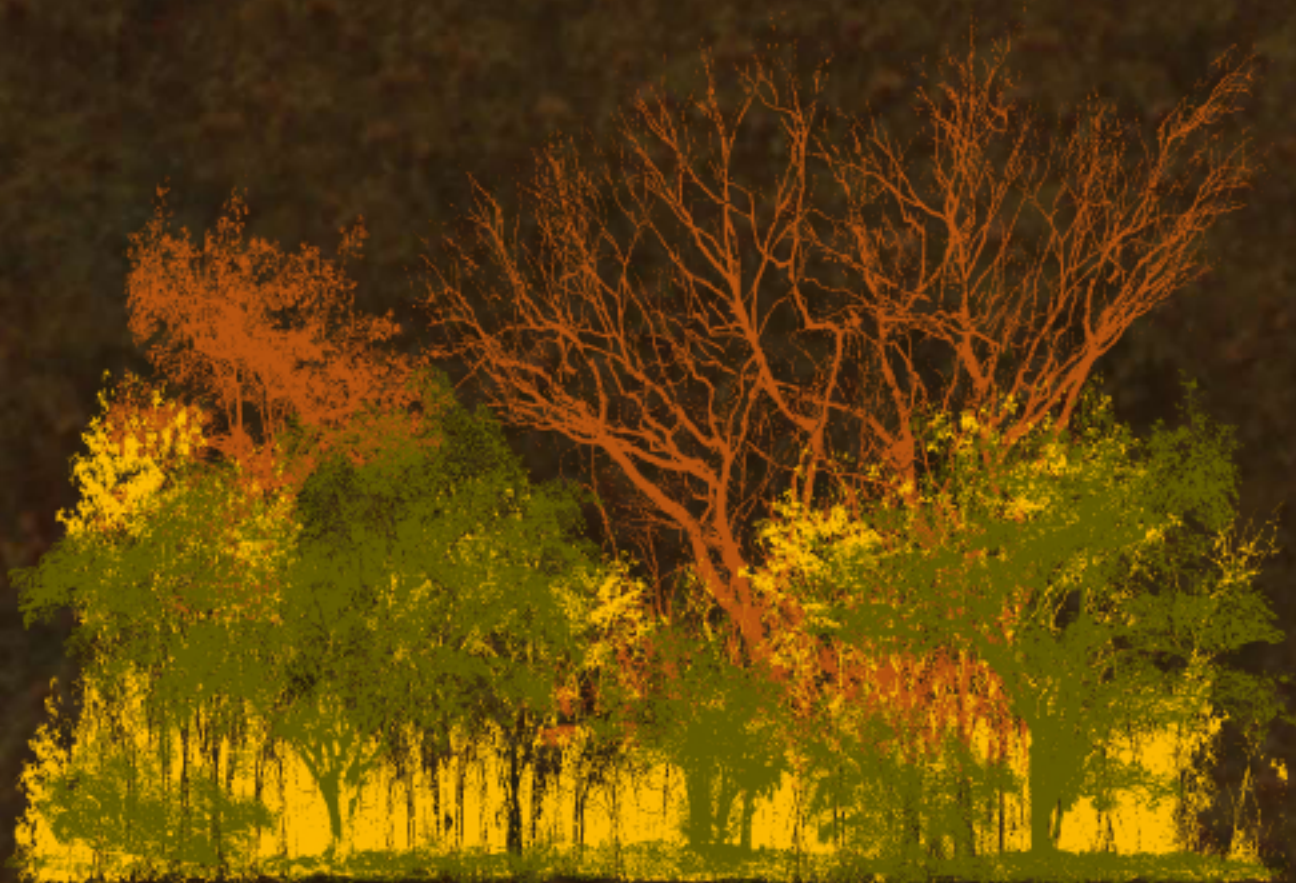


**MANUAL OF METHODS**

# **HUMAN, ECOLOGICAL AND BIOPHYSICAL DIMENSIONS OF TROPICAL DRY FORESTS**



**Editors:** Jafet M. Nassar, Jon Paul Rodríguez,  
Arturo Sánchez-Azofeifa, Theresa Garvin,  
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Instituto Venezolano de Investigaciones Científicas

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Coordinación editorial: Pamela Navarro

Fotografía: Tropi-Dry.

Diseño y diagramación: Pascual Estrada

Impresión: Gráficas Lauki C.A.

Depósito legal: If66020085744073

ISBN: 978-980-261-103-4

Caracas, Venezuela

2008

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**A collaborative Research Network for the study of  
Tropical Dry Forests in the Americas**

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## PREFACE

Current processes of global climate change, combined with the fast rate of modification of natural landscapes throughout the tropical belt of the American continent, have finally brought about a scenario forecasted and repeatedly presented for more than half a century by academic institutions and scientists. The situation is such that public opinion is pressing governments to take action for conservation of remaining natural areas, restoration and rehabilitation of deteriorated ecosystems, and implementation of agricultural production framed within ecological guidelines. There are no easy solutions for the ecological instability determined by diminished agricultural productivity, environmental disasters caused by increased variability in rainfall distribution and intensity, and socio-political problems associated with human population growth and increasing economic inequalities.

The recognition of the global dimension of ecological disturbances led in the Americas to the development of environmental projects that, due to the complexity of the problems to be tackled and their geographical extension, had to be approached on the basis of international cooperation. Pioneering efforts in this direction were the Amazonas project developed by IVIC in cooperation with US (University of Georgia) and German institutions (Max Planck Institut and University of Hamburg)<sup>1</sup>, and international programs launched by the International Union of Biological Sciences (IUBS) (Responses of Savanna Ecosystems to Stress and Disturbance<sup>2</sup> and Soil Biology and Global Change<sup>3</sup>). Within the Amazon basin in South America a large scale project with wide ranging objectives was the still ongoing Large Biosphere-Atmosphere Experiment led by Brazil with the strong cooperation of scientists and institutions from throughout the world<sup>4</sup>. Those projects were designed to understand scientifically the nature of changes taking place in large areas at a continental scale. However, the approach did not include the human component in the final analysis the element responsible for the orientation, or lack thereof, of the processes of land-use change in the tropics. The establishment of the Interamerican Institute for the Study of Global Change (IAI)<sup>5</sup> constituted a change of paradigm, as it was focused, from the beginning, on the integration of this component into the framework of the natural sciences approach.

The lack of a common methodology that allows result comparability is among the many difficulties to overcome when establishing cooperative international projects to the analysis of similar systems in different geographic areas. The present book provides a methodological framework for the research program to be developed within one of the multinational projects currently funded by IAI (Collaborative Research Network Program II). The book describes a wide range of methods that encompass ecological procedures (ecosystem structure, soil microbiology, phenology, biotic interactions), remote sensing procedures (spatial information, image processing) and social science procedures (human-ecosystems relationships, socio-economic indicators, and environmental policy). In all sections, emphasis has been laid upon the dynamics of the systems investigated, so that the analyses conducted on superimposed scales provide a sequential picture of the processes of deterioration and/or recovery of heavily disturbed systems. The methodological philosophy embedded in the book combines observations at the local, small scale level (communities, populations, people and towns) with landscape evaluations provided by remote sensing analysis. The book includes also guidelines for the presentation of the results that should be exchanged between groups and sites.

The wide range of methodological topics covered in the book were not all touched with similar depth, probably reflecting the strengths of the participating groups and the priorities in the IAI project itself. Nevertheless, the descriptions are supported by well over 100 references that certainly complement the material included in the book.

This book was developed as a manual of methods designed specifically for tropical dry forests with emphasis on the topics included in the IAI project, but it has wide application potential, as the global change questions addressed with these methods are similar to other terrestrial ecosystems such as savannas, wet forests, or paramos. Therefore, this book represents a welcome and useful contribution to the study of tropical ecosystems.

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## WHY A MANUAL OF METHODS FOR TROPICAL DRY FOREST RESEARCH?

Tropi-Dry is a collaborative research network sponsored by the Inter-American Institute for Global Change Research (IAI) under their Collaborative Research Network (CRN-2) program.

Tropi-Dry's goal is to bring together researchers in conservation biology, ecology and evolution, remote sensing and geographic information systems, sociology, anthropology, policy analysis, and forestry to develop a comprehensive "state of the art" understanding of the status of tropical dry forests (primary and secondary) in the Americas. Tropi-Dry's research focuses on developing a common multidisciplinary strategy in collaboration with local and national policy making organizations in order to produce comprehensive and comparative land use/policy studies in tropical dry regions in the natural and social sciences.

At present, Tropi-Dry incorporates researchers and institutions from Brazil, Canada, Costa Rica, Cuba, Mexico, United States of America and Venezuela. Active study sites have been established in Brazil, Costa Rica, Cuba, Mexico and Venezuela, though we aim to gradually expand our study site network to other tropical regions within the Americas, as well as to Africa and Asia.

From the natural sciences perspective, Tropi-Dry works towards the development of a comprehensive and standardized protocol for recognizing and comparing tropical dry forest successional stages and some key faunal groups associated with these stages. This characterization will enable a first assessment of the structure and composition of existing tropical dry forests in the Americas. Secondary successional stages are likely to be the most frequent state of tropical dry forests in the next decades, but at present no standardized method exists to assess their status, complexity and relative value to society or how these forests can be monitored using remote sensing.

From the social sciences perspective, Tropi-Dry seeks to develop innovation linkages between governments, scientists, and communities. While many traditional ecological research approaches focus solely on the natural world, researchers are increasingly recognizing the importance of incorporating the human dimension of forest use as a key component for understanding forest management. In Tropi-Dry, each study site is directly linked to both a local community of forest users and a cohort of decision-makers and policy experts. The knowledge of these groups of people forms a key component of our research. By incorporating their views, perspectives and knowledge, we seek to integrate both lay and expert approaches to environmental management.

Together, these two efforts will provide a robust data set integrating expert and lay perspectives with developing scientific knowledge. At the integration level (natural and social science to policy making) researchers work closely with key policymakers and institutions at the national and local levels. Integration therefore is a key element of the work conducted by TROPIDRY. One of our key goals is to facilitate interaction and knowledge transfer between and amongst scientists, policymakers, and the public.

The main purpose of this guidebook is to provide all Tropi-Dry researchers and field assistants with detailed and complete methodological protocols

for all the research aspects considered in the IAI-funded project "*Human, ecological and biophysical dimensions of Tropical Dry Forests*." The multiple localities covered and the numerous research teams involved in this investigation demand the use of standardized protocols across sites and research teams. By standardizing all methodologies, we will be able to perform valid comparisons of results across localities and propose regional patterns for some of the phenomena studied. We expect this manual to also become a primary reference for dry forest research throughout the tropics, allowing us to replicate our field research well beyond the boundaries of Tropi-Dry's current study sites. We also see this as a living document, and welcome feedback and suggestions for improving future editions.

The project, as well as the manual, is divided into three components: ecological, remote sensing and social. The objectives and methods of each component were developed by experts in different scientific disciplines, including plant, animal and soil ecologists, plant taxonomists, conservation biologists, social scientists, and remote sensing experts, among others. These experts participated in three workshops organized by the Tropi-Dry Research Network between the years 2004 and 2006, sponsored by the Interamerican Institute for Global Change Research (IAI) and the University of Alberta. These institutions are also funding the project for five years, starting in June 2006. For each research component, one discussion group was created with the task of exchanging ideas and experiences regarding the most appropriate and logistically feasible methods that could be implemented in the field and in the laboratory to achieve the goals proposed. After extensive and productive discussions, each group came to an agreement and organized methods according to the different aspects to be investigated, and also according to chronological sequences in which they should be conducted. The methods were described with the maximum detail possible, with the idea that any researcher should be able to follow the same procedures in any locality where the study is being conducted. When available, literature supporting the methods proposed is included.



## ECOLOGY PROCEDURES



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## INTRODUCTION

Tropical dry forests (*Tdf*) are one of the most threatened ecosystems on the planet and are particularly vulnerable to land use cover change. Various political and economic factors have increased anthropogenic stresses in these ecosystems, leading to severe disturbances and widespread clearing. *Tdfs* have historically been preferred zones for agriculture and human settlement in Mesoamerica, the Caribbean, and South America. The current use and abandonment of these ecosystems will make secondary succession the most frequent state of tropical dry forests in the upcoming decades, but at present, no standardized methods exists to assess their status and complexity. The main purpose of this section is to develop a comprehensive and standardized protocol for recognizing and comparing tropical dry forest successional stages and some key faunal groups associated with these stages. Our aim is to develop a methodology to characterize the structure and composition of existing tropical dry forests in the Americas. Our study comprises a comparative analysis considering tropical dry forests of four countries along a latitudinal gradient: Mexico, Costa Rica, Venezuela, and Brazil.

We will examine five biophysical components of the *Tdf* that are dynamic in nature and define the different successional stages that can be distinguished in this type of ecosystem: vegetation, soil, plant productivity, phenology, and biotic interactions of major influence on forest succession. For analysis purposes, we have defined four successional stages: pastures (cleared forest starting to recover), early stage (3-5 year old abandoned pastures), intermediate stage (10-15 year old abandoned pastures), and late or mature stage (> 50 year old forest). Starting with the vegetation analysis, we will characterize and compare the floristic composition and vegetation structure of the four successional stages, considering herbaceous vegetation, shrubs, trees, and lianas. Besides estimating standard plant community parameters, we will also determine the Holdridge complexity index, which will be used to contrast our results with values reported in other studies. With respect to soil, we will study its physical-chemical properties, mycorrhizae (AMF) and *Rhizobium* activity and diversity. Plant productivity will be estimated based on an analysis of litterfall that comprises not only leaf litter production, but also nutrient estimations. Vegetative and reproductive phenophases will be monitored during three years at two levels: plant community and species. Besides defining the temporal reproductive patterns of the different successional stages, we will characterize the reproductive and dispersal attributes associated with each successional stage based on the plants' sexual attributes, and their pollination and seed dispersal syndromes. Regarding biotic interactions, we will focus on two types that we consider play major roles in the configuration of the different successional stages of *Tdfs*: seed dispersal and herbivory. Seed dispersal patterns will be described by conducting periodic inventories and fecal analyses of two key faunal groups that contribute to the regeneration of *Tdfs*: birds and bats. Herbivory patterns will be characterized in each successional stage considering three herbivore categories (gall-inducing insects, leaf miners, and folivores) and two strata (canopy and ground level).

All analyses will be conducted across the four successional stages and across a variable temporal schedule, depending on the analysis. The different sets of data obtained from the ecology component will be linked to the remote sensing data recorded for the experimental plots during the entire duration of the project. In this way, the relationship between the hyperspectral information obtained from the plots and the biophysical parameters estimated within them can be determined.

# ECOSYSTEM COMPOSITION AND STRUCTURE

## Goals

- Characterize and compare the floristic composition and structure of successional stages of tropical dry forests (*Tdfs*) across a latitudinal gradient in the Neotropics.
- Link the structure and composition of successional stages to satellite images.
- Determine Leaf Area Index (LAI) and Woody Area Index (WAI) for successional stages and link this information to remote sensing analysis.
- Determine plant growth across the different successional stages over 4 years.

## Successional stages and plot design

### Definition of successional stages

We have defined four successional stages of interest (Fig. 1):

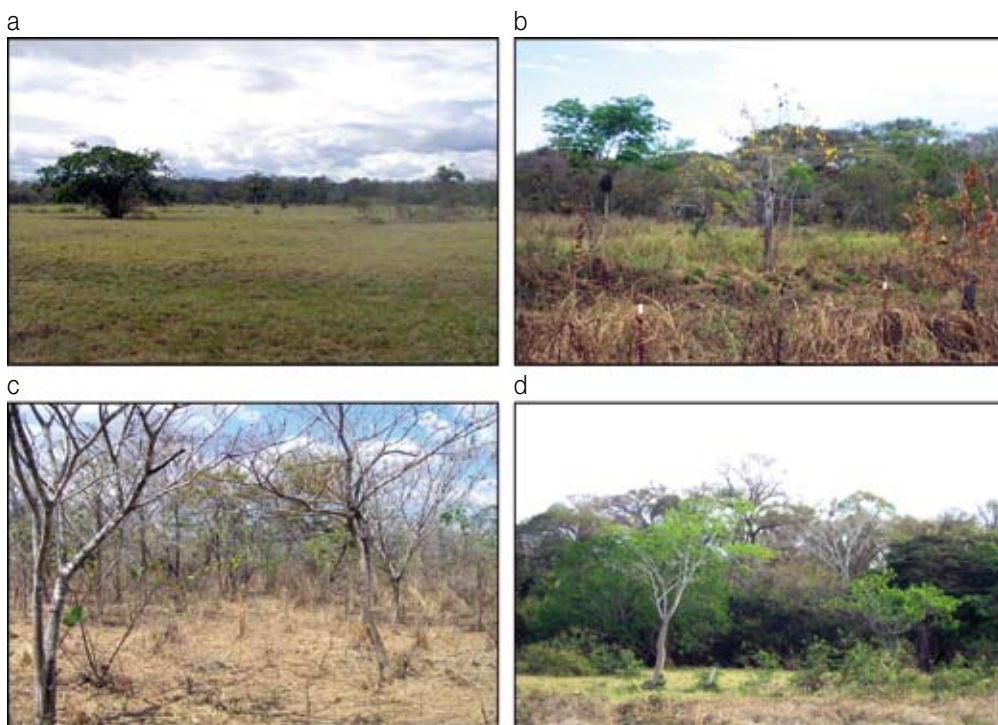


Figure 1. Photographs of the four successional stages of tropical dry forest considered in the study: pastures (a), early (b), intermediate (c), and late stage (d). Pictures correspond to sampling localities at Hato Piñero, Cojedes, Venezuela.

- a) Pastures: Areas used as pastures for cattle before the beginning of the project, now protected from fire with fire breaks and with fences to keep cattle out. These areas are dominated by herbaceous vegetation that is often a composite of native and exotic domesticated plants.
- b) Early stage: Areas originally used as pastures for cattle or for agricultural purposes that have been protected with fire breaks and fences to keep cattle out; under natural regeneration for the last 3 to 5 years. These areas contain herbaceous vegetation, bushes and trees, with many open spaces.

- c) Intermediate stage: Areas originally used as pastures for cattle or for agricultural purposes that have been protected with fire breaks and fences to keep cattle out; under natural regeneration for the last 10 to 15 years. These areas contain mostly bushes and trees.
- d) Late stage: Mature forest or forests more than 50 years old.

### Plot design

For each successional stage, three 60 x 30 m plots must be established, for a total of 12 plots (see Appendix I for plot nomenclature and site description data sheets, *p. 106-107*). These plots must be located within a matrix of vegetation of the same kind, with all margins between the plot and a different vegetation type measuring at least 50 m. Inside each plot, a subplot of 50 x 20 m (0.1 ha) must be delimited (Fig. 2), following Gentry's plot size design to survey plant biodiversity in Neotropical ecosystems (Gentry, 1982). All aspects related to floristic composition, vegetation structure and remote sensing will be conducted in the 50 x 20 m subplots. Using these plot dimensions allows the comparison of values of the Holdridge Complexity Index calculated in this study (see below) with values previously reported for various ecosystems, including tropical dry forests (Kalácska *et al.*, 2004). In addition, this subplot size fits well within the 60 x 30 m plots used to estimate Leaf Area Index. Some of the sampling protocols that require more space, such as bat and bird sampling, will be conducted in the 60 x 30 m plots and surrounding margins, if necessary. The plots should be located between 100 and 1000 m (measured from the edge of the plot) from an area of older successional stage, to allow for significant propagule input. For each plot, the distance to the nearest source of propagules must be determined; this should be estimated from the satellite images.

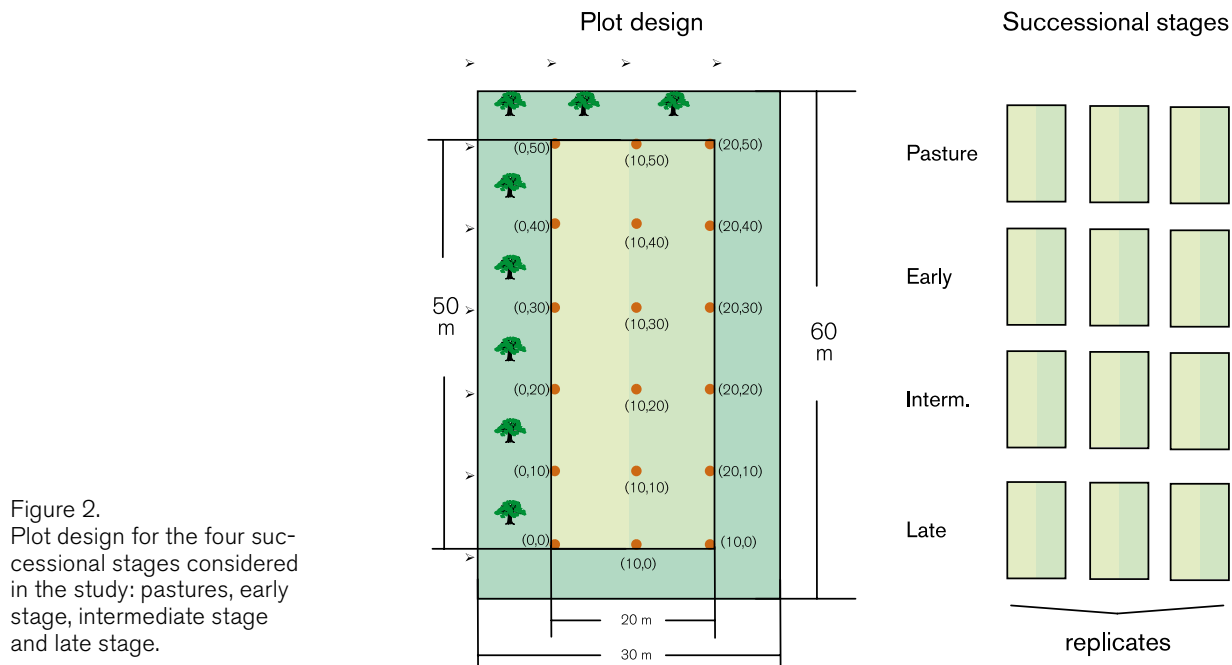


Figure 2.  
Plot design for the four successional stages considered in the study: pastures, early stage, intermediate stage and late stage.

Sites representing different successional stages should be geo-referenced and evaluated using satellite images. An evaluation of the tones, textures, forms and size of vegetation polygons will assist in the accurate classification of sites and selection of plots; the criteria to be considered include



continuity, homogeneity and shortest distance to the edges of a particular vegetation matrix.

### **Land use history and climate data**

The Social Science team in each country will obtain the history of the different study sites (*p. 85*). The determination of the age and history of land use of each site is of utmost importance, as the intensity of prior use can significantly alter the current composition and functioning of vegetation. The history of use at each site must therefore be established and linked to the current forest structure and composition. Local geology, geomorphology and climate of the study sites (rainfall patterns, temperature, humidity, etc.) must also be understood. In order to get this information, meteorological stations should be set up to measure precipitation, photosynthetic active radiation, ambient temperature, relative air humidity, and relative soil humidity. In each study area at least one meteorological station should be installed, with sensors positioned at least 3 m above the vegetation mass. For example, in a late successional stage site with a 15 m height canopy, sensors should be installed in a tower at least 18 m tall. In addition, the tower must be located within the vegetation matrix representative of that successional stage, not near the periphery.

### **Floristic composition and structure**

In this section all procedures required to examine floristic composition, structure and the successional dynamics of a tropical dry forest during five years of monitoring are described.

Floristic data should be entered into a database created in Microsoft Excel. This database should include all fields concerning floristic composition (Appendix II, *p. 108*) and structure (Appendix II, *p. 109*). If possible, each species collected and identified should be accompanied with digital pictures of the plant, leaves, flowers, fruits and bark.

Botanical collections should be conducted following common techniques used in floristic studies (Jain, 1977; Hicks and Hicks, 1978; Womersley, 1980; Lot and Chang, 1986). Plant specimens should be collected, preferably in reproductive state, representing each species found inside the plots and the surrounding margins, with sufficient duplicates to allow distribution to national and regional herbaria, and if possible, to international herbaria linked to the Tropi-Dry project. Plant samples should be labeled in the field according to the numbering sequence of each collector, preserved in newspaper misted with 50% alcohol and placed in plastic bags. Once in the lab, pressed samples should be dried in the oven, then identified with labels containing the following information: information recorded in the field, locality, date of collection, and data of collector. The samples should then be identified in herbaria using the appropriate botanical keys.

