford Univ, Oxford.

Appendix A – working group report

Appendix B – analysis of possible storage time effects on morphological leaf traits

Appendix C – leaf morphology data form

## Appendix D – trial rehydrations performed by JP Drury in October 2007

JP Drury collected branches from the crane and put them in a sealed “Ziploc” bag on ice. In the lab, he took three leaves from the branch and got their fresh mass and area immediately. He then put each of the remaining 12 leaves on the branches in a small cup (about the size of the cups you have to pee in at the doctors) with their petioles submerged in water and the lamina exposed to the air. He then placed each cup in an individual ziploc bag and sealed it. This was all done in the closet of your office where the filing cabinets are. The closet was darkened with a sign on it so no one entered. He took three leaves out of the closet and removed them from the water at one hour intervals over the next four hours and immediately took the fresh mass and the area of each leaf. The leaves were dried at 60° for more than three days. The rehydration ended after four hours because leaf lamina had obvious dark spots indicating air spaces were filling with water.

Results: SLA was unaffected by rehydration (Fig. D1). LDMC was unaffected for TRIO but appears to have increased for BONM (Fig. D2). This might reflect overhydration for BONM. Additional rehydration experiments are needed though.

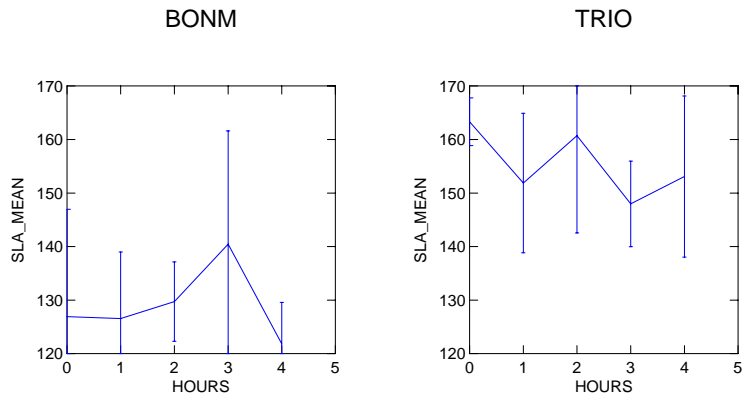


Figure D1. The relationship between SLA and leaf rehydration time (hrs). Error bars are standard errors.

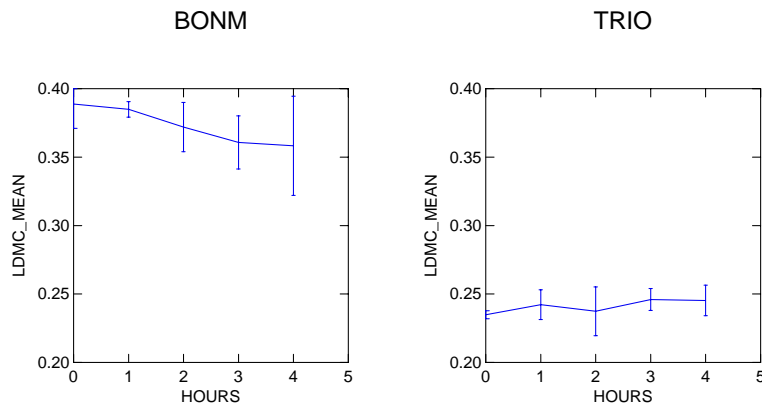


Figure D2. The relationship between LDMC and leaf rehydration time (hrs). Error bars are standard errors.