If digital photographs of each species are taken, they must be obtained from fresh material in the field and dried material preserved in the herbarium. Photograph resolution should be at least 600 dpi, following a format similar to Lorenzi (2002), including images of: the complete plant body, inflorescence with leaves, fruits and seeds (Fig. 3). These digital images can then be used to create a digital catalogue of the floristic diversity found in each plot, study site and country. This information will certainly be very useful once the project



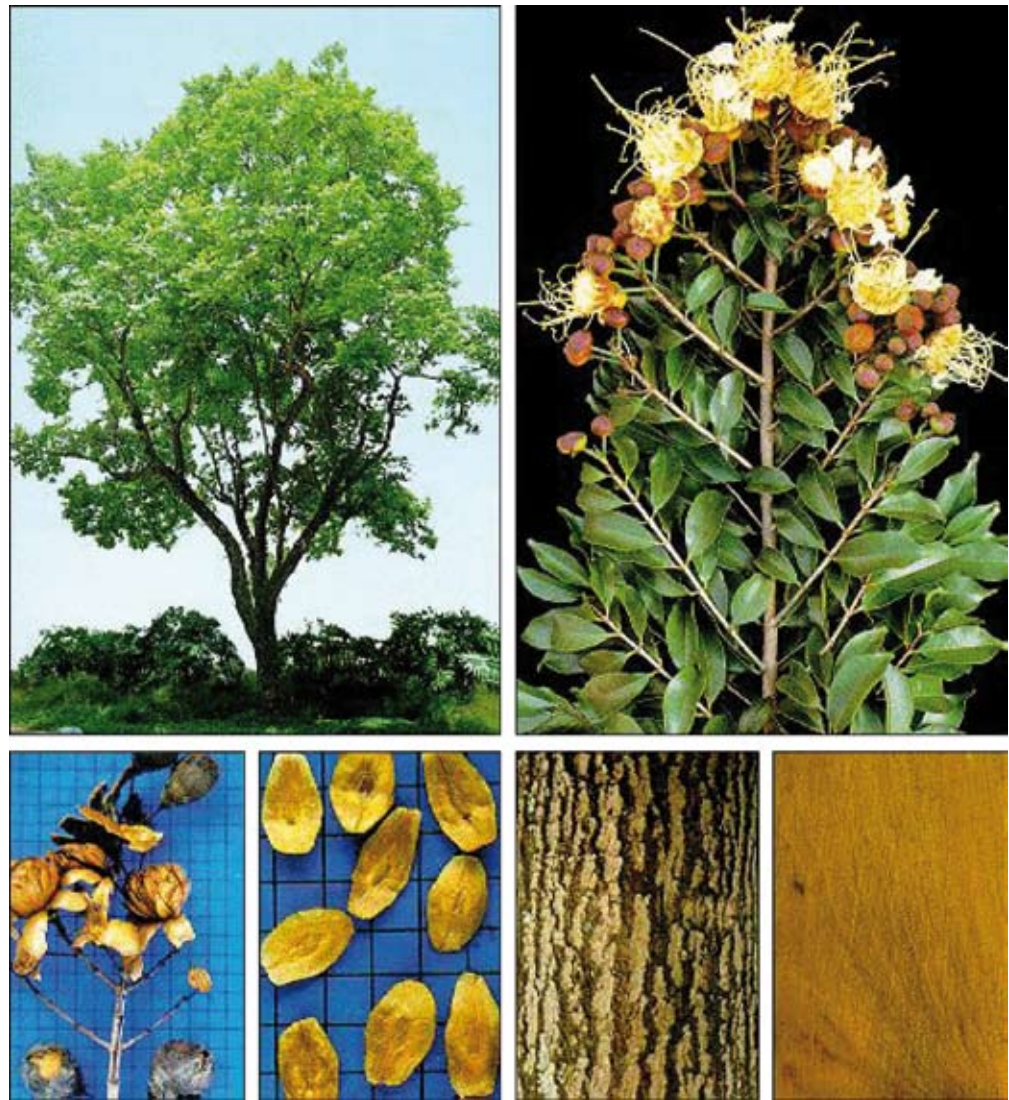


Figure 3.  
Example of illustrations  
of a botanical specimen  
(*Lafoensia glyptocarpa*)  
following the format used  
by Lorenzi (2002) in his  
botanical guides of  
Brazilian trees.

advances and the number of botanical specimens collected increases, especially to identify newly collected material and to exchange information with other Tropi-Dry teams conducting floristic surveys in other countries.

To examine vegetation structure, physiognomy and plant recruitment, individuals should be marked with aluminum labels and with fluorescent paint at 1.30 m above ground level. Inside each 50 x 20 m plot, each individual shrub and tree larger than 5.0 cm DBH should be marked with appropriately coded labels. All lianas larger than 2.5 cm DBH should also be marked. Data should be recorded using the data forms presented in Appendices II (*Woody vegetation species level data*, p. 110 and *Woody vegetation plot level data*, p. 111) DBH for each labeled tree and liana, and trunk height for every labeled shrub and treelet, must be determined annually. In the case of plants with more than one trunk, DBH should be measured for all trunks; the DBH value for each trunk should be recorded in a line corresponding to the same individual tree. In addition, in each plot the height of the ten tallest trees must be measured with a clinometer. These measurements must be taken during the wet season. Dead individuals and recruitment of new individuals must also be recorded annually. The cause of death should be classified as: physical damage, animal damage, disease, and/or other. The plants labeled in each plot will be mapped establishing their position in a coordinate axis determined by the two perpendicular sides of the plot.

The ecological importance of the woody vegetation will be determined for each plot using relative density, frequency and dominance parameters, according to the Importance Value Index formula:

$IVI = De \% + Fr \% + Do \%;$  where

$De$  (density) % = (total # of ind. of species x / total # of all ind.) x 100

$Fr$  (frequency) % = (# of subplots in which species x appears/ total # of subplots) x 100

$Do$  (dominance) % = (cover area of species x / cover area of all species) x 100.

An overall IVI can be calculated by pooling the data of the three plot replicates of each successional stage.

Complexity of each successional stage can be estimated using Holdridge's Index of Complexity (CHCI, Holdridge 1967, Holdridge *et al.*, 1971), for which the following parameters per plot must be estimated: height in meters (H), stem density (D), number of species (S) and basal area (G). These variables are then integrated in the following equation:  $CHCI = H \times G \times D \times S / 1000$ .

In addition, the following analyses will be conducted to help explain forest dynamics:

- Appearance and disappearance of species and individuals (demography)
- Curves of diameter growth
- Changes in relative importance of species
- Species cumulative curves between years 1 and 5

The study of herbaceous vegetation should be conducted annually at the peak of both the rainy and dry seasons, in randomly placed 2 x 2 m subplots. Two subplots should be placed in each replicate of each successional stage. This adds to a total of six subplots for each successional stage per country. Ecological importance of herbaceous species is determined by: number of stems, number of species, and percent area cover. This information will be linked to the patterns of appearance/disappearance of woody species in each successional stage each year. Herbaceous vegetation data should be recorded using the data form in Appendix II (*Herbaceous vegetation species level data*, p. 112).

## **SOIL, MYCORRIZAE AND RHIZOBIUM CHARACTERIZATION**

Holistic research approaches applied in field experiments are likely to yield abundant and highly pertinent information on the functioning of soil as a biological system. In the context of plant nutrition and ecosystem function and stability, the key soil biological components are: fine roots (fine, terminal, un-lignified, absorbing roots); AMF (arbuscular mycorrhizae); PGPR (plant growth promoting rhizobacteria), including nitrogen fixing and phosphate solubilizing bacteria (Hamel *et al.*, 2004); and earthworms (Whalen and Hamel, 2004). Most plant root systems are composed of roots and mycorrhizal fungi (Smith and Read, 1996). AMF are known to alter plant community composition and productivity (Bever, 2002), and are also major soil bioindicators, as they are the most common plant root symbionts (Trappe, 1987).

## Goals

- Characterize soil composition and texture in the different successional stages of *Tdfs* across a latitudinal gradient in the Neotropics.
- Characterize mycorrhizae and *Rhizobium* in the different successional stages of *Tdfs* across a latitudinal gradient in the Neotropics.
- Examine the relationships between these aspects and structure, floral composition, plant growth and phenology.

## Physical-chemical analysis of soil

Sampling will be conducted in year 1 and year 4 in each country, at two times: time of (i) moisture sufficiency and (ii) moisture deficit. Ideally, soil samples obtained from all countries should be stored appropriately and shipped to a single lab, where all analyses should be conducted. If this is not possible, then analyses should be conducted in one lab per country, always following the same protocols, described below.

For soil physical-chemical characterization, a soil profile should be opened in the central part of each 60 x 30 m plot. Diagnostic horizons of each soil profile should be identified in the field using the Munsell table. From each diagnostic soil horizon, four soil samples are to be taken for physical-chemical analysis. The collected samples should be air dried and passed through a 2 mm soil sieve. Total C should be determined by the Walkley and Black (1934) method. Available P should be extracted according to Tiessen and Moir (1993) and then determined by the colorimetric method of Murphy and Riley (1962). Total N should be measured following the Kjeldahl method (Jackson 1976). Soil pH should be measured in 1 M KCl. Exchangeable cations (K, Mg, Ca) should be extracted in 1N ammonium acetate (Thomas 1982) and determined by atomic-absorption spectrophotometry. In soil samples with pH values < 5, exchangeable Al should be extracted with 1 M KCl and determined by titration with NaOH (McLean 1965). Soil texture should be determined by the hydrometer method (Day 1965). All physical-chemical characterizations should be recorded in the data form presented in Appendix II (*Soil, Mycorrhizae and Rhizobium Characterization*, p. 112).

## Soil analysis for presence of mycorrhizae and *Rhizobium*

This study focuses on AMF and PGPR, especially the relevant roles of AMF and PGPR in plant nutrition, soil quality enhancement and crop production improvement. Specifically, we will examine: (1) the influence of plant community structure on soil microbial communities, AMF diversity and abundance, and soil functioning; (2) the influence of soil on soil microbial communities, AMF diversity and abundance; and (3) components determining soil aggregation.

The primary aim in this part of the study is to discover AMF and PGPR functioning at the ecosystem level in tropical dry forest environments. Plant cover throughout secondary *Tdf* successional stages in Venezuela, Mexico, Brazil and Costa Rica is provided mainly by grasses (pasture), scattered trees, rosette plants (agaves), lianas and palms. Sampling should be carried out in plots representing all four successional stages, as described in the Introduction section (p. 17). Note that in this case, samples (both plant and soil) should not only be taken just to the side of the tree, rosette

plant, liana or palm, as grasses often do not grow there; rather, sampling should also include the areas surrounding the plants where grasses are found, as demonstrated in Fig. 4.

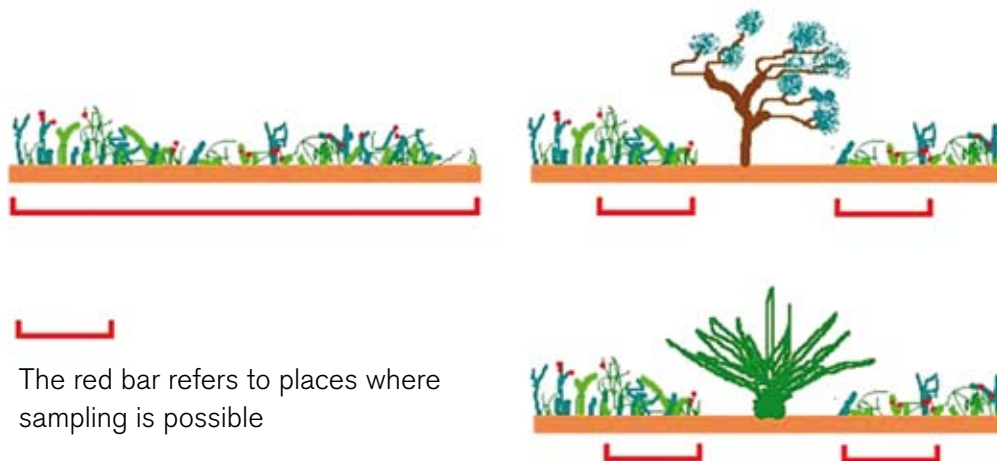


Figure 4.  
Sampling protocol for arbuscular mycorrhizal fungi (AMF) and the associated plant growth promoting rhizobacteria in the study plots.

## General conditions

As previously described, three replicate plots should be established in each of the following:

1. Pasture
2. Early succession
3. Intermediate succession
4. Late succession (mature forest)

Each plot will be sampled in year 1 and year 4, during two time periods:

- (i) Moisture sufficiency
- (ii) Moisture deficit

## Plant sampling

At each sampling time in each plot, the percentage of soil coverage provided by every present plant species should be evaluated in five 0.25 m<sup>2</sup> quadrates randomly distributed in a plot of about 40 m<sup>2</sup>. This 40 m<sup>2</sup> plot should be randomly located within the larger 60 x 30 m plot. In pastures, the point intercept method should be used to determine soil coverage by plant species. The data will be comparable between sites as each can be converted into estimates of relative cover of different species.

Plants litter should be collected, dried and weighed for mean above-ground mass determination. Plant material collected from each of the five 0.25 m<sup>2</sup> quadrates should be thoroughly homogenized to build one composite sample for each plot. From each homogenate, 5 g will be shipped to an appropriate laboratory for tissue analysis (carbon, macro- and microelements; Si; lignin; phenolic compounds).

## Flagging the sites

Use pin-flags (or other flags) to mark the areas sampled in order to easily find the location at time of the next sampling (for both dry season and wet season samples). Each new sampling should be conducted in the area immediately adjacent to sampling area 1.



## Soil sampling

There will be three sample sets (samples A, B, and C) from each of the 12 plots (three replicates each of pasture, early, intermediate and late succession):

- **Soil sample A** - For the determination of soil variables such as granulometry, soil fertility and extra-radical phase and physiological status of AM fungi, either ten soil cores of 2.5 cm diameter (or any number of any other cores of known diameter) or five 10 x 10 cm monoliths cut out with a knife, should be taken to a depth of 20 cm from each plot. The cores from each plot are to be mixed together (creating a 400 g composite sample for each plot), weighed, and air-dried. The soil samples must be placed in plastic bags; bags must be kept in a cooler in the field and refrigerated at 4 °C once in the lab. About 400g should be collected.
- **Soil sample B** - A 5 cm diameter by 20 cm long core should be taken from each plot for soil water stable aggregates, moisture at sampling time, and bulk density. This core should be pushed into the soil using a piece of wood and a hammer, or any other appropriate field equipment. The core is then removed and the soil exceeding the lower rim of the core cut off using a knife, resulting in a precise volume of soil. The cores are then closed with plastic caps at both ends, kept cool and wrapped in plastic until they are processed. Total fresh weights of soil (containing rootlets) for each composite sample should be assessed prior to any other measurements.

Soil samples will be sent to an appropriate lab for processing and analysis.

## Root extraction

In order to separate rootlets, 200 g of soil samples taken from A should be collected with a shovel and mixed thoroughly with tap water. The supernatant is then poured through a 140- $\mu$ m sieve, with the sieve sloped at an angle of 45° or greater. This process is repeated several times until floating rootlets are no longer visible. Rootlets remain trapped in the sieve; none should pass through the sieve. Afterwards, the entire sieve content is re-mixed with water and poured onto a 40- $\mu$ m sieve (this time very carefully). In this manner, rootlets receive a final washing. At the end of this process, the material (rootlets + organic material) is recovered, air-dried and weighed. The soil that has been washed off can be discarded.

Finally, an aliquot of the dried material is placed on the side of a large piece of white paper or thin cardboard. Then, through elutriation (blowing slowly with the mouth), dry rootlets can be separated and segregated relatively easily.

## Soil sample processing

**Soil sample A** – Must be weighed.

- The soil volume ( $\text{cm}^3$ ) sampled should be calculated as: g of soil / bulk density ( $\text{g cm}^{-3}$ ).

**Soil sample B** – Soil in cores of known volume should be weighed fresh, dried at 105 °C to a constant weight, and then weighed dry.

- Soil bulk density should be calculated as: g of soil / core volume in cm<sup>3</sup>
- Soil moisture should be calculated as: [(g moist soil – g dry soil) / g dry soil] x 100

Soil samples should then be air-dried in a safe and aerated place. Before drying, large root pieces must be separated from the soil and placed in a labeled envelope. The soil samples are then sieved through 2-mm mesh and homogenized, and the roots remaining on the sieve are pooled with the roots previously recovered by hand. Lignified rootlets are discarded. Un-lignified (fine, absorbing) rootlets from each composite (plot) sample are placed in a small envelope and stored in a paper bag.

## Labeling

Containers should be labeled as indicated in Fig. 5 (labels may also be placed on the cap, but must always be placed on the container). In addition, the label should be written on a piece of paper in graphite pencil (standard pencil) and placed inside the container; this label is permanent. Label components include:

Country: e.g., Brazil, Costa Rica, Mexico, Venezuela.

Time: D (dry season) or W (wet season)

Site (block of pasture, early, intermediate and late succession): 1 to 4 (make sure sites are numbered on a field map so their location can be identified)

Sampling date: day month year

Description of the sample.



Figure 5.  
Example of label with  
required information for soil  
samples

## Microbial data

All samples of soil and roots collected from A should be processed via wet sieving and decanting. Mycorrhizal spores should be separated and: 1) mounted in PVLG or PVLG-Meltzer on glass slides; or 2) separated for molecular analysis. Morphologic characteristics should be determined for all mounted vouchers. Results will be used to prepare taxonomic reports based on morphology for each country and community analysis using multivariate and classical statistics software.

### (a) Arbuscular mycorrhizal data.

Each soil sample should be assessed for:

External mycelium (ectophyte – EC) for each sieved fraction: EC1, 40 – 140 µm; EC2, 140 – 500 µm; EC3, 500 – 2000 µm.

Each bag with rootlets should be assessed for: mycorrhizal colonization rate (%MC), mycorrhizal visual density (%VD), root hairs (RH), rootlet phytomass (based on a soil volume concept, rPhy/dm<sup>3</sup>; this



data should be provided for each composite sample coming from each country), endophyte (ED, estimated on a %VD base using rPhy data), and external hyphae still attached to rootlets (EC4). Based on EC1 to EC4 data, total EC for each composite sample is estimated. Finally, EC:ED and EC:rPhy ratios are estimated.

- (b) Total microbial biodiversity should be evaluated using phospholipid fatty acid profiling; Glomalin, by ELISA; dehydrogenase activity should be measured on fresh soil (this must be done on fresh soil).
- (c) Plant analysis (N and P).
- (d) Soil chemical properties (total organic C and N on 5 g of soil; pH and electrical conductivity on 100 g of soil; and resin extractible N, P, K, Ca, Mg, Fe, Mn, B, Cu, Zn, and Cl on 50 g of soil).
- (e) Analysis of dry aggregate distribution and analysis of water stability of aggregates.
- (f) Molecular analysis of AM fungal community composition based on the LSU. This would be done from extracting DNA from roots – presumably this is best done from the composite samples. DNA should also be extracted from spores of individual fungal species present in the samples. These will help inform the analysis of sequences from roots and may be useful for descriptions of undescribed fungal species.
- (g) Assessment of physiological groups of microorganisms. Total heterotrophs, cellulolythics, diazotrophs, fungi, actinomycetes and phosphate solubilizing bacteria, morphologic taxonomy and community analysis based on morphology.

All mycorrhizae and microbial characterization should be recorded in the data form presented in Appendix II (*Soil, Mycorrhizae and Rhizobium Characterization*, p. 112).

### Statistical analysis

The effect of the treatments (“successional stage”) on the variables measured can be determined by analysis of variance. Multivariate analyses should be used to relate microbial profiles and system components to successional stage. Integration of data from different countries can be achieved by pooling and parsing per study subjects.

### Soil mycorrhizal potential

An infectivity assay can be a convenient approach to examine soil mycorrhizal potential. Trap plants are grown in the soil under examination for 2 to 4 weeks, a period of time long enough for colonization to occur but short enough to prevent mycorrhizal development from reaching its full potential, a point at which plants may become uniformly colonized (see McGonigle 2001). Brundrett *et al.*, (1994) proposed to grow trap plants in undisturbed cores to maintain an intact AM mycelium in the soil under evaluation. In contrast to other methods, the intact core method accurately evaluates soil infectivity. The limitation of this method is that its end result is not a convenient number of propagules, but the percentage of colonization of a trap plant after a num-

ber of days. This method is appropriate to compare the mycorrhizal potential of soils or plots within the framework of an experiment. Furthermore, AM fungi are filamentous, a type of growth which makes it impossible to determine where a propagule starts and ends. Thus, rate of colonization expresses soil AM infectivity more realistically than number of propagules.

## Materials and Reagents

1. Appropriately labeled steel cylinders with a tapered and sharpened lower end, which sends the pressure outward and preserves the soil core when it is pushed into the soil. These cylinders serve as growth containers. As many cylinders as there are plots multiplied by the number of desired sub-samples per plot are needed. At least 4 sub-samples should be taken.
2. Small wooden board to cover and push cylinders into the soil.
3. Trowel to lift the cylinders.
4. Knife to level off the lower end of the soil core.
5. Nylon mesh and high gauge rubber bands to close the lower end of the cylinder while allowing drainage.
6. Growth chamber to control temperature, humidity, and lighting conditions, to allow repetition of the assay at other times.
7. Perforated plastic trays with corrugated bottoms that will allow excess water to drain while maintaining the soil in place when watering soil cores.
8. Germinated seeds or seedlings of the desired species of trap plant. Note that more than one species can be grown simultaneously in each core. The number of plants of each species should be kept constant among growth cylinders. Clover seedlings inoculated with *Rhizobium* (Brundrett *et al.*, 1994) have been used previously, but any mycotrophic plant species may be used.
9. Polyester wool may be used to cover the surface of the cores, particularly in the case of soil rich in clay, in order to protect the soil surface structure from water damage during watering events.

## Procedures to be conducted on samples A or B

1. Cover the steel cylinder with the wooden board and push it into the soil until filled.
2. Carefully dig the cylinder out of the soil.
3. Invert the cylinder holding the upper surface against your palm or the wooden board, level off the lower surface of the core, cover it with the nylon mesh and secure this mesh with a high gauge rubber band. Avoid exposing the cores to excessive heat during the collection process.
4. Carefully carry the cores to a clean greenhouse workbench.
5. Insert germinated seeds or plantlets in the center of the cores. Seeds can be placed in pairs and thinned to one per pair after a few days.
6. Cover the soil surface with polyester wool to protect the soil structure.
7. Place in corrugated trays in a growth chamber at required preset settings.
8. Water very gently to field capacity when required, to avoid soil structure degradation.
9. Grow the trap plants for about 2 to 4 weeks (see comment below).
10. Clear, stain and assess the roots for their percentage of mycorrhizal colonization and total root length using the grid-line intersect method, as discussed by McGonigle, 2001.

It is worthwhile having extra cores available to periodically assess the status of root colonization prior to harvest and insure that trap plants have an adequate amount of mycorrhizal development.

## Evaluation of the extra-radical phase and physiological status of AM fungi

Several methods have been used to quantify the extra-radical mycelium of AM fungi. Soil chitin measurement has been used to estimate AM fungal biomass in soil (Bethlenfalvay and Ames, 1987). However, chitin measurement does not give an estimate of the active AM extrarradical hyphal biomass. Chitin is also abundant in invertebrates' exoskeletons and in zygomycetous fungi.

The spread of AM fungi extrarradical hyphae in soil was studied using root exclusion chambers. Sequential sampling in compartmentalized growth containers allowed the comparison of the spread of AM fungal species from a root barrier into a hyphal compartment (Jakobsen *et al.*, 1992; Schuepp *et al.*, 1987). A rotating wire system was proposed to facilitate the task of extracting extrarradical hyphae from mineral soil samples with low clay content (Vilarino *et al.*, 1993; Boddington *et al.*, 1999). The method of root exclusion chambers has been the most popular way to evaluate the size of the extrarradical AM hyphae in soil, even though there are some limitations to direct measurement of hyphae extracted from soil. AM fungal hyphae are generally non-septate and some researchers found certain morphological features, such as hyphal size (Ames *et al.*, 1983), angle of branching, and wall characteristics, to be typical of AM mycelia. But considering the different morphologies found among AM genera, the range of possible AM extrarradical hyphae sizes (from  $< 1\mu\text{m}$  in the fine endophytes to  $18\mu\text{m}$  in *Glomus manihotis*, Dodd *et al.*, 2000) and the diversity of other soil fungi, one must conclude that the identity of coenocytic hyphae extracted from a soil sample is at best uncertain, especially in hyphal pieces bearing no branching. Also, much of the AM hyphae extracted from soil are neither viable nor functional and vital staining techniques must be applied if the amount of active hyphae is sought.

Wright and Upadhyaya (1999) and Balaz and Vosatka (2001) have proposed inserted membrane techniques in which a membrane of a material resilient to decomposition is inserted in the soil to trap the hyphae that will cross it. These methods are simple and rapid, and give a measure of total hyphae cross section plates. This method can be used with immuno-detection of the AM fungi-specific protein glomalin (Wright and Upadhyaya, 1999; Wright, 2000) to assess the proportion of AM and saprophytic fungi. Inserted membrane techniques should be considered where data on hyphal density are not necessary.

Fatty acids are often specific to taxonomic groups. The phosphate group of phospholipids, the lipids making up membranes, is rapidly cleaved in soil, and phospholipid fatty acid (PLFA) measurement reflects the occurrence of living or recently dead organisms. The measurement of AM fungi PLFA indicators thus provides information on the functionality of the organisms. The phospholipid fatty acids (PLFA) 16:1 $\omega$ 5, 18:1 $\omega$ 7, 20:4 and 20:5 have been proposed as indicative of AM fungi (Olsson *et al.*, 1995; Olsson, 1999), while the fatty acid 16:1 $\omega$ 5 is the preferred indicator of AM fungal biomass (Balser *et al.*, 2005). Arbuscular mycorrhizal fungi do not have completely specific fatty acids; 20:4 and 20:5 are present in algae and protozoa but are rare in non-AM fungi and bacteria, and 16:1 $\omega$ 5 and 18:1 $\omega$ 7 occur in some bacterial genera but are not normally found in other fungi. Background levels of the PLFA 16:1 $\omega$ 5 ranging from 30 to 60% have been attributed to the presence of bacteria in the soil (Clapperton *et al.*, 2005).

The fatty acid 16:1 $\omega$ 5 is dominant in many AM fungal species although it was absent from several *Glomus* species and from most *Gigaspora* species (Graham *et al.*, 1995). The whole cell fatty acid (WCFA) 16:1 $\omega$ 5, which is a more specific indicator of AM fungi than PLFA 16:1 $\omega$ 5, has been correlated with hyphal length, though the relationship varied seasonally (Gryndler *et al.*, 2006). A non-mycorrhizal control can be used to correct for background levels of the PLFA 16:1 $\omega$ 5. PLFA data on bacterial biomass, which can be generated simultaneously, can be used to interpret changes in the abundance of the PLFA 16:1 $\omega$ 5. Neutral fatty acid (NLFA) 16:1 $\omega$ 5 should also be monitored as it highly dominates reserve fatty acids of all AM fungi tested, and as bacteria produce very few neutral lipids. The measurement of NLFA 16:1 $\omega$ 5 can be used to support observation on the variation in fatty acid 16:1 $\omega$ 5 from the PLFA fraction.

The method of Clapperton *et al.*, (2005) for lipid extraction from soil and measurement of PLFA and NLFA 16:1 $\omega$ 5 is given below.

## Materials and reagents

### (a) Extraction

1. Weighing boats
2. 35 mL glass centrifuge tubes
3. Dichloromethane (DMC)
4. Methanol (MeOH)
5. Citrate buffer
6. Saturated NaCl solution
7. 7 mL glass vials
8. Pipette
9. Nutating shaker
10. Centrifuge
11. N<sub>2</sub>-gas flow drying manifold (we use a Reacti-Vap III)
12. Hot plate at 37°C (we use a Reacti-Therm III)

### (b) Lipid-class separation

13. Clamp-holder construction with 10 clamps (to hold columns)
14. Pasteur pipettes filled with silica gel up to 2 cm from the top (columns)
15. Pasteur pipettes fitted with pipetting bulb
16. 4 mL glass vials
17. DCM
18. Acetone
19. MeOH
20. N<sub>2</sub>-gas flow drying manifold (we use a Reacti-Vap III)
21. Hot plate at 37°C (we use a Reacti-Therm III)

### (c) Transmethyl esterization

22. N<sub>2</sub>-gas flow drying manifold (we use a Reacti-Vap III)
23. Hot plate at 37°C (we use a Reacti-Therm III)
24. Pasteur pipettes fitted with pipetting bulb
25. Micropipette with tips
26. MeOH
27. H<sub>2</sub>SO<sub>4</sub> (concentrated)
28. Water bath

29. Hexane
30. Vortex mixer
31. Ultra pure water
32. Methyl nonadecanoate (19:0; Sigma Aldrich)
33. 200  $\mu$ L glass syringe with needle
34. 100  $\mu$ L tapered glass inserts and gas chromatograph (GC) vials

(d) Gas chromatography measurement of fatty acids

1. 16:1 $\omega$ 5 standard fatty acid (from MJS Biolynx #MT1208)
2. Gas chromatograph with flame ionization detector (FID). We use a Varian 3900 GC equipped with a CP-8400 autosampler, helium as carrier gas (30 mL min<sup>-1</sup>) and a 50-m Varian Capillary Select FAME #cp7420 column.

## Procedure on samples A

(a) Extraction

1. To extract total soil lipids, shake 4 g (dry weight equivalent) of frozen or fresh soil in 9.5 ml DMC : (MeOH) : citrate buffer (1:2:0.8 v/v) for 2 hours in glass centrifugation tubes.
2. Add 2.5 mL of DMC and 10 mL of a saturated NaCl solution to each tube and shake for five more minutes.
3. Centrifuge tubes at 1000 g for 10 minutes.
4. Pipet the organic fraction into clean vials.
5. Add 5 mL of DCM:MeOH (1:1 v/v) to the tubes.
6. Shake for 15 minutes.
7. Centrifuge for 10 minutes at 1000 g.
8. Combine the organic fractions in the corresponding vials and dry under a flow of N<sub>2</sub> at 37°C in the fume hood.
9. Dissolve samples in 2 mL of DCM.
10. Samples can be stored at -20°C for a short time, if necessary.

(b) Lipid-class separation

Lipid-class separation is conducted in silica gel columns made with Pasteur pipettes.

1. Using a pipette, load samples onto columns washing the vials twice with a small amount of DCM and adding the wash to the columns. Care must be taken to keep the solvent level above the silica gel at all times.
2. Elute the neutral lipid fraction first by leaching columns with approximately 2 mL of DCM, collecting the eluent in 4 mL vials.
3. Elute the glycolipid fraction by leaching columns with approximately 2 mL of acetone, collecting the eluent in other 4 mL vials.
4. Elute the phospholipids fractions by leaching columns with approximately 2 mL of MeOH, collecting also the eluent in 4 mL vials.
5. Discard the glycolipid fraction.
6. Dry the neutral and phospholipids fractions under a flow of N<sub>2</sub> at 37°C in the fume hood.
7. Dissolve the dried fractions in a few mL of MeOH (for PLFA) or DCM (for NLFA), which has been stored previously at -20°C.

### (c) Transmethyl esterization

Fatty acid methyl esters are created through mild acid methanolysis as follows:

1. Dry neutral and phospholipids fractions under a flow of N<sub>2</sub> at 37°C in the fume hood.
2. Add half a Pasteur pipette full of MeOH/H<sub>2</sub>SO<sub>4</sub> (25:1 v/v) to the vials.
3. Place vials in an 80°C water bath for 10 minutes.
4. Cool to room temperature.
5. Add 1 Pasteur pipette of hexane, vortex vials during 30 seconds, and leave to settle for 5 minutes.
6. Discard the lower fraction.
7. Add 1 mL of ultra pure water, vortex vials for 30 seconds, and leave still for 5 minutes.
8. Discard the aqueous fraction entirely.
9. Add 10 µL of methyl nonadecanoate, the internal standard.
10. Dry samples under a flow of N<sub>2</sub> at 37°C in the fume hood.
11. Wash vials with 50 µL of hexane using a glass syringe.
12. Transfer the samples into 100µL tapered glass inserts, and placed inside a gas chromatograph (GC) vial.

### (d) Gas chromatography measurement of fatty acids

1. Sample (2 µL) injection should be in 5:1 split mode.
2. Hold the injector at 250°C and the FID at 300°C. Hold the initial oven temperature, 140°C, for 5 minutes, then raise it to 210°C at a rate of 2°C min<sup>-1</sup>. Next, raise it from 210°C to 250°C at a rate of 5°C min<sup>-1</sup>, and finally hold it steady for 12 minutes.

### (e) Peak identification

Identification of peaks is based on the comparison of retention times to a known 16:1ω5 standard. Amounts are derived from the relative area under specific peaks, as compared to the 19:0 peak value, which is calibrated according to a standard curve made from a range of concentrations of the 19:0 FAME standard dissolved in hexane. The abundance of individual PLFAs is expressed as micrograms PLFA per gram dry soil. The amount of fatty acid is calculated with the following formula:

$$16:1\omega5 = (A_{16:1\omega5} / A_{istrd}) * C_{istd} * D$$

16:1ω5 is the calculated concentration of the AM fungal indicator (moles or weight per unit volume), A<sub>16:1ω5</sub> is the GC area of the AM fungal indicator, A<sub>istrd</sub> is the GC area of the internal injection standard as determined by the GC data system (unitless), C<sub>istd</sub> is the concentration of the internal injection standard given, and D is the appropriate dilution factor.

Solvents used throughout the procedure should be HPLC grade, tubes and vials should be made of glass and their screw-top caps lined with Teflon. Organic solvents are toxic and must be handled in the fume hood. The use of parafilm is prohibited. Care must be taken to avoid contamination with extraneous lipids. For example, the use of gloves is recommended. A bacterial and fungal saprobe fatty acid indicators mix (Supelco Bacterial Acid Methyl Esters #47080-U) can be used in conjunction with the AM fungal indicator 16:1ω5 to simultaneously obtain information on the whole soil microbial community.



# VEGETATIVE AND REPRODUCTIVE PHENOLOGY

## Goals

- Determine vegetative and reproductive phenological patterns of successional stages in *Tdfs* across a latitudinal gradient in the Neotropics.
- Establish relationships between phenological patterns and remote sensing data (i.e. MODIS).
- Characterize plant reproductive systems, pollination, and seed dispersal syndromes in each successional stage.

## Vegetative and reproductive phenology

Phenology will be evaluated in the early, intermediate and late successional stages, for a total of nine plots per country. In the case of the pastures, phenological data should be recorded after shrubs and trees appear in the plots.

### Plot level phenology

In each 60 x 30 m plot, two 50 x 3 m transects should be established flanking the edges of the 50 x 20 m plot contained within it (Fig. 6). The transects should be set up in such a way that recording of phenological data does not affect the survival of plants contained within the 50 x 20 m plots. In each transect all individuals with a DBH above 2.5 cm should be identified and marked. The trunk of each labeled individual should be marked with acrylic fluorescent paint at 1.30 m above ground, where DBH will be measured. In addition, each individual should be labeled with aluminum plates nailed to the trunk with aluminum nails or tied with wire.

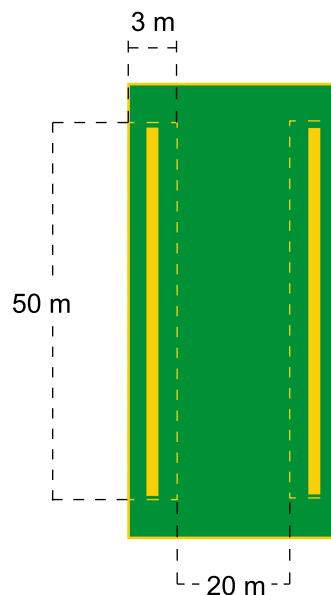


Figure 6.  
Transect design for leaf  
and reproductive phenology  
monitoring.

Every month, during three consecutive years of observations, all individuals marked within the phenology transects should be monitored for presence/absence of each of the following phenophases: green leaves, dead leaves, floral buds, flowers, unripe fruits and ripe fruits. In addition to recording presence/absence data, the percentage of coverage of each phenophase should be estimated according to the following categories: I (0%), II (1-25%), III

(25-50%), IV (50-75%) and V (75-100%). With this information, periodicity, frequency and duration of each phenophase can be described. When feasible, lianas should be included in the phenological surveys with the help of binoculars. For data input, see Appendix II (*Plot level phenology*, p.113).

### Species level phenology (optional)

For early, intermediate and late successional stages, select the 5-10 most abundant woody species, and mark at least 10 adult individuals of each species. The trunk of each labeled individual should be marked with acrylic fluorescent paint at 1.30 m above ground, where DBH will be measured. In addition, each individual should be labeled with aluminum plates nailed to the trunk with aluminum nails or tied with wire. Individuals may be located outside of the plots for this part of the study.

Every month, during three consecutive years of observations, all marked individuals should be monitored for presence/absence of each of the following phenophases: green leaves, dead leaves, floral buds, flowers, unripe fruits, and ripe fruits. In addition to recording presence/absence data, the percentage of coverage of each phenophase should be estimated according to the following categories: I (0%), II (1-25%), III (25-50%), IV (50-75%) and V (75-100%). With this information, periodicity, frequency and duration of each phenophase can be described. For data input, see Appendix II (*Species level phenology (optional)*, p. 114).

### Leaf Area Index (LAI)

Leaf area index will be determined in early, intermediate and late successional stage plots, for a total of 9 plots examined per country. Pasture plots will not be considered for this part of the study. LAI should be measured with a Plant Canopy Analyzer (PCA) LAI-2000 (LI-COR, 1992) *sensu* Kalackska *et al.*, (2004). This instrument records the amount of sun radiation that penetrates the canopy using a hemispheric lens (Planchais and Pontailier, 1999). Reference measurements should be recorded above the canopy before and after recording radiation within each plot. These reference measurements can be taken in open spaces, where the forest does not appear in the field of vision of the lens.

LAI measurements should be recorded according to the sampling scheme indicated in Figure 7 (*see also LAI under Remote Sensing*). This sampling scheme covers maximum surface within the plots without overlapping measure-

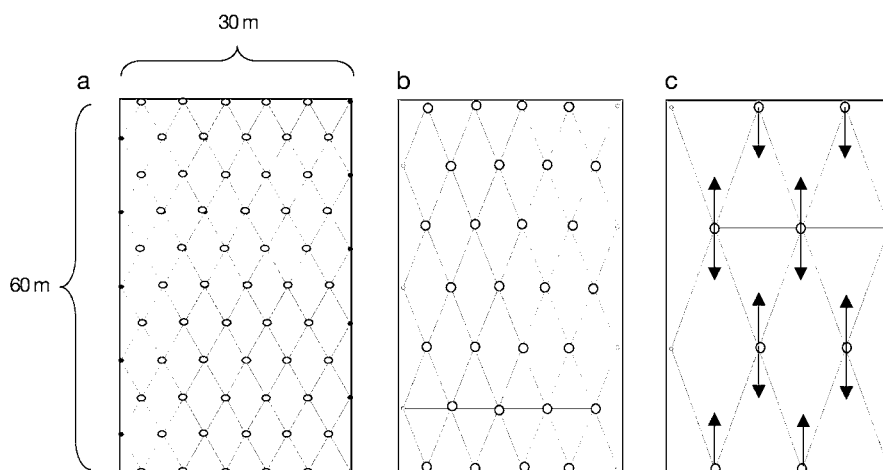


Figure 7.  
Sampling design to estimate LAI using the LICOR in the experimental plots.

ments. The scheme of measurements varies between successional stages, because the area measured by the LICOR varies as a function of the height of the canopy. The dots within the plots represent positions where the measurements should be taken. In order to evaluate the LICOR's precision several measurements can be taken in the same position (up to 10) to verify that no significant variation exists between measurements. In each point of the plot three LAI records should be obtained. For data input, see Appendix II (*LAI*, p. 114).

## **Reproductive attributes**

### **Floral biology**

Reproductive attributes will be described for most plant species identified in the plots. During their flowering season, a minimum of 15 flowers and floral buds of each species should be collected, preserved in 70% ethanol or propanol and appropriately labeled for subsequent morphological characterization.

At the time of collection, flowers should be photographed and characterized in terms of: (a) position in the plant, (b) organization (solitary, inflorescence), (c) number of flowers per inflorescence, (d) odor and (e) color. Preserved flowers should be analyzed to determine the sexual expression of the plants: hermaphroditic (male and female parts in the same flower), monoecious (separate male and female flowers in the same individual), or dioecious (male and female flowers separated in different individuals). Other sexual variations, if they can be identified, should also be indicated (e.g. androdioecious, gynodioecious, trioecious, etc.). Basic morphological measurements should be taken, including: total length (mm), internal length of floral tube (mm), external corolla diameter (mm), internal floral tube diameter, number of stamens and number of ovules. For data input, see Appendix II (*Floral biology attributes*, p. 115).

### **Pollination syndromes**

Pollination syndromes of all plant species will be inferred based on floral traits, information obtained from the literature, when available, and capture of floral visitors in the field. If possible, floral visitors should be captured while visiting the flowers, with entomological nets in the case of insects. General categories of visiting insects include: small, intermediate and large bees, beetles, butterflies, moths, hawk moths, flies and wasps. For plant species pollinated by vertebrates, only the general category of floral visitors should be determined (terrestrial mammal, hummingbirds, other birds and bats). For data input, see Appendix II (*Floral biology attributes*, p. 115).

### **Seed dispersal syndromes**

Ripe fruits of all species should be collected, examined, photographed and preserved in 70% ethanol if necessary for further analysis. Seeds should also be stored in an alcohol. The seed dispersal syndromes of each species should be defined by combining examination of the fruit and seeds with information obtained in the available literature. General seed dispersal syndromes include: anemochory, ballistic, ectozoochory and endozoochory (birds, terrestrial mammals and bats). For data input, see Appendix II (*Floral biology attributes*, p. 115).

# CALIBRATION OF THE LICOR-2000 WITH LITTERFALL DATA

## Goals

- Use leaf litter estimates to calibrate Leaf Area Index.
- Establish relationships between leaf phenological patterns and remote sensing data (i.e. MODIS).
- Estimate plant productivity through litterfall estimates.

A LICOR-2000 will be used to estimate PAI (Plant Area Index) at the peak of forest foliage coverage (e.g. October or November in Costa Rica) and every month thereafter until the end of the dry season, when there are few or no leaves left in the forest plots (*see LAI in the Remote Sensing chapter for the LICOR-2000 methodology*). In this way, the manner in which PAI changes from the time of highest foliage coverage to the time of lowest foliage coverage can be assessed.

PAI can be assumed to decrease each month as the dry season advances, since deciduous species will start to lose their leaves. By measuring the amount of leaves that are lost from month to month and calculating the LAI represented by that month-to-month loss, we can examine the relationship between LAI as determined by the optical readings (taken with the LICOR 2000) and by the litter trap measurements, and use that relationship to calibrate the LAI-2000.

The contribution of the wood component (WAI or wood area index) to PAI can also be measured, and subtracted from the total PAI value to obtain the foliage contribution to PAI. Hemispherical photos are taken during the dry season, when most of the forest canopy is void of leaves and therefore represented almost entirely by the woody component (branches, trunks, etc.; for methods, see LAI in the Remote Sensing chapter). With these photos, the contribution of the WAI to the PAI can be estimated; this information can then be used to determine the true LAI for every month.

## Number of litterfall traps

Only one plot per successional stage need be sampled. Fifteen litterfall traps per plot should be set (Fig. 8), though this number may be increased if

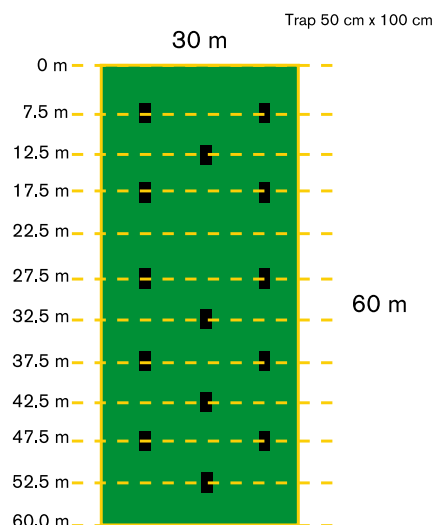


Figure 8.  
Litterfall sampling design for  
LICOR-2000 calibration.

deemed necessary, upon examination of the precision and accuracy of the mean litter mass (Kalácska *et al.*, 2005) and the number of species found per trap, as determined by the ICE estimator (Colwell, 2005).

### **Trap size**

Traps should measure 50 x 50 cm; using a trap larger than 50 x 50 cm would result in more biomass to be sorted and measured, with no increase in the precision or accuracy of the analysis (Kalácska *et al.*, 2005).

### **Litterfall collection**

Leaf litter should be collected at monthly intervals until sites are leafless (at the end of the dry season). To reduce decomposition, and if possible, leaves should be collected every two weeks. This will also reduce the total amount of leaves to be processed at any given time. If leaves are collected every two weeks, the LICOR-2000 must also be used at that time interval (*see LAI in the Remote Sensing chapter for the LICOR-2000 methodology*).

### **Litterfall processing**

In each trap, litterfall must be sorted by species or pseudo-species and subsequently dried to constant weight using a forced air oven at 80°C. Prior calibration in Costa Rica showed that 2.5 hours was necessary to reach constant weight for all samples (note however, that these leaves were already partially dried from exposure in the litter traps). Each country must determine the time required to reach constant weight, which will depend on the type of fan and the moisture content of the sample.

Weight should be recorded for each species separately. All unidentifiable remains should be grouped into one category and weighed separately from the known species. These unidentifiable samples are used to create a generic calibration equation for the unknown remains. For data input, see Appendix II (*LAI calibration and litterfall*, p. 116).

### **Specific leaf area per species**

In each of the three plots in which litterfall traps have been located, fifteen fresh leaves should be collected from all individuals with a DBH equal to or greater than 5.0 cm and which represent species for which litterfall was found in the traps. The area of the leaves should be measured with the LICOR-3000, a portable leaf area meter, before they are dried to constant weight (at 80°C). Subsequently, the leaves are weighed and specific leaf area (SLA) values are calculated for each species. Please note that the leaves should be packaged individually, as once dried they become very difficult to separate.

For the unknown category and those species for which SLA values are not obtained, the general leaf weight/leaf area relationship calculated from the pooled sampled species should be used. For data input, see Appendix II (*Specific leaf area per species*, p. 117).

## LICOR-2000 calibration

For calibration of the LICOR-2000 estimates, total leaf area for the leaves of each species is first calculated from the unique SLA values. Leaf area is then converted to LAI by dividing by the area covered by the 15 traps. LAI for the first time period is the sum of the LAI values from each collection. For each subsequent time period, the total LAI from the previous collection is subtracted. With the LAI values for each collection, the relationship between the LAI-2000 estimates and the litter traps can be examined through least-squares linear regression.

## Litterfall plot-level sampling

The phenological sampling design can be reproduced for the litterfall plot-level sampling. Litter traps should be arranged along two transects that parallel the phenological sampling transects (Figure 9). If possible, the traps should lie within the phenology transects, so that the phenodata corresponds directly to the information gathered from the traps. Litterfall should be collected at the same time that the phenological observations are made (see below). If the litterfall traps or collection are deemed too intrusive, the traps should be placed as close as possible (adjacent) to the phenology transects.

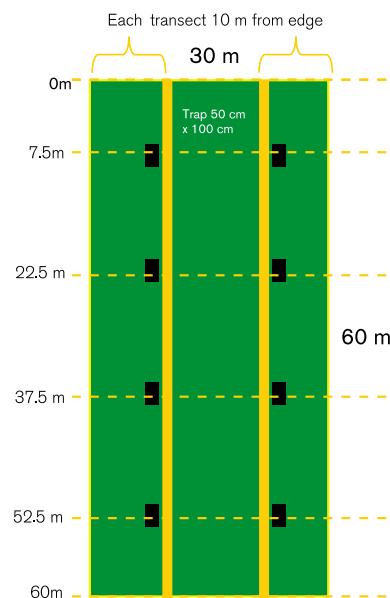


Figure 9.  
Litterfall sampling design  
for litterfall site-level  
estimations.

Each transect begins at the 30 m side of the 60 x 30 m plot. A litter trap is placed 7.5 m from the edge and every 15 m thereafter (at 22.5 m, 37.5 m and 52.5 m) (Figure 9). This spacing maximizes independence of the replicates, as none of the tree crowns, even in mature forest, are likely to overlap two traps. Each trap should be 50 x 100 cm (0.5 m<sup>2</sup>), yielding a litter sampling density of > 22 m<sup>2</sup>/ha of plot area. This sampling intensity is greater than that found in 68% of published litterfall studies (70% are under 42 m<sup>2</sup>/ha, modal intensity is 7-10 m<sup>2</sup>/ha; D. Lawrence, personal communication).

The traps should be made of 1-mm fiberglass mesh (typical window screening) fitted over a PVC frame. The trap should rest on top of four PVC corner posts at a height of 20 cm above the ground (this allows for the contribution of herbaceous vegetation, which is especially important in the youngest stands). The trap can be shaped either like a box or a basket, but the mesh must have a cone shape with a depth of 15 cm at the deepest point; a soft

basket shape rather than a rigid frame allows heavier objects (e.g. fruit) to fall in without bouncing out.

For complete data clarity, each trap should be assigned a unique number across all countries. Unique numbers are always much easier to track than codes. With 8 traps per plot and 12 plots per country, the numbers should be in batches of 96. The numbers have been categorized in a series from south to north, beginning with 0: Brazil will therefore use the numbers 001-096 and 101-196 (there are two sites in Brazil); Venezuela will use 201-296; Costa Rica, 301-396; and Mexico, 401-496. Litterfall should be collected every four weeks. Large leaves and pieces of wood that hang over the edge of the litterfall trap should be cut so that only the part falling within the trap area is counted.

Samples from each trap should be placed in separate paper bags (labeled by trap number) and dried at  $< 70^{\circ}\text{C}$  for several days, or until constant weight is reached (the time will depend on the volume of material). The bags should be moved around and shaken every day or so, to ensure the samples dry properly.

Once dry, the weight of each sample (each trap) should be recorded separately. First, the weight of the sample and the bag should be recorded, and then the bag should be emptied and weighed alone. The difference between these two values equals the dry weight of litter per  $0.5\text{ m}^2$  trap. For data input, see Appendix II. (*Litterfall at the site level*, p. 117). After weighing the bag for each individual trap in a plot, the litter from all traps (within one plot) should be combined into one pile and mixed thoroughly. This thoroughly mixed composite sample is then divided by repeatedly halving it and randomly rejecting one half or the other, until a sample of approximately 80-100 g remains. This sample should be ground in a Wiley mill to a size small enough to pass through a 1 mm mesh screen. The sample should then be divided into two sub-samples. Each sub-sample should be sealed in a plastic bag and labeled with permanent black marker (at least twice) with the plot number and date. One archived sample is to be preserved for later nutrient analysis as part of a cross-country comparison. The other archived sample may be used for other purposes by the local team. The litterfall samples obtained using this protocol will also be used to study seed dispersal by wind.

## BIOTIC INTERACTIONS

### Goals

- Identify seed dispersal vectors and describe seed dispersal patterns in successional stages of *Tdfs* across a latitudinal gradient in the Neotropics.
- Identify herbivore groups and describe herbivory patterns in successional stages of *Tdfs* across a latitudinal gradient in the Neotropics.
- Establish temporal relationships between seed dispersal and herbivory patterns with phenological patterns in the different successional stages.

### Seed dispersal

In order to determine which seed dispersal agents contribute with propagules in each successional stage, surveys should be conducted based on assemblages of frugivorous bats and birds present in the successional plots. Even though



we recognize the potential contribution of non-flying mammals and ants as seed dispersal agents in both fragmented and undisturbed tropical forests (Bonaccorso *et al.*, 1980; Janzen 1986; Smith *et al.*, 1989; Auld and Denham 1999; Vander Wall *et al.*, 2005; Griscom *et al.*, 2007), we will limit our study to bats and birds, the two groups of seed dispersers from which the most information has been recorded in neotropical forests (Charles-Dominique, 1986; Foster *et al.*, 1986; Medellín and Gaona, 1999; Galindo-González *et al.*, 2000; García *et al.*, 2000; Martínez-Garza and González-Montagut, 2002; Barrantes and Pereira, 2002; Arteaga *et al.*, 2006; Lozada *et al.*, 2007). This survey includes: (1) four census events per year (approximately every three months), (2) collection of fecal samples from birds and bats, and (3) identification of seeds.

### Study of frugivorous bats

#### Census

The four censuses should be scheduled based on the occurrence of the dry and rainy seasons in the study sites: one month after the first rain (beginning of rainy season: BRS), three months after the first rain (peak of rainy season: PRS), one month after the rains stop (beginning of dry season: BDS) and three months after the rains stop (peak of dry season: PDS). The dates at which these climatic phenomena occur will vary among countries and years, but it is important to program the censuses based on the expression of these four seasonal conditions in the different countries and not on fixed dates of the year for all countries.

During one census period, bat captures should be conducted one day in each of the three plots of each successional stage, for a total of 12 plots surveyed over 12 days. One of two possible sampling sequences should be employed: Pasture (day 1) → Early (day 2) → Intermediate (day 3) → Late (day 4), or vice versa. Within the sequence chosen, the replicate of each successional stage to be sampled on any given day should be chosen randomly; for example, a possible sequence could be: Pasture replicate 3 (day 1) → Early replicate 1 (day 2) → Intermediate replicate 2 (day 3) → Late replicate 1 (day 4). This sampling sequence should be repeated a total of three times to include all 12 plots in the survey. Employing this sampling design ensure that two plots of the same successional stage are not sampled over contiguous days. Sampling should be avoided if possible on moonlight nights and rainy days. Overall, one complete census period will take a minimum of 12 days of captures if no capture events have to be cancelled due to rain or other factors (Figure 10).

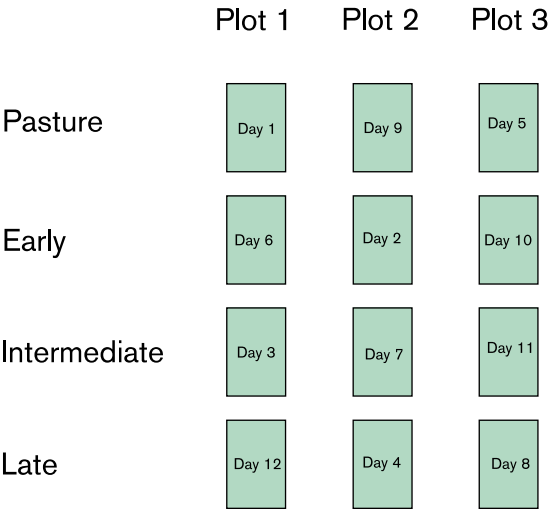


Figure 10.  
Example of sampling design  
for one census of the bat  
community associated with  
each successional plot.

Frugivorous bats (all belonging to family Phyllostomidae) should be captured using ten 38 mm mist nets, 12 m long x 2.5 m high. Mist nets should be placed in flyways inside or close to the plots. Several species of phyllostomid bats disperse seeds in the Neotropics and forage close to the ground (Moreno y Halffter, 2000). Mist nets should be opened at sunset and remain open for 5 hours (Fenton y Kunz, 1977); the nets should be checked for bats at least every 30 min. During the first hours of capture bats tend to fall in the nets very often. Researchers should take this into consideration and try to visit the nets as frequently as possible. Trapped bats should be removed from the nets and temporarily stored in cotton bags. In processing captured bats, the following information should be collected: species identification, weight, gender, relative age and reproductive condition. Species identification should be conducted with the help of taxonomic keys and photographs taken in the field. Age classes should be determined by illuminating the dorsal surface of the extended wing to examine the area of fusion between epiphysis and diaphysis of the fourth metacarpel (Anthony, 1988). Those specimens that show an open epiphysis should be considered subadults, and those with a fused epiphysis should be considered adults. Reproductive condition in females can be determined by examination of the abdominal region and breasts (Racey, 1988). Four reproductive conditions will be considered: pregnant, lactating, post-lactating and non-reproductive. In the case of males, reproductive condition will be based on size of the testicles. Each individual should be weighed with a Pesola spring scale. For data input, see Appendix II (*Seed dispersal by bats*, p. 118).

### ***Fecal samples***

Captured individuals must be kept in cotton bags during approximately 30 min to obtain fecal samples. In addition, while removing bats from mist nets, check for feces on the net or ground. A plastic sheet may be placed below the nets to facilitate fecal collection. Feces must be stored in small paper bags for later processing. In the lab, feces will be examined for seeds. Seeds must be removed from feces as soon as possible in the lab in order to avoid fungal growth on them. Seeds should be carefully cleaned with paper towels, washed with ethanol, air-dried and stored in glass vials appropriately labeled for later identification. Some of these seeds can be germinated to obtain pictures of the seedlings; these pictures can help to determine the species identity of the seeds.

### ***Seed identification***

In order to identify the seeds obtained from the fecal samples, a reference collection of seeds must be prepared. This reference collection is based on the seeds obtained from the fruits collected within the experimental plots during the fruiting season of each species. The seeds must be removed from the fruit pulp, washed, cleaned with paper towels, air-dried and stored in appropriately-labeled glass vials. Unidentified seeds obtained from the feces should be classified as morphospecies temporarily. The seeds obtained in each fecal sample should be quantified by species and morphospecies. This information, combined with the ID of the bats from which the seeds were obtained can be used to generate a list of plant species being dispersed by bats in each successional stage (*see Appendix II List of plant species dispersed by bats and birds*, p. 120). Part of the referential collection of seeds should be germinated to obtain seedlings that can be compared with the seedlings produced by germinating the seeds obtained from the fecal samples. This procedure will help in the identification of all seeds present in the feces.

## Study of frugivorous birds

### Census

In order to optimize the time and effort invested in the study of seed dispersers, surveys of frugivorous birds present in the study plots should be done during the same days and in the same plots that bat surveys are being conducted. As mentioned before, the four censuses should be programmed based on the occurrence of the dry and wet seasons in the study sites: one month after the first rain (beginning of rainy season: BRS), three months after the first rain (middle of rainy season: MRS), one month after the last rain (beginning of dry season: BDS) and three months after the last rain (middle of dry season: MDS).

During one census period, bird captures will be conducted one day in each of the three plots of each successional stage, for a total of 12 plots surveyed. Overall, one census period will require a total of 12 days of captures (Figure 10).

Frugivorous birds should be captured using ten 38 mm mist nets, 12 m long x 2.5 m high. Mist nets should be opened at sunrise and remain open for 5 hours. Mist nets should be checked for birds every 15-30 min. Trapped birds should be removed from the nets, individually marked with numbered aluminum bands and temporarily stored in cotton bags. In processing captured birds, the following information should be collected: species identification, gender (for some species), age, reproductive state, and molt status. Species identification should be determined with taxonomic keys and photographs taken in the field. Sex can be determined for some species using plumage characteristics. Age can be determined as juvenile or adult using plumage characteristics and iris color (Ralph *et al.*, 1994). Reproductive females (nesting) can be recognized by checking the reproductive patch in their abdomen. Molt status can be recognized by checking for new quills on the body and wings. For data input, see Appendix II (*Seed dispersal by birds*, p. 119).

Each day of the mist net census, point counts must be conducted in the area immediately surrounding the plots of each successional stage, within the matrix that contains the same habitat type. Five variable radius point counts of 10 minutes each should be conducted along a 500 m transect to estimate relative abundance of birds. Birds should be registered and identified based on sight and sound. For data input, see Appendix II (*Identification of bird species based on point count surveys*, p. 120).

### Fecal samples

Captured individuals must be kept in cotton bags during approximately 30 min to obtain fecal samples. Seeds should be stored in vials with 70% alcohol for later identification. In the lab, feces will be examined for seeds. In addition to getting fecal samples, stomach contents should be obtained from birds that do not defecate in the bags using the tartrate emetic method (Tomback, 1975; Poulin *et al.*, 1994).

### Seed identification

Seed identification should follow the methods proposed for bats. For data input, see Appendix II (*List of plant species dispersed by bats and birds*, p. 120).

## Seed dispersal by wind

Besides animal-dispersed seeds, wind-dispersed seeds must be studied at each successional stage. To estimate species identity and relative abundance patterns of plant species dispersed by wind in each successional stage, the same samples obtained from the litterfall study should be used. Wind-dispersed seeds should be separated from the litterfall obtained in each litter trap, labeled and stored. In the lab, seeds should be separated and identified per bag and plot, using a reference collection of wind-dispersed seeds that is developed from collections of seeds obtained in the field. The reference collection should be developed from the same samples used for the phenology and reproductive attributes.

## Herbivory

### General pattern

The same two transects per plot used for phenology (50 x 3 m; see *p.* 34) should be used to quantify herbivore patterns for gall-inducing, leaf mining, and leaf chewing insects. Sampling should be conducted for trees and shrubs greater than 2.5 cm DBH and extend from the upper to the lower branches of each individual. For those species that have more than 10 individuals per plot, 10 individuals should be chosen at random among the two transects. Three or more branches should be arbitrarily collected from the top, intermediate, and bottom of each plant individual for all plant species, to provide a sample of no less than 30 leaves from each plant stratum (if the leaves are composite then collect leaflets instead). Ten leaves should be randomly collected from each plant stratum to quantify the impact, and whenever possible the abundance and richness of gall-inducing, leaf mining and chewing herbivores.

### *Galling insects*

For each of the fifty leaves collected, measure the total leaf area (see below), record the number of and digitally photograph each gall morphotype. In addition, record the area of the leaf damaged by gall-inducing insects. To estimate the leaf area affected by galling insects, first obtain a digital image of each leaf and then estimate the total area of the leaf and the area affected using Sigma Scan Pro software. To control for plant size, DBH should be measured on each tree and shrub sampled. Galls from each plant species may be collected and incubated in the laboratory for proper identification of the gall-inducing organism and associated fauna.

### *Mining Insects*

In each of the 30 leaves collected, the abundance and the frequency of leaf miners should be recorded. The leaf area affected by leaf miners should be recorded using a digital image of each leaf, and measuring the total area of the leaf and the area affected by the mining using Sigma Scan Pro software. To control for plant size, DBH should be measured for each tree and shrub sampled.

### *Folivorous insects*

For each of the 30 leaves collected, record the levels of herbivory caused by folivores using a digital image of each leaf. The total leaf area and the area removed by the folivores should be evaluated by the use of the Sigma Scan

Pro software. To control for plant size, DBH should be measured for each tree and shrub sampled.

**General observation.** Many species (such as in various legumes and Anacardiaceae, among others) possess compound leaves of various sizes. In cases in which the leaflets are large ( $\geq 4$  cm length) they may be treated as a leaf unit. In cases in which the leaves are double compound (many legume species) of small sizes, the entire leaf must be used. In any case, a clear specification must be made in the data protocol.

For data input, see Appendix II (*Herbivory general pattern*, p. 121).

## Canopy herbivory

### Sampling design

Sampling should be performed in five points of each 50 x 20 m plot: at the center and next to each corner (Fig. 11). At each point, the emergent (tallest) individual is located. If it is a tree it should be used for climbing. Samples

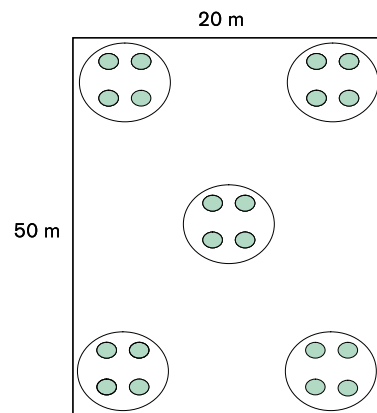


Figure 11.  
Localization of the five sampling sites at each sampling plot. Canopy samples.

should be collected in the four closest neighboring plants with circumference at breast height (CBH) equal to or higher than 15 cm (Fig. 12). If an individual with multiple stems is found, a judgment of its final CBH must be performed. The emergent tree can be one of the four sampled trees, but this is not mandatory. The host plants should be identified to the highest

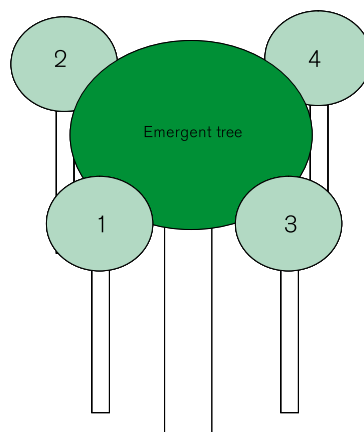


Figure 12.  
Schematic representation of the sampling individuals in relation to the emergent tree individual.

possible taxonomic level. Herbivory samples should be collected once year during two years, at the end of the wet season. This schedule allows for herbivory to accumulate throughout the period when leaves are present.





Figure 13.  
Tree climbing and  
entomological umbrella use.  
a: Tree climber using a  
rope; b, c and d: net hitting  
technique with the help of  
a stepladder on the ground,  
and at the canopy level,  
respectively.

### Canopy samples

The canopy in the intermediate and late successional stages can be accessed through the “free climbing” technique, using a rope (Fig. 13a). In the early succession stage, trees are not usually robust enough to allow climbing. In this case, the canopy should be accessed using a 7 m stepladder. Insect herbivores should be collected through the hitting method. This method consists in using an entomological umbrella (Fig. 13bcd), following the standards proposed by Basset (1999), and modified by Ribeiro *et al.*, (2005). Once in the canopy of the emergent tree, the collector gathers a group of shoots (of variable size and amount) and hits them 10 times with a wooden stick, collecting the insects that fall from the shoots inside the entomological umbrella, which has been positioned directly below the group of shoots. This procedure should be repeated for three different groups of shoots on each tree, therefore totaling 30 hits per individual plant. All sampled insects should be stored in properly labeled glass or plastic recipients containing 70% alcohol. Each labeled recipient should contain information on the successional stage, plot number, sample number (1 to 5), and plant species or morphospecies or its field number (1 to 4). In the laboratory, collected specimens should be separated into morphospecies (operational taxonomic unit) and identified to the highest possible taxonomic level to determine herbivore richness and abundance per host plant. Unidentified species must be sent to a specialist for identification. Later, the herbivorous insects will be grouped into two guilds: sap-suckers and chewers. For data input, see Appendix II (*Canopy herbivore insects*, p. 122).

Finally, 10 stems (shoots of the present growth season) of each sampled plant individual must be collected. The length of these stems must be measured with a ruler and the number of leaves (simple or compound) recorded. For each leaf, the abundance of each morphospecies of insect galls and mines

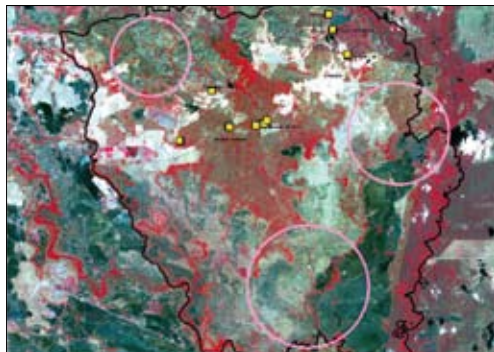


should be recorded. Researchers should be sure to inspect the stem for galls as well as the leaves, as galls on the stem may be less conspicuous than leaf galls. At least one specimen of each gall and mine morphospecies should be stored in 70% alcohol for future identification. Taking photographs of the different galls and mines is strongly suggested, as they may lose their coloration in the alcohol. Five mature leaves from each stem must be collected to determine leaf area lost to herbivores, totaling 50 leaves per individual plant. Leaves can be photographed in the field (with an indication of scale) or scanned in the laboratory. Total leaf area and the area removed by chewing insects should be determined using the appropriate software. For data input, see Appendix II (*Canopy branch herbivory*, p. 123).

### ***Understory samples***

Samples in the understory are to be conducted only in the late successional stages, as in earlier successional stages the understory is often not clearly distinguished or not well developed. Samples must be conducted in the same five areas of the 50 x 20 m plots where the canopy samples are performed. Samples should be taken from 4 randomly selected individuals with CBH equal or higher than 5 cm and shorter than 3 m high, following the same protocol used for the canopy (i.e., through the use of the entomological umbrella). For data input, see Appendix II (*Understory herbivore insects*, p. 124).

## REMOTE SENSING



## **WORKING GROUP**

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## INTRODUCTION

Tropi-Dry's research protocols are designed so that they integrate methods from the three components. The expectation is that each component will address questions relative to the component itself, but also provide tools and knowledge for addressing questions from the two other components. Participants in the three components regularly meet to exchange ideas in order to assure that their research does not proceed in disciplinary isolation.

Remote sensing meets several general Tropi-Dry goals, such as the provision of maps showing the extent of dry forest and other types of land cover, and the characterization of their change (fragmentation levels and rates of deforestation), conservation status, stand age, and disturbance history. It also provides a common/uniform spatial platform for the purposes of data integration and design of field campaigns. Remote sensing can be used to address ecological questions of biodiversity and ecosystem function (e.g. impact of disturbance on species diversity, functional diversity, or ecosystem productivity), as well as social and policy questions relative to the spatial distribution of people and their economic activities (e.g. the impact of a particular land use policy on forest structure and function). Questions that integrate spatial modeling of land cover change under different policy scenarios, with predictions of human demographic growth, in the context of the provision of ecosystem services, for example, are where the remote sensing component can make its biggest contribution.

A major contribution of Tropi-Dry will be the development of a database of processed satellite images at various spatial and temporal scales, available to all project participants. For each site and its area of influence, we will acquire data at high spatial (QuickBird) and high spectral (Hyperion) resolution, nested within time series of medium spatial (Landsat) and coarse spatial (MODIS) resolution. Researchers will be able to move between 1-m spatial resolution QuickBird images, to the decade-long time series of Landsat data, while also being able to examine the nature of land cover reflectance at each field site, using the 220 spectral channels offered by Hyperion. Integration of social sciences with natural sciences will add another unique dimension to the application of remote sensing to the study of tropical dry forests. When possible, data will be granted open-access status and published on the Tropi-Dry website for anyone to download. But even if data owners do not allow their data to be released to the public, we encourage all those interested in using the data to establish contact with Tropi-Dry researchers and seek a collaboration.

## SPATIAL INFORMATION

### Goals

Provide uniform geographic coordinates and base maps for guiding fieldwork, and facilitate data integration, analyses and display.

All cartographic products at the level of the specific field sites will be generated using the Universal Transversal Mercator projection (UTM), Datum WGS-84, with a specification of the Zone and reference to north or south. Coordinates (DD.DD) will be reported with six decimal points. Digital base maps at each site must include, at the very least, a local road network distribution (roadmap), river networks, and other easily recognizable landscape features. These are essential for orthorectification, determining distance from roads, and other georeferencing requirements.

## Tasks

(1) Generation of base maps of each field site using ArcGIS. (2) Generation of global equal area projection base map using ArcGIS.

## Deliverables

(1) Site-specific shape files showing roads, rivers, topography and human settlements. (2) Global shape file displaying the world's countries in an equal area projection, using geographical coordinates.

## REMOTE SENSING DATA ACQUISITION AND PROCESSING

Remote sensing products exist at several “spatial resolutions” (pixel sizes), here identified as “high”, “medium” and “coarse.” Additionally, “spectral resolution” ranges from “multi-spectral” (few, broad bands) to “hyper-spectral” (many narrow wavebands) .

In the long-term, it is expected that Tropi-Dry study sites will build an exhaustive collection of satellite images, using the following products and/or sensors:

Multi-spectral data:

- High resolution: QuickBird (2.4 spatial resolution; [www.digitalglobe.com](http://www.digitalglobe.com)).
- Medium resolution: Landsat [Multispectral Scanner (MSS), Thematic Mapper (TM) and Enhanced Thematic Mapper (ETM+); <http://landsat.gsfc.nasa.gov/>] and Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER; [asterweb.jpl.nasa.gov](http://asterweb.jpl.nasa.gov)).
- Coarse resolution: Moderate Resolution Imaging Spectroradiometer (MODIS; [modis.gsfc.nasa.gov](http://modis.gsfc.nasa.gov)).

Hyper-spectral data

- Hyperion ([edc.usgs.gov/products/satellite/eo1.html](http://edc.usgs.gov/products/satellite/eo1.html)).
- CHRIS/PROBA ([earth.esa.int/proba/](http://earth.esa.int/proba/)).

Because of the cost associated with high resolution and hyper-spectral data, however, Tropi-Dry will initially focus on the acquisition of a time series of medium and coarse resolution imagery for all study sites. High resolution and hyper-spectral data will be acquired when possible.

We begin, by illustrating the use of high resolution and hyper-spectral data, and then proceed to detailing our protocols regarding medium and coarse resolution, multi-spectral data.

## Image acquisition and processing

### High spatial resolution data – QuickBird (available on demand)

QuickBird data sets are commercially available from Digital Globe ([www.digitalglobe.com](http://www.digitalglobe.com)). The sensor has two spatial resolutions: 0.60 m in panchromatic mode and 2.4 m in multispectral mode. Four bands (red, green, blue and near infrared) are present in the image. Images for this sensor are used specifically for plot location and development of basic cartography.

**Objectives:** The purpose of acquiring QuickBird imagery is to develop detailed base maps (especially at sites where official cartography is unavailable or outdated), characterize community structure (e.g. detailed vegetation mapping, identifying populations of individual trees), and provide a support tool for field sampling.

**Tasks:** QuickBird data must be identified, purchased, and georeferenced. (QuickBird data is not atmospherically corrected.)

**Deliverables:** Georeferenced imagery by site according to agreed precision and accuracy.

### **High spectral resolution – Hyperion (available on demand)**

Hyperion is a sensor part of the EO-1 NASA Satellite system currently in orbit. Hyperion collects 220 unique spectral channels ranging from 0.357 to 2.576 micrometers with a 10-nm bandwidth. The instrument operates in a pushbroom fashion, with a spatial resolution of 30 m for all bands. The standard scene width is 7.7 km. Standard scene length is 42 km, with an optional increased scene length of 185 km.

**Objectives:** The purpose of acquiring Hyperion imagery is to provide maps of stand structure, age, composition, ecosystem complexity (e.g. Holdridge Index), leaf area index, and ecosystem function.

**Tasks:** Hyperion requires at least two acquisitions per year (one during dry season, and one during wet season). These images must be georeferenced and atmospherically corrected.

**Deliverables:** Maps of stand structure, age, composition, complexity, leaf area index and function can be produced from Hyperion.

### **Medium resolution data – Landsat (available for the time period 1972 to April 2003)**

The Landsat Program is a series of Earth-observing satellite missions jointly managed by NASA and the U.S. Geological Survey. Since 1972, Landsat satellites have collected information about Earth from space. The fundamental goal of Landsat MSS, TM and ETM+ images is to provide a comprehensive view of the dynamics of land use/cover change at each one of the Tropi-Dry Sites.

**Objectives:** The primary use of Landsat imagery will be to construct maps of land cover change and successional stage.

**Tasks:** Landsat data must be downloaded, georeferenced and atmospherically corrected. In some cases, data must be purchased.

**Deliverables:** Maps of land cover, successional stage and forest cover/land use change.

### ***Atmospheric Correction***

All images acquired by the project must be atmospherically corrected, allowing a detailed analysis of vegetation reflectance as a function of successional stage. The University of Alberta's Earth Observation Systems Labora-



tory (EOSL) will conduct the atmospheric correction of the images using Atcor, the Erdas Imagine atmospheric correction module.

### **Georectification**

Imagery and digital elevation model (DEM)

Imagery and DEM data used in the production of Tropi-Dry products should be of the highest resolution possible. Having said that, not every field site will be able to acquire high resolution imagery or high resolution DEM information – the minimum allowed scale will be 1:50,000. Unless higher resolution data is available, all Tropi-Dry sites should develop their DEM for orthorectification using data from the Shuttle Radar Topography Mission (SRTM). SRTM data can be downloaded from the website of the Consultative Group for International Agricultural Resources (CIGAR) Consortium for Spatial Information (<http://srtm.csi.cgiar.org/SELECTION/inputCoord.asp>). For more information on the accuracy of these data, as well as access to the entire dataset, visit the SRTM website (<http://www2.jpl.nasa.gov/srtm/>).

Orthorectification process

Orthorectification can be facilitated using a number of remote sensing packages, such as Geomatica, ERDAS, and ERMapper. Each of these programs has a rectification process, requiring the image to be rectified, a suitable DEM and correction points. An explanation of the overall procedure for the orthorectification processes and the integration of the DEM (especially for hyper-spectral datasets) can be found in Feng *et al.*, (2003).

The process involves matching identifiable features in the image with those known on the ground, using a DEM to correct for distortion in the world. It is not within the context of this document to teach the process of image orthorectification. It should be noted that the practitioner should have sufficient experience with the software and production process that any resulting errors are not due to operator mistake.

Ground Control points

When orthorectifying the imagery, care should be taken in the choice of ground control points (GCP) for the process. One should avoid features with short-term stability or points that are temporal in nature.

The features that are best for orthorectifying are those that can be easily seen in the image of interest and that have good positional information. It is not advisable to use hydrological features, because although they can be easily seen, their active nature will often create errors because the position of the feature when mapped is no longer the position of the feature today. Simply put, the river has changed course and is no longer in the same position as on the map. Roads, intersections and stable physical features can be used. They must be easily recognized in the imagery as well as accessible for survey on the ground.

GCPs can come from mapped products, preferably digital, or from newly collected information from field surveys. The more accurate the position of these features, the more accuracy there will be in the final product. Where possible cadastral map features should be included in the pro-

cess. Such data are often available from governmental agencies, however are not always easy to get. Where such map features are not available, ground survey of correction points is the only alternative. Correction points can be collected using middle to upper end GPS units (e.g. such as Garmin). However care must be taken to ensure that the points surveyed are those that are identifiable on the image. A careful review of the image features is advisable prior to field collection – better yet, it is advisable to print a color copy of the highest resolution image available, and take it to the field for ground truthing.

To ensure the maximum spatial accuracy of the resulting image, survey points should be collected in the datum and projection of the desired resulting map product (typically datum WGS-84 and UTM projection). In addition, the position of the survey points should be averaged: each point should be collected with a GPS for a minimum of 10 minutes with the average position being used as the point location. These data should be stored in a structured database for post-processing analysis, should there be any problems with the orthorectification process.

Depending on the imagery in question, the number of GCPs will vary, but as a rule of thumb 40 GCPs should be collected. Twenty of these can be used in the process of orthorectifying the image and 20 for post-processing audit (see below). The desired positional accuracy of each survey point should be  $\pm 3$  pixels of the image used, 90% of the time.

#### Audit process

Post processing of the image for final positional accuracy can be accomplished in a number of ways. One of the simplest is that of comparing the orthorectified image to known features that have a high positional accuracy. A program called RecAudit is available for use by Tropi-Dry project members for this purpose. It runs in ArcView 3.2, and was developed by Sanchez-Azofeifa under contract for Alberta Sustainable Resource Development. RecAudit can be obtained from the Earth Observation Systems Laboratory (<http://eosl.eas.ualberta.ca>). The following discussion will not discuss the actual process but will provide some instruction on some of the technical points.

This process provides a root mean square error (RSME) assessment of features within the image (roads, cut lines and such) against the known position of these features on the ground. Those points collected in the field program but not used in the orthorectification process are now used to test the quality of the final image. These and any digital map data that has a reasonably high degree of spatial accuracy can be used.

Comparison of the features seen in the image and their known position provides an easy way to calculate statistics on image positional accuracy. The surveyed GCPs or features within government or county cadastral files can be used. As indicated above, at least 20 points within the image should be assessed, but not the same points that were used in the orthorectification process.

The final assessment should not be less than the above stated quality standard:  $\pm 3$  pixels of the image used, 90% of the time. The use of imagery not meeting the spatial quality standard is a decision left to Tropi-Dry's principal investigators, but must be clearly stated in any analysis that uses that

image. The result of the spatial audit should be included in the metadata for the image.

Figure 1 shows the formula used to calculate the fit of the image to the vectors, and/or the GCPs. The  $x$  and  $y$  positions for each audit point are compared, and a final assessment of all of the differences,  $x$  and  $y$ , are presented.

$$R_x = \sqrt{\frac{1}{n} \sum_{i=1}^n XR_i^2}$$

$$R_y = \sqrt{\frac{1}{n} \sum_{i=1}^n YR_i^2}$$

$$T = \sqrt{R_x^2 + R_y^2} \quad or \quad \sqrt{\frac{1}{n} \sum_{i=1}^n XR_i^2 + YR_i^2}$$

Where:

R <sub>x</sub> = X RMS error	$i$ = the GCP number
R <sub>y</sub> = Y RMS error	XR <sub><math>i</math></sub> = the X residual for GCP $i$
$T$ = total RMS error	YR <sub><math>i</math></sub> = the Y residual for GCP $i$
$n$ = the number of GCP's	

Figure 1.  
RMSE formula for the  
RecAudit process.

It is important to note that this process assumes that:

1. The auditor is familiar with the technical process of running an audit and is comfortable working with the system.
2. The vector information is spatially correct.

If the auditor is not careful in choosing the appropriate points, is in error when placing the cursor on the point, or the vector/GCP data itself is in error, then the resulting calculation of positional error of the image will be higher and may not be a true reflection in the spatial position of the image.

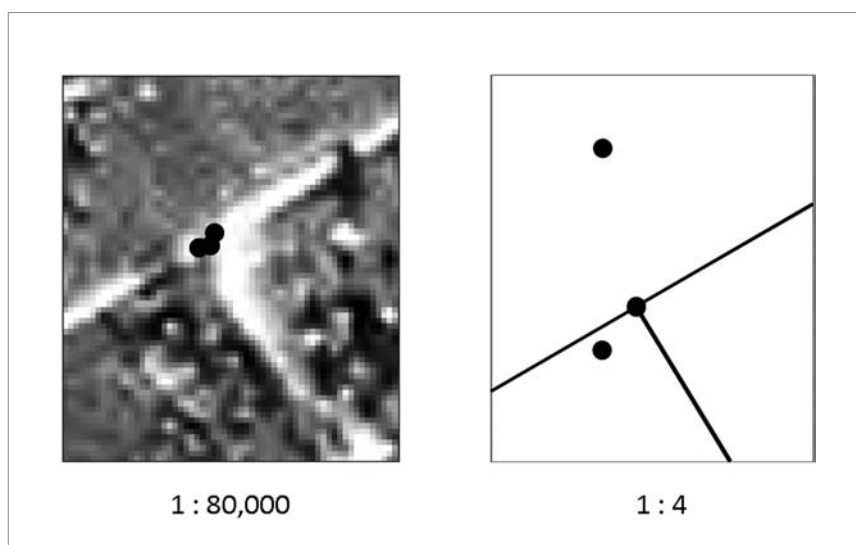
### Choice of Audit Points

As in the choice of GCPs, the features that are best for auditing are those that can be easily seen in the image to be audited and that have good positional information: roads, intersections, and stable physical features are acceptable, hydrological features should not be used (see above).

When choosing audit points, both the image and the vector data should be enlarged to a comfortable viewing scale. The larger the scale of representation, the better. This is dependent on the resolution of the imagery. The optimal scale of 1:1,500 (aerial photography) is not always achievable due to low image resolution. The operator will have to determine the optimal scale of representation through trial and error.

Figure 2 shows an example of the results of choosing a vector point at two different scales of representation: 1:80,000 and 1:4. At each scale, the intersection of the same two lines was used. Clearly, the larger the scale of representation is, the better. Using a larger scale will reduce operator error in determining the actual position of the feature used.

Figure 2.  
Choosing the appropriate  
scale of representation.



While using a scale of 1:4 would provide the best location of the intersection, the time required to do this for all the points would be substantial. It will be up to the operator to decide what scale works best for the vectors and imagery.

Of particular importance is that the vector file be a clean, simple intersection, such that no guesswork is required to estimate the point of intersection. The same is true of the intersection of the image. If the image and the vectors are correct there should be very little difference in their respective  $x$  and  $y$  positions. This is a good point for auditing the image.

Figure 3 shows the intersection of two roads where the intersection is clearly visible and a point can be put confidently within its center. In addition, the features intersect at a 90° angle (in both the image and the vector). Interpretation of the center point is simple.

Figure 3.  
Example of a good audit  
point.

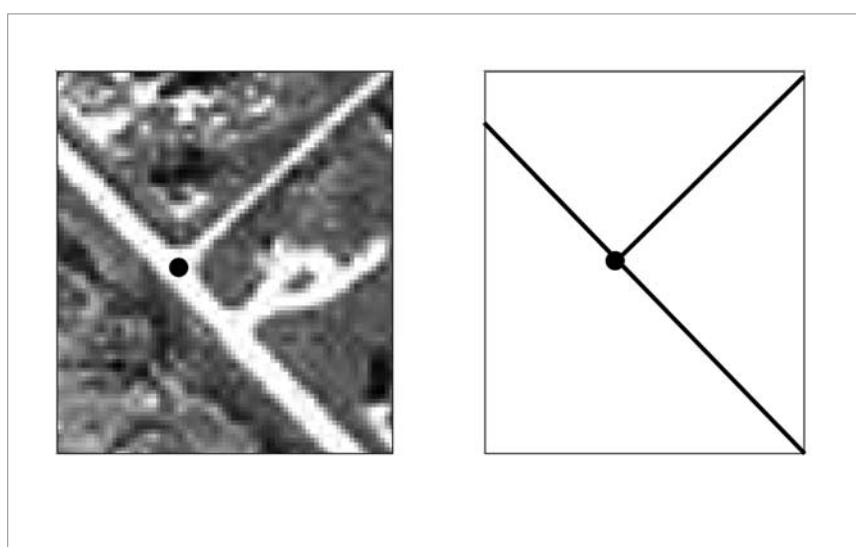


Figure 4 depicts a somewhat similar situation, however there is not a good intersection of the vector information, nor is the intersection at right an-

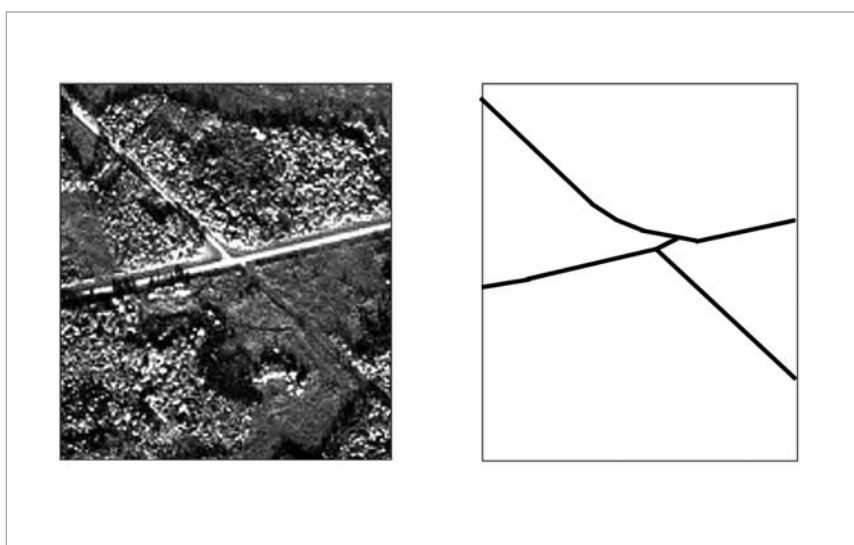


Figure 4.  
Poor choice of an audit  
point.

gles. The intersection is clearly visible on the image, but there is not a clean bisection of the lines on either the image or the vector. The operator is forced to make a best guess of the actual intersection. In this situation, the operator error would most likely be too large, rendering this a poor choice for an audit point.

#### Coarse resolution data – MODIS (available from 2000 to present)

The MODerate resolution Imaging Spectroradiometer (MODIS) on board the Terra and Aqua satellites is an optical sensor designed to deliver spectral information of the earth's surface in 36 spectral bands ranging in wavelength from 0.4  $\mu\text{m}$  to 14.4  $\mu\text{m}$  at a pixel resolution of 250-m, 500-m or 1-km. MODIS Imagery has been made free and readily available to the scientific community and has already been used to model global ecosystems distribution and conservation status (Friedl *et al.*, 2002, Muchoney *et al.*, 2000; Giri and Jenkins 2005). Terra's orbit around the Earth is timed so that it passes from north to south across the equator in the morning, while Aqua passes south to north over the equator in the afternoon. Terra MODIS and Aqua MODIS are viewing the entire Earth's surface every 1 to 2 days.

**Objectives:** MODIS imagery will be used to provide maps of global extent of tropical dry forests, characterize broad (e.g. latitudinal) gradients in forest structure, and provide time series of key variables (e.g. NDVI, EVI, etc.) for phenological analyses and linkages with SpecNet (<http://specnet.info>). MODIS subsets ("cutouts" for specific sites) will be used for detailed time-series analyses of specific sites, and to link synoptic remote sensing products (e.g. forest type maps) to field studies (e.g. phenological analyses).

**Tasks:** Data must be downloaded from any MODIS NASA data provider website and re-projected using the MODIS reprojection tool (downloadable at <http://edcdaac.usgs.gov/landdaac/tools/modis/index.asp>). Data must be clipped to include potential dry forest extent (e.g. using potential vegetation cover as indicated by Olson and Dinerstein 2002). MODIS cutout data (time series for specific locations) can be downloaded separately from the Oak Ridge National Laboratory (<http://www.modis.ornl.gov/modis/index.cfm>), though not all Tropi-Dry study sites are available.

**Deliverables:** Maps of tropical dry forest extent and time-series (e.g. phenological) products will be the chief MODIS deliverables.

### **MODIS Products**

#### MODIS Level 1: Spectral information

The objective is to extract MODIS surface reflectance data (Bands 1-7) at 500-m pixel resolution that potentially represents the Tropical Dry Forest Ecosystem in the Meso- and South American region. This objective will be achieved by:

- a) Downloading MODIS surface reflectance imagery (Cloud cover <30%) from the NASA LP DAAC (Land Processes Distributed Active Archive Center; <http://edcdaac.usgs.gov/main.asp>) for the Mesoamerica and South America regions, from the wet and dry season.
- b) Limiting the potential distribution of Tropical Dry Forests using previously defined ecoclimatic information that defines tropical biomes distribution (Olson and Dinerstein, 2002). Following the approach of Miles *et al.*, (2006), MODIS cells are to be extracted that fall within the Olson and Dinerstein (2002) biome classes: "Tropical and Subtropical dry broadleaf forest", "Mediterranean Forest, woodland and scrub", "Desert and Xeric Shrubland" and "Tropical and Subtropical Grassland, savanna and shrub".
- c) Deriving MODIS single scenes for the dry season and wet season with <5% of cloud cover produced using map algebra and several images available for each period (wet and dry). For each country, analysis of wet and dry seasons should first be accomplished by an analysis of forest phenology (e.g. using NDVI).
- d) Further identifying the extent of Tropical Dry Forest by performing an automated classification (Decision Tree Algorithms/Machine Learning) using the spectral bands from MODIS subsets, training sites and other ancillary data (e.g. elevation, slope, aspect and spectral indices). For further information on this method, refer to Giri and Jenkins (2005). The method will allow us to map the distribution of Tropical dry forest and potentially identify differential patterns of leaf-on/leaf-off activity within the ecosystem.

The Tropical Dry Forest map will allow us to monitor spectral information within its extension and derive products to understand Tropical Dry Forest optical and ecological dynamics across the Americas.

#### MODIS Level 2: MODIS Products: NDVI, PAR, LAI, EVI and SIWSIs

At selected Tropi-Dry sites at different latitudes, we will explore how the MODIS vegetation products quantify the phenology along a latitudinal gradient. Using MODIS reflectance and vegetation products, it is possible to use the following indices to obtain the inter-annual patterns and compare phenology as a function of latitude:

1. Normalized Difference Vegetation Index (NDVI)
2. Fractional Photosynthetically Active Radiation (FPAR)
3. Leaf Area Index (LAI)
4. Enhanced Vegetation Index (EVI)



5. Two Shortwave Infrared Water Stress Indices (SIWSIs): SIWSI (6,2) and SIWSI (5,2)

The following MODIS products must be downloaded from NASA LP DAAC:

1. MOD 13: Normalized Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI)
2. MOD 15: Leaf Area Index (LAI) and Fractional Photosynthetically Active Radiation (FPAR)
3. MOD 09: Surface Reflectance: the reflectance of band 2, 5 and 6 will be used to calculate the two SIWSIs:

$$SIWSI(6, 2) = \frac{\rho_6 - \rho_2}{\rho_6 + \rho_2} \quad SIWSI(5, 2) = \frac{\rho_5 - \rho_2}{\rho_5 + \rho_2}$$

Data over a 3-year period should be plotted against time. Erroneous data must be removed according to MODIS' Quality Control Flags. A cubic spline interpolation should be applied to replace missing values (Zhang *et al.*, 2003). The first derivative of the indices plotted against time can be used to determine the beginning and the end of the growing period, and the stable LAI period. The student's t-test can be used to assess whether the means of any two phenological patterns from the four sites are statistically different.

## FIELD DATA COLLECTION FOR CALIBRATION AND VALIDATION

Remotely sensed data must always be verified in the field, to assure that interpretation is accurate and assigned land cover categories actually reflect landscape features. This is especially important for tropical dry forests, which are subject to extreme fluctuations in their spectral properties throughout the year, as their phenology changes in response to fluctuations in precipitation and temperature. In the dry season, a tropical dry forest may be hard to distinguish from scrub or savanna vegetation, while in the wet season it may appear like a humid forest.

### Goal

To provide ground information ("ground truthing") to validate and calibrate the various remote sensing products.

### Tasks

The primary field tasks of the remote sensing group are to provide spatial information (GPS coordinates and plot boundaries) for accurate registration of images and for integration of remote sensing with ecological and social science data.

### Deliverables

GPS points will be provided for the following:

- i. Distribution of sample points for the generation, calibration and validation of land use/land cover maps.
- ii. Spatial location of plots used by the ecology group.
- iii. Spatial information of sampling points within plots.

- iv. Relevant infrastructure and features for base map construction.
- v. Social community based information used by the social science group.

## Field Data Collection

Due to the nature of the field activities and the distance to assistance, it is very important that field operations be well planned. A four-hour ride to a plot can be made very frustrating, for instance, if upon arrival you find that instrument batteries are not fully charged.

### Field Form

Prior to setting out, a field form should be drafted to be used in the operation. This will not only provide a paper copy of the information collected but also necessary metadata on the operation. It should include at minimum:

1. Date
2. Project Name
3. Investigators
4. Objectives
5. Weather conditions
6. Reason for investigation
7. Comments
8. Pictures taken
9. GPS Coordinates
10. Accomplishments

### Conditions of the Existing Equipment

If a field form is created prior to the field trip, all objectives for the excursion can be articulated. This is important in clarifying to personnel what will be accomplished and what will not be accomplished. Clarifying the objectives of the field operation in advance will help make things flow smoothly.

### Field Equipment

Prior to leaving for the field it is valuable to *make a list* of all of the equipment needed for the operation.

A good suggestion is to have one person be responsible for all of the equipment. Equipment maintenance can be distributed among the team members but one person should be responsible for all of it. This is particularly important for equipment that is sensitive in nature and/or requires power. Batteries should be recharged nightly and if possible, spare batteries should be acquired. Cords and cables for electrical equipment should be labeled and if possible duplicates should be acquired.

Before each sojourn, the person responsible for all of the field equipment should review the equipment list (operation objectives), and ensure that all required pieces are present and are in working order. This could take place the morning of departure but is best done the night before. Should some piece of equipment fail or something go faulty, there may be time in the morning to correct the situation.

In addition, a small tool box of the most commonly used tools should be put together and carried in the vehicle; equipment should include (at mini-

mum): hammer, screwdrivers, duct tape, level, nails, and an assortment of screws. This will allow the field team to make minor repairs in the field and could save considerable time. A shovel, a car jack and towropes are essential safety equipment should a vehicle get stuck, and should be carried in the vehicle at all times.

For plot establishment and monitoring, the following items are suggested:

1. Sample point markers – colored flags, PVC pipe cut to  $\sim 1\frac{1}{2}$  ft., etc.
2. Spray paint – three colors: fluorescent orange, blue and red
3. Measuring tape: 2 x 50 m and 1 x 100m
4. Flagging tape
5. Cord
6. Compass
7. GPS units
8. Camera, digital preferred
9. Permanent markers
10. Water
11. Lunch

## Records

### *Field Notes*

For obvious reasons, notes on field activities should be captured in the field. This will ensure the greatest probability that the notes are correctly annotated and recorded. A diary is recommended as it provides the ability to keep all of the notes in a one place, making them easy to locate later. A good practice is to do as much recording in the field as is possible before the plot is departed. This will reduce the need to return for missed information.

### *Photographs*

Some digital cameras will provide the actual number of the picture when the picture is taken. Many do not. It is very valuable to record on a form the number of the picture taken relative to the situation or plot and the total number of pictures taken. It is sometimes handy to photograph a blank image between plot images to allow the reviewer to separate them easily. Many plots will look the same and this makes it difficult to separate the images and organize them according to plot.

It is best to create a separate metadata form for photography wherein the plot and sample point can be identified and the photos taken there recorded. It need not be complicated and can be as simple as this:

Plot	Sample Plot	Objective	Images
CDN 1	A1	Hemi pictures	DSC001-003
		Field shot	DSC004
CDN 1	B2	Hemi Pictures	DSC005-008

With hemispherical pictures, a close-up picture of the plot number/sample point number printed on a white card, taken just before or after the photos of that plot/sample point are taken, will help ensure that those photos are attributed to the correct plot.

When back in the office, it is good practice to copy the images to CD as soon as possible. Memory cards can fail and, from time to time, cameras can fail as well. Duplicating the images quickly to a back-up source is key to preserving the images should anything happen to the memory card or to the camera.

Images captured during a field exercise should be catalogued as quickly as possible. There are several software packages that can be used to do this. One shareware program that is handy is "RenameIt", though there are several others easily available from the Internet. A good practice is to append the project name to the beginning of each image file name, for example:

DSC001 becomes CDN\_1\_A1\_DSC001

In this way, all of the imagery for the project CDN can be easily found and sorted from all of the other images, and from all of the other projects.

### ***GPS Records***

The addition of a GPS unit is a great tool for recording sites and incidents. There are many types of units, and they all have their own operating systems. What is most important is that one become comfortable with the unit being used.

Of particular importance is the datum and projection of the position information that will be captured (for Tropi-Dry it would typically be datum WGS-84 and UTM projection). These two parameters are often required for future use of the data. For example, if the data are to be used to correct satellite imagery, the data should be collected in the datum, projection and format of the software that will be used for the image correction. Data can be collected in other datums and projections, but they may have to be translated into their final form, and this will add another step to the process.

The format of the position data is also important. To bring data into ESRI products such as ArcGIS, for example, the data must be in decimal degrees. If the position data are collected in degrees-minutes-seconds, they will have to be translated back into decimal degrees to allow loading into ESRI map products.

Like photos, the GPS is a means of recording project information. Therefore, data should be collected and catalogued according to the project, as described for photographs. Waypoint data and route data should be recorded and catalogued according to the project, for example, CDN\_01\_AB1\_wave\_01.wav. In this case, the first wave point for the first sub-sample point of project CDN\_01 is identified. Data should be named and archived as soon after the field expedition as possible.

### ***USGS – FGDC Metadata Standard***

Each image should be processed according to protocol along with the production of appropriate metadata. It is suggested that Tropi-Dry image metadata follow the USGS-FGDC Metadata model (<http://www.fgdc.gov/metadata/csdgm/>). This file should contain all the relevant information about the image, its attributes and the results of the orthorectification and audit processes. Diligence in completing this last element of the production process will provide valuable data for the project in general and for those who may use the imagery for other products in the future.

## Linear Feature Extraction

Mutually agreed upon linear features must be extracted from the imagery acquired. Linear feature vector datasets should be created using Auto Sync, the Erdas Imagine 9.7 digitizing module. Supplementary vector data acquired either in the field using a GPS or digitized from maps using other software should be re-projected to a common projection and used to extract the linear features by recoding the data accordingly.

## GENERATION OF FOREST SUCCESSION AND STRUCTURE MAPS: LINKING REMOTE SENSING TO ECOLOGY AND SOCIAL SCIENCE

Being able to accurately distinguish tropical dry forest successional stages from mature dry forests and from other land cover is a central objective of Tropi-Dry. This capacity would provide a clear picture of the status of tropical dry forests in the Americas, and would also allow the assessment of their potential to deliver ecosystem services. For example, a correct quantification of the carbon sequestration potential of the different successional stages of tropical dry forests via remote sensing would provide society with a relatively inexpensive and expeditive tool for negotiating in the international carbon credits market.

### Goal

Provide land use and land cover change maps which support the ecology and social science activities of Tropi-Dry. These maps also provide a basis for detailed analysis of ecosystem function (e.g. productivity, carbon sequestration). This activity will allow the exploration into how remote sensing can be used to understand the dynamics of secondary tropical dry forests across latitudinal gradients.

### Tasks

Utilize artificial intelligence and inverse modeling techniques to develop maps of forest structure and composition.

### Deliverables

Primarily, change-detection maps over 3 time periods (1980s, 1990s, and 2000) with the following classes: deforestation, secondary growth, and stand age. In addition, forest structure and composition maps for each Tropi-Dry site.

Preliminary analyses conducted in Costa Rica (Arroyo-Mora *et al.*, 2005) have shown that successional stages based on forest structure rather than age have statistically distinct spectral characteristics. The focus on structure is important because it is the canopy that interacts with the incident radiation to produce a spectral signature. Age and structure are not correlated because of the high degree of variation in land use history in tropical dry forests (Arroyo-Mora *et al.*, 2005, Kalácska *et al.*, 2004). Chronological age since abandonment cannot be used to choose plots because from a remote sensing perspective, the results will be misleading and will not cover a wide enough range of spectral characteristics (Figure 5). When plots are chosen based on structure, successional stages can be distinguished using vegetation indices such as NDVI (Figure 6, Arroyo-Mora *et al.*, 2005)

Figure 5. Scatter plots of reflectance in bands 3 and 4 of pixels from sites with known ages (top plot) and the same sites classified by successional stage (bottom plot). The classes for the ages are: ca 5-10 yrs, 19-22 yrs, 25-30 yrs and Never Cleared (NC) (from Arroyo-Mora *et al.*, 2005).

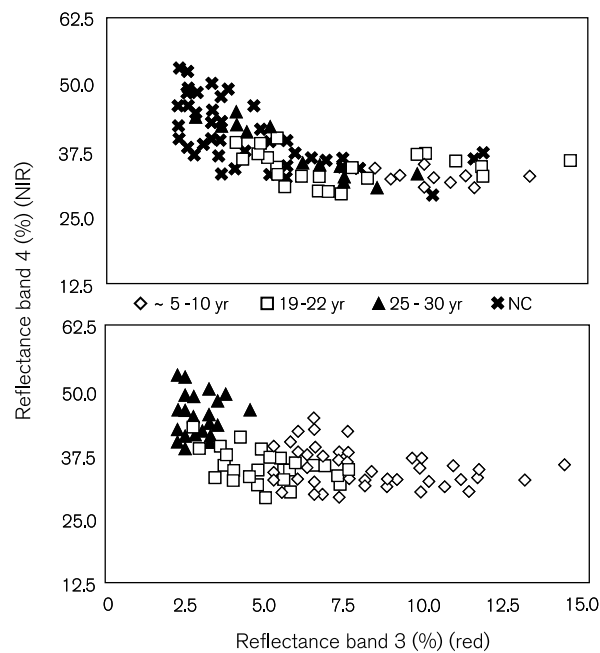
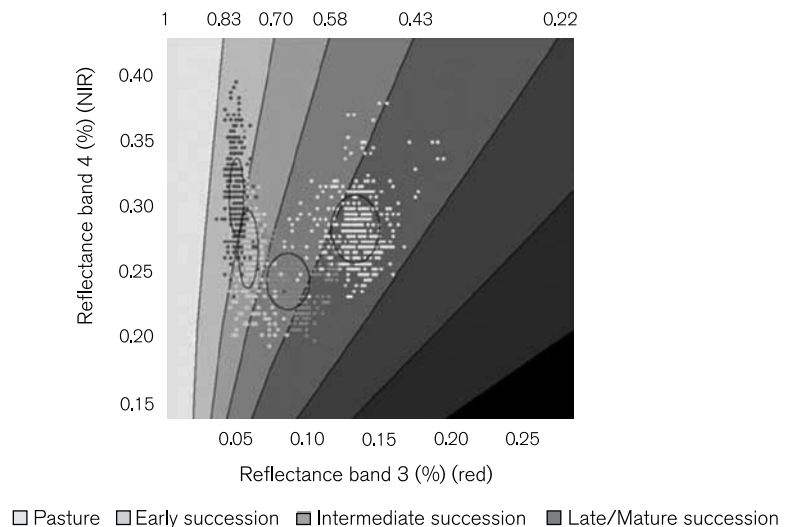


Figure 6. NDVI for Landsat ETM+ image based on successional stages. The 225 pixel values per class, from  $3 \times 3$  kernel window on the Landsat ETM+ image, are displayed over the vegetation index thresholds. Ellipses represent the mean and standard deviation estimated for each successional stage. Gray colors represent uniform classes of each spectral index (from Arroyo-Mora *et al.*, 2005).



Using vegetation index classes, imagery can be classified into forest successional maps to help guide the location and set-up of plots in the field. For each site forest structural characteristics will differ considerably; the following is simply an example of values of different forest characteristics for three different dry forest sites in Costa Rica and Mexico (from Kalácska *et al.*, 2005).

## Mapping Tropical Dry Forest Characteristics from Hyperspectral Imagery

### Motivation

Simple forest/non-forest maps do not offer the level of detail necessary to assess forest degradation or recovery (Ingram *et al.*, 2005). On a regional scale, areas can be more precisely assessed and prioritized for conservation by illustrating the characteristics of the forest than by considering only one attribute (e.g. measuring leaf area index only). The changes in the characteristics of the forest (e.g. biomass, basal area, diversity) are the details needed to properly assess the condition of the forest within a specific area. Maps such as the ones created in this study are a valuable resource for



the management and conservation of tropical forest ecosystems, especially when considered in the context of being a comprehensive spatial snapshot of the forest structure and diversity.

### Field data requirements

For these analyses the following field data should be used, collected during the floristic inventory phase:

- Identification of all stems with a DBH greater or equal to 5cm (0.1 ha plots).
- DBH for all stems at or above 5cm (0.1 ha plots).
- Canopy height (0.1 ha plots).

From these data the following variables are calculated:

- Canopy height
- Basal area
- Shannon species richness:  $H' = -\sum p_i \ln p_i$ , where  $p_i$  is the proportion of individuals found in the  $i$ th species
- Aboveground biomass:  $ATB = \exp\{-1.996 + 2.32(\ln D)\}$  where  $D$  is DBH in centimeters
- Holdridge Complexity Index:  $HCI = \frac{HGDS}{10000}$

where  $H$  is canopy height,  $G$  is basal area,  $D$  is the number of stems and  $S$  is the number of species

- Number of species per 0.1 ha plot
- Number of stems per 0.1 ha plot

NOTE: The following methodology was developed for a field site in Santa Rosa National Park, Costa Rica with 26 plots in three successional stages. The general methodology should be applicable to several other sites, but the models created for Santa Rosa must be created for each site independently. For the Tropi-Dry project, this activity will be coordinated by and carried out primarily at EOSL.

### Methods

The satellite images used in the study from Santa Rosa were three atmospherically corrected Hyperion scenes (220 bands, 30m spatial resolution) acquired in March 2002, December 2002, and January 2003. The pixel values for each of the 26 sites were extracted from each image in a 3x3 pixel window around the center of each plot. Initial pre-processing consisted of the removal of the bands with a low signal-to-noise ratio (<430nm and >2400nm) and the areas of water absorption (centered around 1400nm and 1900nm) resulting in a total of 153 bands for the analysis.

The analysis consisted of a discrete wavelet decomposition followed by a regression model. Wavelet transform is a time-scale signal analysis technique that may reveal features of a signal (e.g. reflectance spectrum) such as trends or discontinuities that other techniques may miss (Misiti *et al.*, 1996). The wavelet transform process breaks up a signal into shifted and scaled versions of the original waveform being used (i.e. mother wavelet). The majority of these waveforms tend to be irregular and asymmetric. With discrete wavelet transforms, signals are analyzed over a set of discrete scales (i.e.  $2^j$ ,  $j=1,2,3,\dots,n$ ). Wavelet basis functions are calculated for spectral data by shifting and scaling the mother wavelet (basis function) across the data. The result is a set of approximation (cAj) (low pass filter) and detail (cDj) (high pass filter) coef-

ficients (Mallat 1989, Misiti *et al.*, 1996). We conducted a discrete wavelet transform of the Hyperion spectra using a mother wavelet from the Daubechies family (db3). This multilevel decomposition (7 levels) was implemented in Matlab v.6.5 resulting in seven detail coefficients (cD1-7) and the largest approximation coefficient (cA7) with a combined length of 182 elements.

Subsequently, a stepwise regression was employed to select which elements from wavelet decomposition to include in the final model to infer the forest characteristics from the images. To test the effectiveness of the various models we used a cross validation method (Isaaks and Srivastava, 1989) to assess the validity of the models. This “leave-one-out” validation technique was used due to the limited number of data points (sites). The withheld data points were sequentially estimated by the models (best fit models reduction – inference combination from best overall models) to produce an overall estimation of the fit. Twenty-six separate models were created for each variable, each with twenty-five data points. These models were then used to estimate the value of the data point that was left out. The model (i.e. data reduction/transformation technique with regression model) with the best fit (from the validation) was then run on a pixel-by-pixel basis over the image (using Matlab v.6.5) to create maps of the biophysical characteristics and floristic diversity.

Specifically for the wavelet decomposition, the wavelength regions of interest were obtained by conducting an inverse discrete wavelet transform. This approach may be “reversed” to reconstruct each decomposition level (starting from cA7 and cD7) by inverting the decomposition step first through upsampling (i.e. addition of zeros between each element in the “odd” locations of the vector) and subsequently convolving the resulting array with reconstruction filters for the given wavelet (db3 in this case). The general reconstruction formula is (Mallat 1989, de Carvalho *et al.*, 2004):

$$a_{j,k} = \sum_l [h(k-2l)d_{j+1,l} + g(k-2l)]d_{j-1,l}$$

for all  $j, k$ , where  $h$  and  $g$  are the high pass and low pass reconstruction filters,  $j$  and  $k$  are the level and location in the array (e.g.  $a_{2,3}$  is the third element in the approximation coefficients at the second level),  $d$  are the detail coefficients and  $l$  is the length of the vector.

For Santa Rosa, Costa Rica, the final overall best model for biomass, Shannon diversity, canopy height, Holdridge Complexity Index, and basal area was with the March image (dry season). There was no suitable model following validation for stem or species densities. However, species density has a relatively strong relationship with Shannon species richness ( $R^2 = 0.79$ ) and could subsequently be mapped from Shannon species richness. These may or may not be similar to results found in other sites.

For the multiple regression, the error from the cross-validation remained fairly constant for all sites regardless of whether or not they were close to the mean. This is an important consideration because there is a broad range for all biophysical characteristics.

In summary, the most robust model for estimating the biophysical characteristics and diversity was the wavelet transform, followed by a stepwise regression from the March (dry season) image. The majority of the features chosen from the wavelet decomposition were from cD1 and cD2. Upon examination of the wavelengths that were associated with the elements chosen for each stage, it was found that for biomass the wavelengths were primarily in the visible (498, 621, 682 nm) and near infrared regions (743, 865 nm)

of the spectrum with only three from the shortwave infrared region (1227, 1589, 2237 nm). For canopy height, HCI and basal area, all wavelengths were from the near infrared and shortwave infrared (743-2257 nm). Finally, for Shannon species richness all wavelengths were from the shortwave infrared (1206-2237 nm) except for one from the visible (621 nm). These may be similar to the wavelength regions chosen at other sites or they may be somewhat different, depending on the forest structure at each site.

## **FRACTION OF PHOTOSYNTHETICALLY ACTIVE RADIATION ABSORBED OR INTERCEPTED (FPAR)**

The fraction of photosynthetically active radiation (PAR) absorbed or intercepted by the canopy (FPAR) is an important measure of vegetation structure that is closely related to remotely sensed vegetation greenness indicators (e.g. NDVI) and leaf area index (LAI). FPAR provides a fundamental measurement of light absorption that is often linearly related to NDVI and is the fundamental starting point for most vegetation growth and carbon uptake models. For light-use efficiency models that calculate carbon flux from remote sensing, it is essential to have an estimate of PAR absorbed by the vegetation canopy (FAPAR). In some cases, it is impractical to calculate FAPAR, but more practical to calculate the fraction of PAR *intercepted* (FIPAR), which provides a close approximation of FAPAR. Ideally, these measures of canopy light absorption are corrected by the amount of green vegetation present to yield a “green FPAR” (or fraction of light absorbed by photosynthetically active tissues). This situation is analogous to the plant area index/leaf area index contrast described below (p. 72). Just as it is helpful to sample LAI through the seasons (to isolate the green leaf fraction of plant area index), it is helpful to sample FPAR through the seasons (to identify the portion of FPAR attributable to photosynthetic tissues).

### **Goals**

The goal of FPAR measurements will be to provide measurements of canopy light absorption and forest structure. These measurements have several purposes, including validation of remotely sensed indices (e.g. NDVI), assessment of forest structure (e.g. for evaluating successional stage), and independent assessment of LAI. Furthermore, as a fundamental biophysical measurement of light absorbed by the forest canopy, FPAR provides a starting point for modeling forest carbon uptake and productivity.

### **Tasks**

Field plots or transects must first be established (p. 73). Using plot or transect sampling methods, a spatially representative set of below-canopy measurements must be made, and these must be referenced to above-canopy PAR measurements to derive FPAR as described below. Depending upon prevailing sky conditions, it may be necessary to establish continuous, automated above-canopy PAR measurements for this purpose (p. 80).

### **Deliverables**

The final product will be a spatially and temporally explicit set of total FPAR and “Green FPAR” values for each Tropi-Dry site, as well as spatially explicit maps of FPAR for each plot through time.

## Sampling design

Determination of FPAR requires a comparison of above-canopy PAR readings to below-canopy PAR readings. Thus, an above-canopy PAR sensor (*p. 80*) provides an essential reference value, and usually a single above-canopy sensor will suffice. In some cases, this may be accomplished by the establishment of weather stations at each Tropi-Dry forest.

Due to the variable light fields underneath a forest canopy, many below-canopy PAR readings are required. Thus, some kind of spatially distributed sampling protocol is needed to gather a spatially representative sample of below-canopy PAR. The sampling design defined for leaf-area index (*p. 73*) would be suitable. Following this design provides the additional advantage of a direct link between the FPAR and LAI sampling, allowing a calibration between the two measures of canopy structure. An alternative below-canopy sampling strategy is to select a long transect (or transects) through the forest, and to sample each meter along each transect. Transect sampling is particularly suitable for rapid or automated measures of radiation. Examples of automated PAR sampling methods include the TRAC system and the tram system (Gamon *et al.*, 2006). In either case, the number of below-canopy samples must be sufficient to gather a representative measure of light penetration for a given forest plot. To enhance the number of below-canopy points, light bars (e.g. LI-COR, Lincoln NE, or AccuPAR, Decagon, Pullman, WA) are often used. These are essentially a series of 80 to 100 quantum sensors in a line that simultaneously measure many points at once, greatly facilitating the task of collecting representative below-canopy PAR measurements.

## Calculating FPAR

The fraction of PAR absorbed (FAPAR) is calculated as follows:

$$\text{FAPAR} = 1 - t - r + tr_s$$

Where  $t$  is the fraction of radiation transmitted through the canopy,  $r$  is the fraction of radiation reflected by the canopy, and  $r_s$  is the reflectance of the soil surface. These terms are obtained by measuring downwelling PAR above the canopy ( $S$ ), the upwelling PAR reflected from the canopy ( $R$ ), the downwelling PAR below the canopy ( $T$ ), and the upwelling PAR below the canopy ( $U$ ). The terms  $t$ ,  $r$  and  $r_s$  are then calculated as follows:

$$t = T/S$$

$$r = R/S$$

$$r_s = U/T$$

Since it is sometimes impossible to obtain the above-canopy reflected radiation ( $R$ ), the fraction of PAR intercepted (FIPAR) is often calculated instead, using the following equation:

$$\text{FIPAR} = 1 - t$$

To calculate  $t$ ,  $r$  and  $r_s$ , it is necessary to measure downwelling PAR above the canopy. Since it is often impractical to carry a sensor above the canopy for each below-canopy reading, the above-canopy reading is often best made by continually and automatically monitoring above canopy PAR with a quantum

sensor (*p. 80*). For this to be comparable to the below-canopy readings, above-canopy PAR readings should be closely synchronized with the below-canopy readings, and the above- and below-canopy PAR sensors should be carefully cross-calibrated (this is best done by taking a set of simultaneous readings with both sensors measuring the identical light field). Once the sensors are properly calibrated, then each below-canopy reading can be readily compared to each above-canopy reading for the calculation of FAPAR.

A full discussion of the theory and practical aspects of measuring FPAR can be found in the AccuPAR user's manual, available on the Decagon website (<http://www.decagon.com/manuals/LPman12.pdf>).

## LEAF SPECTRAL REFLECTANCE

Inter and intra-species variability, observed using spectral reflectance, is a key element for the development of new approaches for quantifying optical diversity. The most common element of this endeavor is considered to be leaf spectral reflectance, which can be used as an important tool for ecophysiological studies and also as an indicator of environmental stress. In this section we evaluate the necessary protocols for building a comprehensive spectral library of leaf reflectance, which in turn will be used for ecophysiological studies.

### Goals

To implement a latitudinal comparison of leaf spectral properties and leaf traits across tropical dry forests of the Americas.

### Tasks

Collect a library of leaf trait variables for lianas and trees at the leaf level.

### Deliverables

Hyperspectral library of spectral signatures of lianas and trees at the level 400-1100 nm, and associated traits, as follows: chlorophyll a, b and total, carotenoid,  $\Delta$ Photochemical Reflectance Index (PRI), leaf thickness, water content, histology and specific leaf area.

## Sample Collection Protocols for Leaves

Leaf sample protocols follow Castro-Esau *et al.*, (2004, 2006). Manipulation of samples should be done following Foley *et al.*, (2006). *In situ* sampling should be conducted following protocols defined by Gamon and Surfus (1999). In each country, a single collection should be carried out during the wet season.

The fundamental issue is how many species and how many samples per species should be collected. In general it is expected that a total of 35 leaves per species will be collected and processed as follows:

1. Spectral reflectance and histology: 10 leaves
2. Spectral reflectance, leaf thickness and water content: 10 leaves
3. Spectral reflectance and chlorophyll/carotenoid: 15 leaves

## Leaf Histology

A total of 10 leaves/collection/structural group of each liana/tree should be collected. Leaf histology should be processed after spectral reflectance is collected. The steps to follow are:

- Leaves collected at each site are placed in plastic bags and in a cooler for transfer to a processing site. In general, leaf processing must be done within 4 hours of clipping. This time may vary somewhat depending upon the purpose and the species (e.g. some species' leaves dry out more quickly than others – see Foley *et al.*, 2006).
- Three spectra (near the apex, middle and base) per leaf should be recorded prior to shipping. Total time elapsed between collection and arrival at the laboratory should be less than 24 hours.
- Once in the laboratory, three small strips measuring approximately 10 mm in length by 4 mm in width should be cut from the leaf samples (in approximately the same places where spectral reflectance was measured). Prominent leaf veins, especially the midrib, should be avoided.
- The strips should be fixed in formalin aceto-alcohol and placed in a vacuum oven for two weeks under approximately 15 psi.
- After two weeks samples must be run through an ethanol processing center and transferred to a hot paraffin wax bath for embedding into paraffin rings.

For each sample, thin sections (5  $\mu\text{m}$ ) must be cut and mounted onto microscope slides. Slides are then stained using a sequence of timed immersions into chemical baths containing Harris' Haematoxylin and acid Eosin. Thin sections are then photographed using a confocal microscope, with emphasis on areas with unbroken cell structures and good focus. Finally, images must be cleaned of background noise surrounding the tissue cross-section and color-enhanced using Photoshop 7.0. A scale bar and a species name label should be added to the final product. Final cross sections should be analyzed using IMAGEJ (<http://rsb.info.nih.gov/ij/>). From the classified image, percentage of empty spaces in the mesophyll layer should be estimated for each sample and compared against reflectance at 800 nm ( $R_{800\text{nm}}$ ) from the near-infrared plateau.

## Leaf Traits: Leaf Thickness, Specific Leaf Area, and Percent Water Content

Leaf thickness should be measured for ten mature, healthy leaves per species per collection. Average thickness per leaf should be reported as the mean of six measurements taken from near the apex, middle and base using a Nikon leaf thickness micrometer (to nearest 0.001 mm).

Leaf Area should be estimated using a LICOR-3000A.

Percentage water content should be estimated by comparing the leaf wet weight to the weight after drying at 60°C for 36 hours. Specific leaf area is estimated as the ratio of surface area to dry weight.

## Chlorophyll and Carotenoid Extraction (DMSO)

Chlorophyll *a*, *b* and total chlorophyll plus carotenoid concentrations should be estimated using a Dimethyl Sulfide (DMSO) extract and a SMART spectrophotometer 2000 with a wavelength range of 300-1000 nm and a wavelength



resolution of 1 nm and accuracy of 2 nm. For each leaf sampled, a 1 square inch core should be extracted and frozen (minimum -20°C freezer or if possible -80°C freezer). Frozen samples should be removed from the freezer, placed in a 10.0 ml DMSO solution, and warmed using a pre-heated water bath to 65°C for 20 min. Samples are then removed and allowed to cool. Once cooled, each sample (~3 ml) is transferred to a disposable cuvette, using a disposable sample pipette. Absorbance at 470, 646 and 664 nm should be measured. These wavelengths were selected upon calibration of the spectrophotometer using pure chlorophyll *a* and *b* extracts. Chlorophyll *a*, Chlorophyll *b*, total chlorophyll and carotenoid concentration should be reported by unit area ( $\mu\text{mol}/\text{m}^2$ ).

Note that DMSO may not work for all leaves. In particular, sclerophyllous leaves may be difficult to extract with this method. In this case, there are two alternatives: (1) grind the leaf tissue in DMSO (this is a dangerous option due to the potential for exposure to DMSO, so care must be taken to use gloves, hood, etc.), or (2) conduct an acetone extract.

## Photochemical Reflectance Index (PRI)

To estimate xanthophyll cycle pigment pool sizes, “dark-to-light” experiments following PRI kinetics should be conducted as per Gamon and Surfus (1999). The experiments should be conducted using a UNISPEC spectrometer, as follows:

1. Begin with a fully dark-adapted leaf (e.g. a leaf in its pre-dawn state, which can be extended either by shading the leaf or bringing the plant to a darkened laboratory the previous evening). Note that the leaf must be allowed to fully dark-adapt overnight.
2. Clamp a leaf clip on the leaf, with the instrument shutter closed.
3. Open the shutter, immediately exposing the leaf to a pre-determined light intensity and forcing the leaf to begin conversion to the light state. Upon shutter opening, begin automatic sampling of spectral reflectance at a pre-programmed time interval (typically every 30 seconds).
4. Using the index function of the reflectometer, monitor PRI kinetics during the dark-to-light transition to ensure attainment of a steady state. Because xanthophyll cycle conversion is enzyme-dependent, this transition is temperature dependent. Under typical room temperatures ( $25 \pm 30^\circ\text{C}$ ), a complete transition usually takes c. 5 min, but this can vary depending on the physiological state of the leaf. The rapid response of the instrument (scan time adjustable from several milliseconds to a few seconds) permits the capture of reflectance in the dark state before enzymatic conversion of xanthophyll cycle pigments becomes apparent.
5. Once a steady light state has been attained, calculate delta PRI from the difference between the initial (dark) and diurnal (light) value. This delta PRI can then be explored as a measure of active xanthophyll pigment pool sizes, or can be used to infer environmental or physiological conditions.

## LEAF AREA INDEX (LAI)

### Goals

To study the temporal variability of leaf area index as function of latitude and season.

## Tasks

To collect leaf area index using the LICOR 2000 in the ecology plots every month from the mid dry season to the mid wet season.

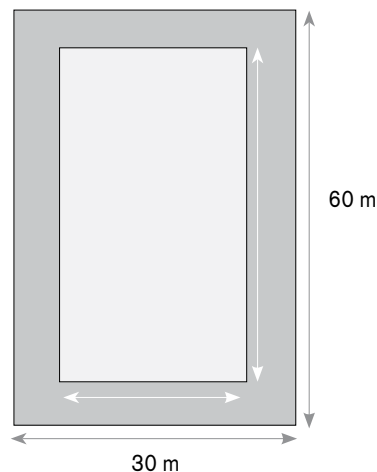
## Deliverables

For each plot (early, intermediate and late): (1) Database of the spatial distribution of LAI for each sampling site, (2) Overall LAI for each plot for each collection, (3) LAI calibration using litter traps.

## Plot design

The design of the sampling scheme is presented in Figures 7 and 8 (after Kalácska *et al.*, 2004). The sampling design, aimed to use the LICOR-2000, is based on tree height.

Figure 7.  
Optical measurements of LAI are taken in the 30m x 60m plot (dark grey). The litter trap measurements (and collection of leaves from the woody species for the specific leaf area during the LAI calibration) and hemispherical photos are taken in the same 30m x 60m plot. The 20m x 50m floristic inventory plot (light grey) is inside the 30m x 60m plot.



## Indirect measurements of LAI using the LICOR-2000

Indirect measurements of LAI should be conducted during the dry season using the LAI-2000 and hemispherical camera (see below for hemispherical camera methodology).

## Sampling procedure

### *Set up and Data Collection (single sensor mode) for Offset Grid Scheme*

- Attach sensor head to the "X" serial port of the data logger.
- Turn on data logger and check serial number (**FCT01**). If there is no serial number, enter the serial number followed by the five appropriate calibration coefficients (**FCT01** for the "X" serial port and **FCT02** for the "Y" serial port).
- Set up acquisition mode: "Single sensor X" for outside canopy "A" measurements (**FCT11**).
- For "A" measurements, set the number of measurements to number desired (3 are usually enough).
- Set prompt for plot number and any additional prompts (**FCT12**).
- Enter data collection mode (**FCT14**) and enter plot number and information for additional prompts if they have been set.

- Collect “A” measurements in an area with an unobstructed field of view of the sky.
- Press “break” to close file (write file to memory).
- Set acquisition mode to “*Remote below X*” for inside canopy measurements (**FCT11**).
- Enter data collection mode (**FCT14**) and enter plot number and information for additional prompts if they have been set.
- Collect below canopy measurements according to sampling scheme detailed in figures 7 and 8.
- Press “break” to close file (write file to memory).

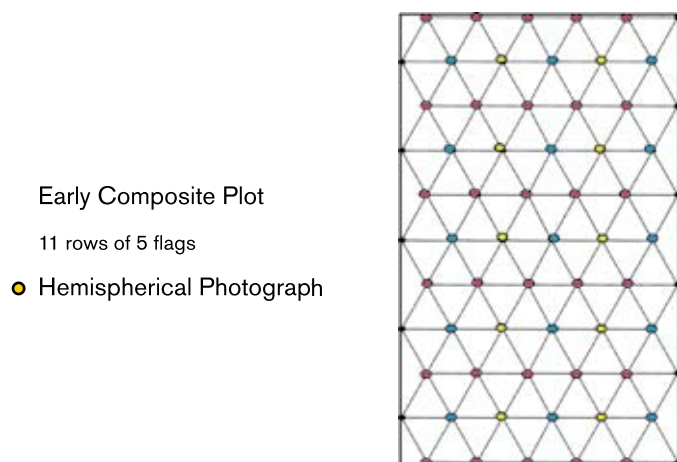


Figure 8.  
Calculation of LAI-2000  
field of view according to  
canopy height.

### Important Notes

- Check memory status after each plot (**FCT12**). Do not let memory drop below 5-10% because the system may lock up. If the data logger locks, it may need to be reset, in which case the data will be lost.
- If two instruments are being used simultaneously, follow the calibration instructions in the manual.
- If a low battery warning is issued, new batteries must be inserted within 5 minutes of removing the used batteries in order for the data not to be lost. Step by step instructions for changing the batteries are located on the inside cover of the back panel of the data logger. Requires 6 “D” batteries.
- Resetting the data logger may delete the calibration coefficients for the sensor. If this happens, re-enter the coefficients with **FCT01** (X) or **FCT02** (Y).
- Measurements must be taken under uniform sky conditions (uniform overcast sky preferred). Early mornings and late afternoons on clear days work well also. Avoid sky conditions of intermittent clouds with bright sun. If measurements must be taken in sunny conditions, the sensor must be shaded.
- The data logger does not write the measurements to memory until the file is closed. Therefore, if more than 150-180 measurements are taken in a single file, the temporary memory will become full, and the instrument will stop collecting data and automatically write the file to memory. If this occurs midway in a plot, a new file must be started.

### Sampling Scheme

- The field of view (FOV) of the sensor is dependent on the view cap and the height of the canopy (Figure 9).

- Figure 9 illustrates the calculation of the FOV of the sensor based on a 45° view cap.

$$A = f\pi H^2$$

A = area on ground represented by the sample (m<sup>2</sup>)

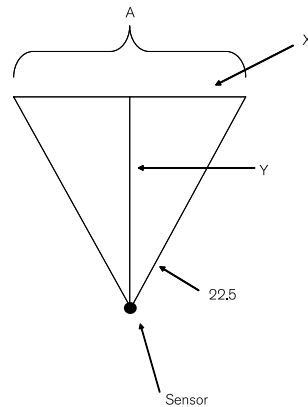
F = view fraction ~0.125 (use of 45° cap)

H = canopy height (m)

Y = Horizontal distance (m)

H = canopy height (m)

X = ½ of lateral distance (m)



Canopy Height (m)	A	Y (m)
2	2	2
4	6	4
6	14	6
8	25	8
10	39	10
12	57	12
14	77	14
16	100	16
18	127	18
20	157	20
22	190	22
24	228	24
26	265	26
28	308	28
30	353	30

Figure 9. Example of flag structure for an early stage plot and location of hemispherical photographs. Red and blue circles represent flags (shown in Fig. 8). Two flag colors are used in order to make orientation within the plots easier.

### Using the LICOR-2000 in the field

Measurements should be made using an offset-grid sampling scheme where the maximum surface area of the plots is covered without overlap of the measurements (Figure 10).

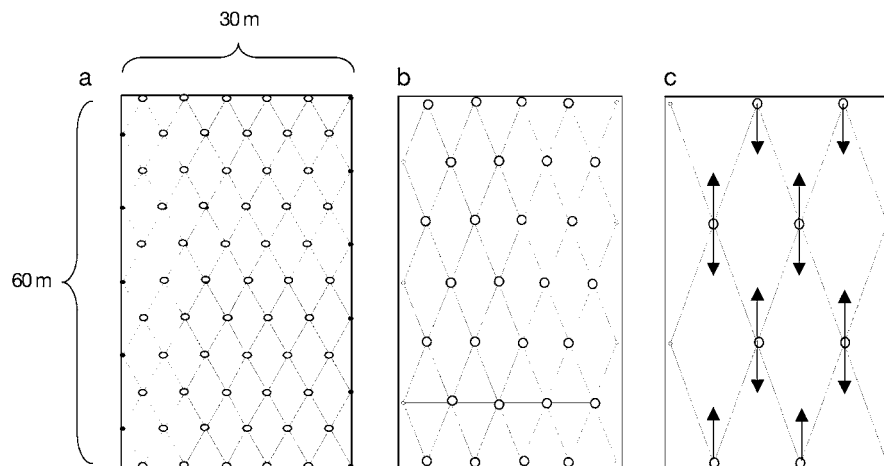


Figure 10. Examples of LAI-2000 sampling method for the (a) early, (b) intermediate and (c) late successional plots. Estimates are taken at the apex of each triangle (o) in the direction of the long axis as illustrated by the arrows on the late stage plot diagram.

In each early successional plot 100 measurements should be taken, each a roughly triangular area of 5 x 6 m, covering 83% of the total plot area. In each intermediate successional plot 36 measurements should be taken, each roughly a triangular area of 8 x 10 m, covering 80% of the total plot area. In each late successional plot 12 measurements should be taken, each covering an area of 15 x 18 m, encompassing 90% of the total plot area. Please note that the size of the area "seen" by the LAI-2000 depends on the height of the canopy; however, unless the height of the canopy is very different from 7.5m ± 2.2 m (early), 10.3 m ± 3.4 m (intermediate) and 15 m ± 2.0 m (late), the above sampling scheme in terms of the number of measurements taken will be sufficient. Reference (above canopy radiation) measurements should be taken both before and after at each site. In addition, the sensor should be fitted with a 45° view cap to eliminate the image of the operator from the field of view and to be consistent with the sam-

pling scheme (Figure 9). All measurements must be taken under uniform sky conditions (diffuse light in the morning or afternoon, or cloudy skies).

The offset grid must be marked in each plot with flags to measure in the same spot every time. Measurements are taken at each flag as shown in Figure 9 (at the apex of each triangle; represented by the white circles in Figure 10) in the direction of the base of the triangles (Figure 10).

## **Hemispherical Photographs**

### **Sampling Procedure**

#### *Set up Prior to Photo Acquisition*

- Write plot number along with date on an index card and take a photograph of the card prior to taking any plot photos.
- Photos are taken as illustrated in Figure 8 (i.e. at every second blue flag for the early and intermediate plot and at every flag in the late plots..

#### *Set up at Each Flag where Photo is Taken*

- Attach camera to tripod.
- Orient camera so North will be on top of photo.
- Level lens with bubble level.

#### *Important notes*

- If using film, all photos in one plot should be on the same roll of film.
- Charge camera batteries every night.

## **Calibration of LAI-2000**

### **General Objective**

To calibrate optical estimates of Plant Area Index (PAI) from the LAI-2000 against LAI as estimated with litter traps. Please note, the methods presented in the following sections are specifically for the calibration and are not the same methods as the litter trap study for ecological analyses.

### **General Procedure**

The LAI-2000 will be used to estimate PAI (Plant area index) at the peak of the forest foliage coverage (for example, October or November in Costa Rica). Thereafter, data are collected every month until the end of the dry season (e.g. April in Costa Rica) when there is no or minimal foliage in the forest plots.

We assume that every month the PAI will decrease as deciduous species lose their leaves. By measuring the amount of leaves that are lost from month to month and calculating the LAI represented by that month-to-month loss, we can examine the relationship between the optical readings and the litter trap measurements and use that relationship to calibrate the LAI-2000.

Hemispherical photos are also taken in the dry season because during that time period, the woody contribution of the canopy (WAI) to PAI can be readily esti-

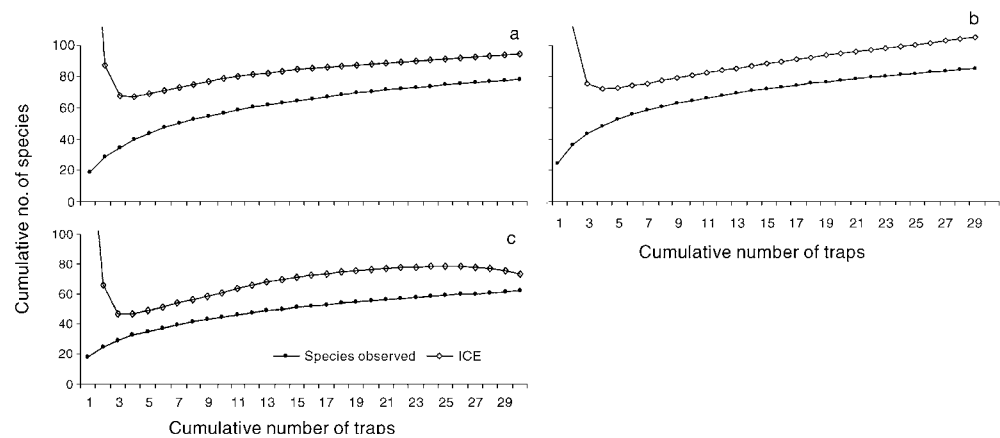
mated from the photos. Once WAI is known, it can be subtracted from the PAI estimates to obtain the foliage contribution to PAI for every month.

## Methods

### Number of Traps

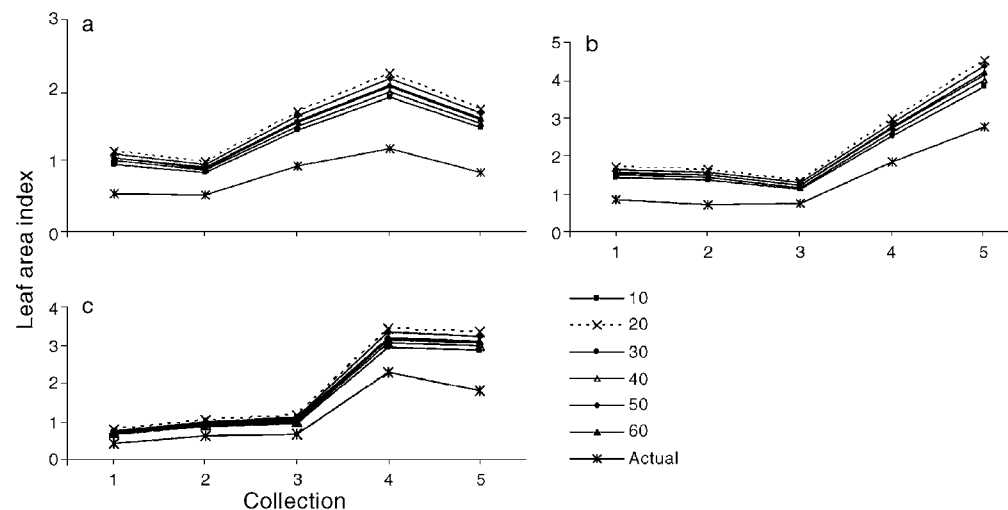
Traps should be established in a total of three plots: one early successional stage plot, one intermediate stage plot, and one late stage plot. In Costa Rica, 30 traps per plot were used. Sampling began in November and was completed in March (5 months). However, upon examination of the precision and accuracy of the mean litter mass (Kalácska *et al.*, 2005b) and the number of species found per trap, in high species rich areas 20 traps was deemed to be sufficient (Figure 11). In low species rich areas, 15 traps are considered adequate, and in monospecific stands, 10 traps are sufficient (Figure 11).

Figure 11. Values of leaf area index (LAI) based on mean specific leaf area (SLA) values derived for 10–60 species compared with actual LAI values based on species-specific SLA values from (A) early, (B) intermediate and (C) late stage successional plots (from Kalácska *et al.*, 2005b). ICE: incidence-based coverage biodiversity estimator.



Since each trap must be collected separately and all collected material sorted by species (or pseudo-species) the amount of work involved is considerable. We suggest using between 15 and 20 traps per plot; the exact number depends on the species richness and, most importantly, the variability in specific leaf area of the species in each stage. However, we *strongly* caution against using fewer than 15 traps because of the high variation in the specific leaf area of dry forest species (e.g. 70.5–637cm<sup>2</sup>/g in Santa Rosa alone). In addition, as can be seen in Figure 12, the sorting of leaves for the purpose of calibration of the LAI-2000 is extremely important.

Figure 12. Cumulative number of species observed per cumulative number of traps based on 500 randomizations and estimates from the Incidence Based Estimator (ICE). (a) Early stage, (b) intermediate stage and (c) late stage successional plots (from Kalácska *et al.*, 2005b).





## *Trap Size*

The size of traps we recommend is 50 x 50 cm. Using larger traps will result in more biomass to sort and measure, yet will not increase the precision or accuracy of the analysis (Kalácska *et al.*, 2005).

## *Litter Collection*

Leaf litter should be collected at monthly intervals until the sites are leafless (at the end of the dry season). To reduce decomposition, leaves may be collected every two weeks; this will also reduce the total amount of leaves to be processed at any given time. If leaves are collected every two weeks, the LAI-2000 *must* also be used every two weeks.

## *Litter Processing*

Litter must be sorted by species (or pseudo-species) and subsequently dried to constant weight using a forced air oven at 80°C. Prior calibration in Costa Rica showed that 2.5 hours was necessary to reach constant weight for all samples (note, however, that these leaves had been partially dried already from exposure in the litter traps). Each site must determine the time required to reach constant weight; this will depend on the type of fan and the moisture content of the samples.

Weight should be recorded for each species separately. All unidentifiable remains should be grouped into one category and weighed separately from the known species (these unidentifiable samples are used to create a generic calibration equation for the unknown remains).

## *Specific Leaf Area per Species*

15 fresh leaves should be collected from all vegetation with a DBH greater or equal to 5 cm in the three plots where the litter traps are located. The area of the leaves should be measured with the LI-COR 3000, a portable leaf area meter, before they are dried to constant weight (at 80°C). Subsequently, the leaves are weighed individually and specific leaf area (SLA) values are calculated for each species. Please note that the leaves should be packaged individually because when they dry, they become very difficult to handle and separate.

For the unknown category and those species for which there are no SLA values, the general leaf weight/leaf area relationship calculated from the unknown remains is used.

## *LAI-2000 Calibration*

For the calibration of the LAI-2000 estimates, total leaf area for the leaves of each species is first calculated from the unique SLA values. Leaf area is then converted to LAI by dividing by the area covered by the traps (i.e. 7.5m<sup>2</sup> if using 30 traps). LAI for the first time period is the sum of the LAI values from each collection. For each subsequent time period, the total LAI from the previous collection is subtracted. With LAI values for each collection, the relationship between the LAI-2000 estimates and the litter traps can be examined through least-squares linear regression.

## Generation of LAI Maps

The purpose of these analyses is to create maps of leaf area index from satellite images using the optical estimates of LAI collected in the field.

### Required field data

Ground-based optical LAI estimated in several plots (30 x 60 m). It is important to state with the metadata of each map whether uncalibrated PAI or calibrated LAI (using the leaf litter trap calibration) is being used.

### Methods

Using atmospherically corrected multi-spectral imagery (i.e. Landsat ETM+), the pixel values in a 3x3 pixel window are extracted around the GPS located centroid of each plot. The values from this 3x3 window are then averaged. Spectral vegetation indices are subsequently calculated (Table 1).

**Table 1. Spectral vegetation indices**

Index	Formula
SR	$\frac{\rho_n}{\rho_r}$
NDVI	$\frac{\rho_n - \rho_r}{\rho_n + \rho_r}$
SAVI2	$\rho_n + 0.5 - \sqrt{(\rho_n + 0.5)^2 - 2 \times (\rho_n - \rho_r)}$
MSR	$\frac{\frac{\rho_n}{\rho_r - 1}}{\sqrt{\frac{\rho_n}{\rho_r + 1}}}$

\*\*\*  $\rho_n$  is near infrared reflectance and  $\rho_r$  is red reflectance

The optical estimates of LAI/PAI are then related to the vegetation indices through a non-linear regression using a Lorentzian Cumulative function. This function is a form of transfer function that will allow us to see which of the indices has the greatest sensitivity to LAI/PAI for each particular study area:

$$y = \frac{a}{\Pi} \left( \arctan\left(\frac{x-b}{c}\right) + \frac{\Pi}{2} \right)$$

where “a” is the transition height, “b” is the transition midpoint, and “c” is the half-width of the transition. The transition height is the range of the SVI values, which are sensitive to changes in LAI. The transition center is the LAI value at the midpoint of the transition and the half-width of the transition is half of the range of LAI values to which the SVI is sensitive. The optimal index for any given site is the one with the broadest transition value (i.e. 2 x “c – the half-width of the transition”). In Costa Rica we found that different indices are more sensitive to different study areas and it is for this reason that several indices must be computed and evaluated using the cumulative function.

Subsequently, the transition function is inverted to solve for LAI:

$$LAI = b - c \tan\left(\frac{\Pi(a - 2 \times SVI)}{2a}\right)$$

where SVI is the vegetation index with the greatest sensitivity to LAI in a given study area; a, b and c are the constants derived from the previous equation. The vegetation index of choice is then calculated for each pixel in the image and this inverted function is then used on a pixel by pixel basis to estimate LAI from the imagery (transformed to vegetation index values).

## **PHOTOSYNTHETICALLY ACTIVE RADIATION (PAR) MEASUREMENTS**

PAR measurements represent an intrinsic variable associated to ecosystem productivity and growth. PAR is also a key proxy for ecosystem structure and succession. In addition, PAR is actively measured by sensors such as MODIS, facilitating the understanding of ecological succession processes in tropical environments.

### **Goals**

To quantify the spatial and temporal dynamics of PAR as a function of successional stage.

### **Tasks**

Measurements of PAR must be taken using a light bar sensor such as the LICOR-LI 190.

### **Deliverables**

PAR datasets for each plot during the peak of the growing season.

## **Field Data Collection**

### **Location, Topology and Seasonality**

PAR must be measured above the canopy. The sensor must be placed pointing vertically upwards, in a location such that higher points are no more than 10 degrees above the plane of the sensor.

If this is not possible, e.g. because of steep slopes, then the sky view factor must be calculated, as follows: from the sensor position, measure the angle from the nadir to the point at the highest angle above the plane of the sensor. From that point, measure 15 additional points, moving 22.5 degrees radially from the previous point with each measurement. For each of the 16 points, calculate the cosine of the angle. Average these 16 values to obtain the sky view factor. The sky view factor is needed to correct for isolation in highly dissected terrain and in applications like PAR mapping from satellite imagery.

The sensor should be located such that the sky view factor is no less than 0.75. The sun should never be hidden from the sensor by topography or other objects between three hours before and three hours after local solar noon. Note that at higher latitudes these conditions must also apply during the local winter, i.e. when the maximum solar zenith angle is minimal.

A single setup can represent a large area ( $\sim 1000 \text{ km}^2$ ) if the terrain is relatively uniform topographically. If there are large topographical variations

(e.g. elevation range, distinct mountain valleys, etc.) then every identifiable unit should have its own sensor installation. The location of the sensor should be recorded with a horizontal accuracy of 50 meters or less, for instance using a GPS.

### **Quantum Sensors**

PAR should be measured using quantum sensors, such as the LICOR LI-190. The sensor must be calibrated no more than one year before measurements are taken. Furthermore, the LICOR LI-190 should be checked weekly for integrity. Common problems include dust, bird droppings, and sensor movement, all of which can attenuate the PAR signal. The measurements may need to be corrected for sensor drift, using the documentation from the supplier of the sensor.

For field campaigns, the logging routine should be as follows: PAR is sampled every second and logged as an average, maximum, minimum and standard deviation, every minute. For long-term, fixed installations with limitations on logging frequency, a logging interval of 5 or 10 minutes can be selected, with a number of PAR samples per logging interval of no less than 30, and at least a recording of average and standard deviation.

Quantum sensors can also be used to measure absorption of PAR by the vegetation (*p. 73*). In this case, multiple quantum sensors above and below the canopy are applied. For the purpose of collecting PAR data, the above-canopy, upwardly-pointing sensor can be used.

### **Analytical Spectral Devise (ASD) Field Spectrometer**

If available, spectrometric measurements can be made to obtain information on the spectral characteristics of incident radiation. The ASD spectrometer must be fitted with a remote cosine receptor (RCR), for which a separate calibration of the configuration spectrometer-fiber-RCR must be available. The RCR has to be pointed vertically upwards and placed with the same restrictions as PAR sensors (*p. 73*).

Continuous measurements must be made to obtain a sample large enough for statistical inferences (e.g. 30 samples minimum). Sets of samples must be collected for different periods of the day (e.g. sunrise, solar noon, sunset) and representing different seasons.

If the spectrometer samples are to be correlated to quantum sensor measurements, then an exact timing difference between the computer running ASD software and the logger of PAR sensors must be established.

### **Generation of PAR Maps**

Calculation and mapping of PAR from MODIS imagery will follow Van Laake and Sanchez-Azofeifa (2004, 2005). MODIS products to be used are MOD03 for the geographical reference, and the atmospheric products MOD04-MOD07. The original, 5-minute swath data must be used, not any of the aggregated products like MODATML. Data from both the Terra and Aqua platforms are needed. The maximum sensor zenith angle is 55 degrees; any data outside of the range -55 to +55 degrees should not be used. If data from either of the images are missing for a particular location, then a single data source may be used.

Certain atmospheric parameters used for PAR calculation are difficult to generate and the MODIS atmospheric products may not report any value, or have a quality flag indicating low quality. In order to facilitate PAR calculation, the following three options are available.

### **Validated Data**

PAR may only be calculated if two complete sets of observations from Terra and Aqua MODIS are available, with quality flags indicating good quality data. An exception is made for the amount of ozone, which can be taken from long-term regional averages or interpolated from MODIS data from a recent period, and for the cloud top pressure, which can be interpolated from nearby data (temporally or spatially). If the cloud optical thickness is lower than 5, atmospheric turbidity data must be available, otherwise it can be omitted.

Validated data will have many “holes” due to missing data in the MODIS products. It can be used both as a stand-alone product and also to correlate with field observations.

### **Preliminary Data**

PAR can be calculated from a single MODIS image, or from a combination of Terra and Aqua images. Quality flags need to indicate that the data are usable, but not necessarily of high quality. Otherwise the conditions for the validated data apply.

Preliminary data may be complete, but it may also have holes, depending on local atmospheric and terrain conditions. It can be used as an indicative product of regional distribution patterns of PAR insolation, and it can also be used to create temporal averages.

### **Working Data**

PAR can be calculated from any combination of MODIS images. In the absence of valid data, parameters can be estimated from regional averages, or interpolated from nearby data (temporally or spatially), with the exception of cloud optical thickness.

Working Data can only be used internally for exploratory purposes and must not be used in subsequent processing, or published externally in any form.

Note that processing a single set of images may yield a map that has all three levels of data mixed together, as the levels refer to sets of data and data quality at the pixel scale. Care should be taken in subsequent processing to separate these different levels.

Maps of PAR data will be generated using the standard Tropi-Dry coordinate systems and mapping procedures.

### **Quality Control**

Calculated PAR values from validated data should be correlated to measured PAR values to establish the accuracy of the calculations. Individual pairs of data may see large differences in values (for a discussion, see Van Laake and Sanchez-Azofeifa 2004), but overall the absolute error should be in the range of 6-8%. When comparing temporal averages (e.g. per month), the accuracy should be in the order of 3-6%.

## SOCIAL SCIENCES





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## INTRODUCTION

The primary goal of the social sciences component of the project is to incorporate an array of knowledge into the management of Tropical Dry Forests (*Tdfs*). At the conceptual level, the human component – as well as the environmental one – is perceived as a set of dynamic, complexly related agents in ecosystem processes. It is understood that these agents both influence and are influenced by each other (Crumley, 1994; Baleé, 1988; Zent and Zent, 2002). However, at present there is no single, universally accepted way of formulating the linkage between social and ecological systems. Using a triangulated research approach (Seale, 1999), the social science component of the project endeavors to understand different situations and to learn from both successful and poor management experiences, through the systematic treatment of cases using common conceptual research approaches (as has been suggested by Berkes F. and Folke, 2000). Therefore, the social science component of the Tropi-Dry project entails a three-stage data collection and analysis process, incorporating social statistics, policy reconstruction, and embedded knowledge of ecology and land use.

Since there is no universally accepted format for investigating the social-ecological systemic relationship, the research outlined here assumes an investigative theoretical stance and attempts to identify and articulate those research questions that might be appropriate for deeper investigation. Much research of this type is conducted as case studies (Yin, 2002) that permit the in-depth investigation of one or more specific examples in order to develop generalizations that can be tested on a broader scale. In short, the social research protocols included in this project are based on inductive, rather than deductive, approaches (Miles and Huberman, 1994).

One of the primary challenges of integrating social science with ecological data is that the spatial contexts of the two do not map easily onto one another. Ecological regions, defined by interactions in bio-physical processes, rarely have the same boundaries as socio-political regions, which are defined by administrative borders and political boundaries (Goodchild and Janelle, 2004). To address this challenge, we defined a socio-political area surrounding the ecological research sites, calling it the Zone of Influence. This Zone of Influence is based on the set of political boundaries of human-occupied areas and is the geographical unit for the collection of the social science data.

The three data sets for the social science component consist of community-level descriptive statistics (quantitative), historical policy reconstruction (policy analysis) and interviews (qualitative and interpretive). A standard set of descriptive statistical indicators will be collected for the Zone of Influence in all research sites. This basic information, based on readily available census and administrative data, will facilitate comparison of indicators across all research sites and will help to develop a standardized picture of the types of communities and economies located in and near Tropical Dry Forest research sites. In order to account for the situated differences in administration, history, and policy development, social science researchers will also develop an historical policy reconstruction for each site. This will consist of a detailed summary and interpretive analysis of the administrative and policy milieu. Finally, a set of key-informant interviews with leaders (influencers) and local residents (users) will provide the rich, in-depth information about land use and local knowledge necessary to fully understand the human-ecological relationship in each site.

Taken together, this triangulated social science research approach will provide basic statistical indicators that can be compared across and between sites throughout the project, as well as the situated policy context and the in-depth local knowledge necessary to begin to understand the interaction of humans with Tropical Dry Forests.

## **BACKGROUND**

### **Human-Ecosystem Relationships**

Humans are increasingly being recognized as an integral part of ecosystems. Human presence transforms landscapes and modifies, often in substantial ways, important ecological processes (Vitousek *et al.*, 1997). Over the last 15 years, scientists interested in understanding the complexity of environmental problems have been advocating for the inclusion of the 'human dimension' in the analysis of ecosystems (Gunderson *et al.*, 1995; Folke *et al.*, 1996; Pace and Groffman, 1998; Holling, 1998; Berkes and Folke, 2000; O'Neill, 2001; ATB, 2004). The need to analyze social and ecological systems as one interconnected entity, instead of separate elements, is increasingly stressed (Gunderson *et al.*, 1995; Berkes and Folke, 2000). There is also growing emphasis on how critical the analysis of coupled human-ecological systems is for the construction of future policy and management alternatives. The challenge, however, is to develop frameworks that facilitate the maintenance of ecosystem health while, at the same time, recognizing and enhancing human livelihoods (Endter-Wada *et al.*, 1998).

### **Situating the Tropi-Dry Social Science Protocols**

As stated previously, the lack of universally accepted protocols for investigating social-ecological relationships has led us to adopt an inductive investigative approach in the Tropi-Dry project. In reality, social research often takes place as an iterative process whereby preliminary (often qualitative) inductive work identifies key issues for further evaluation (Richards, 2006). Once these key questions have been developed, they are often investigated through more quantitative methods that permit statistical evaluation and generalization to a larger population (Creswell, 2003). However, such statistical evaluations often themselves generate unanswered questions that are again best investigated through qualitative work. In this manner, qualitative and quantitative social research is often conducted either in sequence or, increasingly, in parallel through mixed-method approaches (Richards, 2006). Social science research for the Tropi-Dry project will be conducted using a comparative case study, mixed-method research design.

This multi-method, combined qualitative and quantitative research approach (Creswell, 2003) is designed around three parallel investigations. For each research area this will result in the collection and compilation of descriptive social statistics, the construction of an environmental policy history, and the collection and analysis of varied ecological and land use knowledge. Once completed, it is anticipated that this multi-site data set will allow for cross-site comparison and the development of a broad framework for understanding the linkages between social and ecological systems in Tropical Dry Forest environments.

## Working Definitions

There are a number of key terms used throughout the following pages that require definition. The term **Site** is used to identify the location of the plots where ecological and remote sensing data is being collected. In some countries, but not all, the sites are located within state or national parks. **Zone of Influence** (explained in greater detail below) refers to the primary area of human influence around the Site. The Zone of Influence is defined by administrative boundaries (census units, municipalities) in order to facilitate the collection of statistical data. The **Research Area** refers to the combination of the Site and Zone of Influence for each country. The Research Area includes the plots, the area covered by the remote sensing data, and the surrounding human-occupied region.

## Bounding the Research: Explaining the Zone of Influence

One of the primary challenges of integrating social science with ecological data is that the spatial contexts of the two do not map easily onto one another. Ecological regions, defined by interactions in bio-physical processes, rarely have the same boundaries as socio-political regions, which are defined by administrative borders and political boundaries (Goodchild and Janelle, 2004). For this reason an ecological region rarely follows the same geographical boundaries as a census tract or political region. While a challenge, Tropi-Dry social scientists are asked to define a working area for the project that is roughly equivalent to the ecological region but is based on administrative borders such as census units or municipal boundaries. This socio-political area is called the Zone of Influence.

Unlike the ecological region, the Zone of Influence adheres to political and administrative boundaries. Where possible, these boundaries should be as close as possible to the ecological boundaries' however, the fact that these two regions will never be completely synchronized must be recognized. The Zone of Influence, therefore, becomes the socially-defined area of influence surrounding the Tropi-Dry ecological study site. If, for example, the study site is in a national park, the Zone of Influence is the set of political boundaries of human-occupied areas surrounding the park. Since these areas are defined differently in different countries, the selection and definition of the Zone of Influence is at the discretion of the lead Social Science researcher at each research area and should be delineated in conjunction with project members representing the ecological and remote sensing component of the team.

Clear identification of a Zone of Influence serves two purposes. First, it defines the boundaries for gathering indicator data (based on census tracts, political units, regional data, and so on). Second, the Zone of Influence identifies the geographic research area for identifying stakeholders, key informants, and decision-makers for the policy analysis and case studies.

## DATA SET 1: SOCIAL, POPULATION, AND ECONOMIC INDICATORS

### Goals

The first stage of the Social Science component of the project is to compile indicator data to provide contextual knowledge about both the Zone of Influence and the historical context of the research area. The primary goal of

this component therefore is to compile a standard set of numerical indicators that can be compared across Tropi-Dry research areas. These indicators include population, social, and economic data and changes in those measures over time. At the project level, the key research questions for the first data set include:

- Is there a set of standard statistical indicators that hold true across all research areas?
- How do the different research areas compare in terms of social composition and economic activity?

## Methods

Researchers should collect and compile data from multiple sources as indicated in the spreadsheet in Appendix III (*p. 125*). As part of the data collection, researchers must collect data in the smallest area unit available. In some Research Areas this will be at the census unit level, in others at the municipal or regional level. It is likely that at a single research area, some data may be available with considerable geographical detail (for example, population information at the census tract level) while other data may only be available at the municipal or regional level (for example, economic activity by sector). In all cases researchers are asked to collect the data with the greatest geographical specificity possible. The spreadsheet provided in Appendix III (*p. 125*) allows for data to be entered by specific area, as well as to be totaled by region.

## Constraints and Challenges

There are a number of constraints and challenges posed by attempting to collect social data across different countries. While most countries collect some kind of census data at predictable intervals, some countries do not have particularly reliable data – especially in an historical context. While this is recognized, researchers are simply asked to collect the best data possible based on the pre-defined Zone of Influence. Changes in census divisions, revised municipal or administrative boundaries, incomplete data sets and variations in the availability of particular data are all expected to present challenges in cross-comparison of Research Areas. As an exploratory exercise, these challenges are expected and should be noted on the spreadsheet when data is submitted. The greater the geographical detail of the data collected, the less of a problem these challenges will pose in cross-country comparative analyses.

## Data reporting and deliverables

### Zone of influence

The geo-coordinates and boundaries of the Zone of Influence must be reported to the Remote Sensing group by the end of year 1, in order to obtain the required remotely sensed images.

### Data spreadsheet

Table 1 provides an example of a completed spreadsheet including indicator data. A blank copy of the spreadsheet is available in electronic version for

**Table 1. Example of a completed data spreadsheet**

	Study Sites					Mean or total	Year of Data	Data Source
	Ciudad	Arenas	Pena	Valdez	Correa			
1. Economic Indicators								
1.a. Economic Activity by Sector (%)	100	100	100	100	100	100	1992	1992 Census
Primary	15	70	59	64	38	49	1992	
Secondary	32	11	32	188	32	25	1992	
Tertiary	48	19	5	14	29	23	1992	
Quaternary	5	0	4	4	1	3	1992	
1.b. Economically Active Population (%)	100	100	100	100	100	100	1992	Estimated from 1992 Census
Primary	40	85	53	82	38	54	1992	
Secondary	31	8	45	12	31	32	1992	
Tertiary	26	7	2	5	26	13	1992	
Quaternary	3	0	0	1	5	1	1992	
2. Population Indicators								
2.a. Total Population	32,009	26,815	6,292	3,649	7,742	76,509	1992	1992 Census

authorized researchers on the Tropi-Dry website, and a print copy is presented in Appendix III (*p. 125*). Statistical indicators must be delivered by the end of year 1. Metadata requirements for statistical indicators are identified in Appendix III (*p. 131*).

## DATA SET 2: ENVIRONMENTAL POLICY HISTORY

### Goals

The primary goal of the second data set is to reconstruct the policy history of each Research Area. This will provide information to the Ecology and Remote Sensing groups to facilitate placement of their data into a social context, while also allowing for policy cross-comparisons at the project level. The main research questions related to policy history are:

- What is the history and development of policies in the Research Areas?
- What is the current policy environment in the Research Area?
- How have present and past policies shaped land use in the Research Areas?

### Methods

The environmental history of each research area should be reconstructed using historical documents, academic publications and key informant interviews. Reconstruction of the policy history entails a two-part process. First, researchers must conduct an exhaustive search of historical documents and academic publications related to the history of each area. Policy documents pertaining to the following topics should be collected:



- a. Environment (wildlife included)
- b. Agriculture
- c. Forestry
- d. Water
- e. Tourism
- f. Fisheries (where applicable)

Once collected, the documents should be used to construct a preliminary policy history framework that identifies the key changes or developments that have influenced the Research Area. The recommended process for this component is a narrative analysis (Roe 1994), composed of three steps:

*Step 1: Gather documents and construct a preliminary timeline.*

Through the collection and examination of a set of policy and historical documents, researchers can construct a preliminary framework or timeline showing relevant policy developments over time (see Figure 1 for an example). Since the context for each area is different, the timeline for each country may be somewhat different. It is important to remember, however, that a timeline developed from such documents represents an “official” history of policy change. While useful, such documents rarely provide a complete history and often hide important disagreements or undercurrents of contestation (Iannantuono and Eyles, 1999). For this reason, the timeline based on these documents should be considered preliminary.

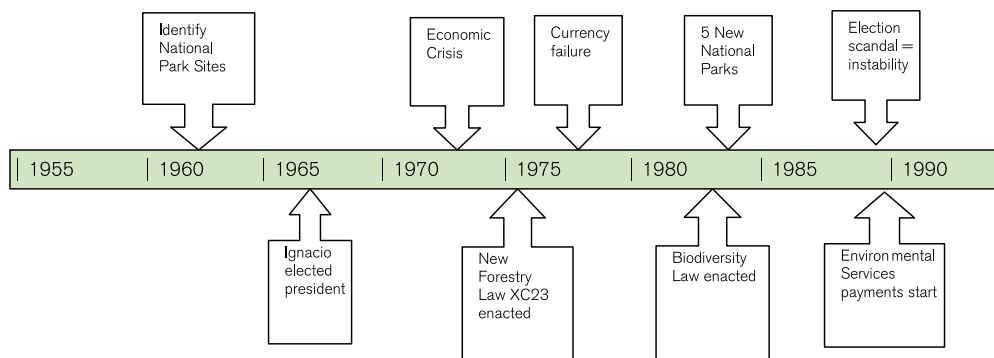


Figure 1.  
Example of a policy timeline.

*Step 2: Interviews with Influencers*

As a component of the case studies, researchers will be conducting in-depth key-informant interviews with policymakers and managers involved in forest management in their areas. Part of the interview will focus on policy history and decisions. Data from the interviews will therefore serve as both a confirmation tool for the document-based historical timeline as well as a source of new, undocumented information that may be useful for the policy history.

*Step 3: Policy History Validation*

Once the data from the interviews has been collected and incorporated into the policy history framework, it can be returned to interview participants for verification. The result is a detailed and confirmed identification of the complex and inter-related nature of environmental policy history for each Research Area. Figure 2 presents one example of what such a framework might look like. Detailed information and instructions on policy analytic procedures and policy reconstruction can be found in Roe (1994), Wildevsky (1987), Pal (1997) and Yanow (2000). Researchers unfamiliar with

policy analysis and reconstruction are encouraged to seek out these sources for more information.

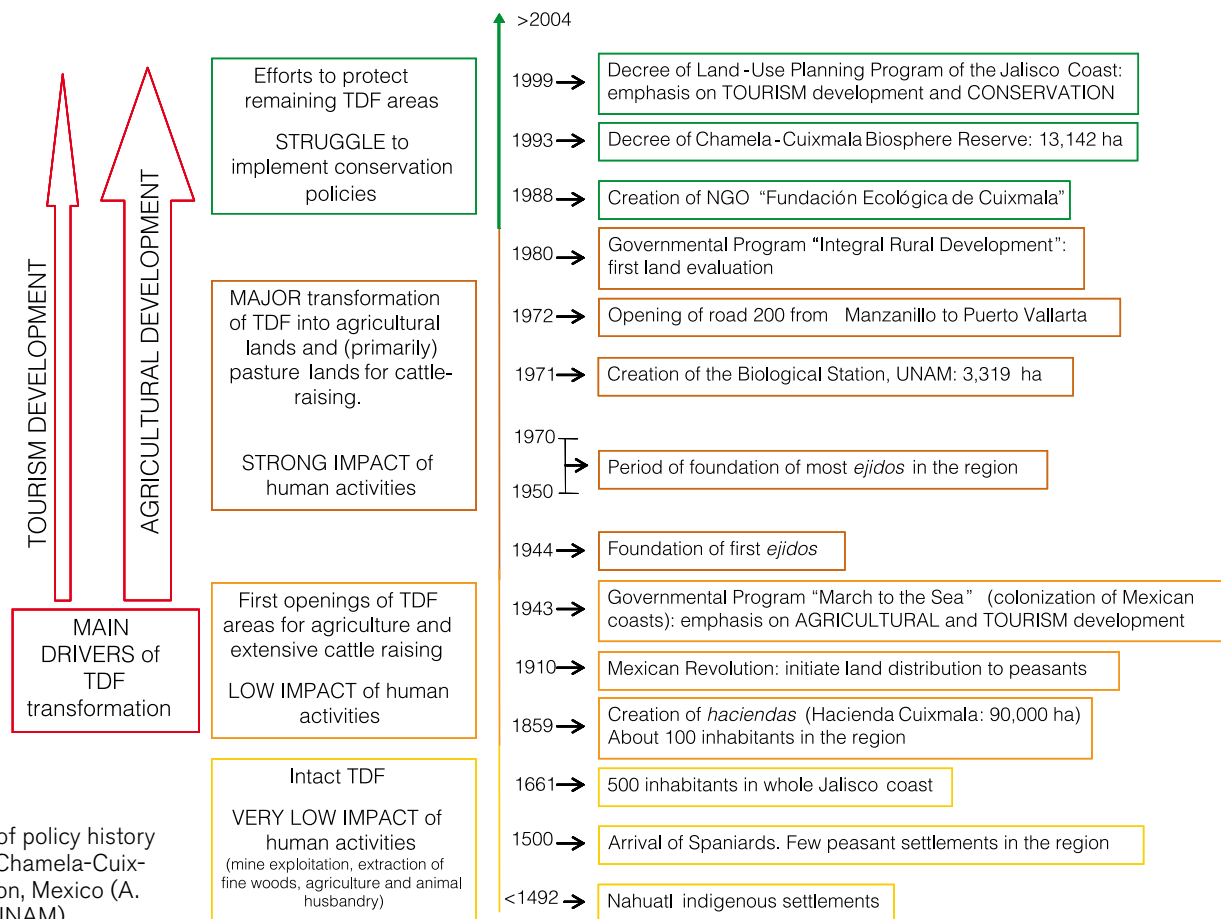


Figure 2. Example of policy history from the Chamela-Cuixmala region, Mexico (A. Castillo, UNAM).

## Constraints and Challenges

It is recognized that policy analyses will be different across temporal and geographical scales. In some countries, environmental policy decisions are made at the national scale (e.g. Costa Rica), while in others they are made by the state or region (e.g. Brazil). In either case, decisions regarding policy scale relevance should be made by the research team as a whole, based on local conditions in the Research Area. Each Research Area will have a different timeline for policy history, and some areas will have shorter policy histories than others. Again, decisions regarding the temporal scale of the policy history should be made by the research team as a whole, based on the specific circumstances in the Research Area. At the project level, it is recognized that influential policies will differ across Research Areas. For this reason researchers are asked to ensure that all policy topics (as identified in methods, above) are examined. Through the systematic examination of all policy topics in all Research Areas, standardization should be maintained.

## Data Reporting and Deliverables

At the end of year 1 (year 2 for some Research Areas), social science researchers must submit to the secretariat a 20-30 page policy analysis and reconstruction identifying and explaining the policy history for the Research

Area. This document should be written in a style appropriate for publication in a peer-reviewed, high quality academic journal in the Policy Sciences. The submission must include a full citation/reference list identifying all the documents that were examined to construct the analysis, as well as all documents cited in the publication. Metadata requirements for the policy studies are available in Appendix III (p. 131).

## **DATA SET 3: CASE STUDIES OF SITUATED KNOWLEDGE**

### **Goals**

This third stage of the research will form the bulk of the work for the social science component of the project. The goal of the third data set is to collect and analyze local knowledge of land use in the Research Area. This entails addressing three important research questions:

- What is the history of land use (and, particularly, Tropical Dry Forest use) in the Research Area?
- What has affected the ways that land use has changed?
- Which policies or practices have, or have not, influenced the ways that the land has been used?

In order to address these questions, researchers must address the importance of situated knowledge in the management of Tropical Dry Forest ecosystems. Recent literature has emphasized the need to acknowledge and use local or traditional ecological knowledge (Berkes *et al.*, 2000; Mauro and Hardison, 2000), particularly where communities interact daily with the ecosystems in which they live. In the case of indigenous groups, this knowledge may reflect culturally transmitted knowledge accumulated over generations (Berkes and Folke, 2000). For indigenous and peasant groups (who frequently have indigenous roots), land has a sacred quality: it is revered and respected and not only considered as an economic resource (Toledo, 2001). Nature is located at the center of culture and the deep bonds between societies and natural elements have resulted in the creation of cosmologies and myths, that may function as mechanisms or institutions that regulate the exchanges and interactions between people and ecosystems (Lazos and Paré, 2000; Toledo, 2001). Where necessary, such belief systems must be incorporated into land use management. Through the study of social perceptions, it is possible to understand how human groups give meaning to their experiences with ecosystems and with other participants involved in environmental decision making (Arizpe *et al.*, 1993; Lazos and Paré, 2000). It is also important to document how local residents perceive environmental issues, policy constraints, and other people involved in managing *Tdfs*. Data on social perceptions and perspectives will contribute to later stages of the project related to policy development as well as to future program development and implementation.

### **Methods**

Researchers are asked to identify two communities or groups of people that use tropical dry forests as the primary case studies. Data collection in the case studies will take place primarily via semi-structured, face-to-face, in-depth key informant interviews. A research assistant and/or the social science director should conduct interviews for each Research Area based on the

semi-structured interview guides presented in Appendices III (*p. 127 and 129*). It is recognized that minor adjustments will be required to accommodate Research Area and regional differences; however the primary goal –to gather situated knowledge about *Tdf* management– will remain the same.

### **Case Study Selection**

As previously stated, researchers must select two ‘communities’ as case studies for the work. Sampling, in this context, should reflect the intent of the research: to identify and document the breadth of views in the Research Area. In this project, the case studies are not meant to generate representative samples that permit generalizable quantitative data at the regional scale (Seale, 1999). Rather, the purpose of examining the two case studies is to understand the detailed, actual lives of respondents living in the two communities, which represent different ways of experiencing Tropical Dry Forests. In most cases, such communities will be small villages or towns located near the research site. Selection of case study communities should be done in collaboration with Team Leaders and affiliated project researchers.

### **Influencer Interviews: Sampling**

For each Zone of Influence researchers must identify Influencers in the region: these are key actors or stakeholders involved in management decisions. Identification of Influencers should take place via a combined snowball and random sampling procedure (Esterberg, 2002; Neuman, 2000). At minimum, researchers should attempt to include Influencers from the following groups:

- NGOs
- Government agencies – municipal, provincial/state, national
- Park Managers
- Scientists
- Associations (if they have an influence on land use and decision-making)

For each research area 5-10 key informant interviews should be conducted with local, regional and/or national Influencers. Respondents should be sampled from the region as a whole, as well as representing the two case study communities. Data gathered in these interviews will follow the interview guide identified in Appendix III (*p. 129*). Details on interview methods are provided below.

### **Users interviews: Sampling**

Users are defined as those people that make day-to-day decisions about how and when the land and forest are used. Users should be sampled using maximum variation to ensure heterogeneity of experiences and expertise. Snowball and purposive sampling (Esterberg, 2002; Neuman, 2000) should be employed to generate a minimum of 15 respondents in each community. Respondents should be selected to represent the following populations:

- Length/type of tenancy
- Age (young and old)
- Sex (men and women)
- Size of holding (large, medium, small)
- Type of activity (type of land use – forestry, farming, ranching, etc)
- Special circumstances (anything else)

## Issues with sampling

Ecosystem or natural resources management practices constitute the interface between social and ecological systems. The aim in the interviews is to gather data describing how those human groups living in *Tdfs* make use of the forest and their perceptions of how forests and lands should be managed. Because the information collected through the interviews is necessarily 'situated,' the selection of respondents is critical. In qualitative and case-based research there is an ongoing discussion concerning sample size (Richards, 2006). Over time, researchers have generally agreed that a minimum of 30 interviews should provide sufficient variability and/or consistency to ensure credible and dependable results. However a more reliable method involves 'data saturation' (Lincoln and Guba, 1985; Denzin and Lincoln, 1994). In this process interviews continue until no new information is obtained. In other words, the collected data has been 'saturated' and no new insights can be gained through conducting further interviews. Tropi-Dry researchers are encouraged to revisit sampling throughout the data collection phase to ensure that new ideas are being generated. At minimum, 35 interviews must be conducted (15 Users in Community 1; 15 Users in Community 2; 5 Influencers). However some research areas might have high levels of contestation and/or within-region differences that may require additional interviews. If data saturation has not been reached with the minimum number of interviews identified above, researchers are encouraged to increase both the sample size and breadth and to continue data collection (interviews) until data saturation is reached.

## Conducting the Interviews

Conducting interviews entails a three-part process. Researchers unfamiliar with interview techniques and procedures in social science data collection are encouraged to examine the wide body of literature on such techniques. While it is possible for almost anyone to collect qualitative data (Richards, 2006), collecting good qualitative data in an ethical manner requires attention to important issues of validity, reliability, personal interactions, and power relations (Lincoln and Guba, 1985).

### *Contacting respondents and requesting participation*

Prospective respondents should be contacted with appropriate respect and their participation ensured without undue pressure or coercion. Power relations in interviewing are of paramount importance and researchers are encouraged to examine the nature of interviewing and some potential problems associated with imbalances of power in the interview process. While the interviews included in the protocols of this book have been pre-tested in some research areas (Wilkinson and Birmingham, 2003), researchers are encouraged to conduct one or two pre-test interviews of their own to assess the validity of the interview instrument – particularly in those research areas where translations from English to a local language could result in changes in question intent. Once approached, respondents should be asked to participate and a time and place set up to conduct the interview. In all cases, conducting such interviews will require considerable thought as to location, personal interaction, and procedures ensuring informed consent. Therefore, researchers are encouraged to ensure that all data collection processes adhere to local ethical requirements. Finally, investigators in each Research Area must adhere to standards ensuring the rigor of qualitative research – including the employment of such quality control strategies as participant feedback – to enhance and ensure credibility, transferability, and confirmability of findings (Baxter and Eyles, 1997; Miles and Huberman, 1994)

## ***Data Collection***

Interviews must follow the interview guides presented in Appendices SS-2 and SS-3, which employ a semi-structured format (Fontana and Frey, 1994). Each interview should begin with the collection of critical ‘metadata’, general information about the respondent which assists in situating both the interview and the respondent’s perspectives. The interview guide should be used as a tool for guiding the conversation, rather than as a rigid format. The goal of the interview is to have the respondent talk about each of the issues identified, rather than to run through the list of topics. Therefore, the interview should be viewed as a conversation between the interviewer and respondent, rather than as a data-gathering or survey method.

Interviews should be 1-2 hours in length and be tape-recorded with the permission of the respondent. Once complete, interviews should be transcribed verbatim into a word processing file. As a final credibility check, transcripts of interviews should be returned to the participants for comment (Baxter and Eyles, 1997). At this stage respondents should be given the opportunity to remove or change any information or commentary in the interview. Once confirmed by the participants, each interview must be edited to replace any identifying names with pseudonyms and then entered into the qualitative data analysis software program (N-Vivo 7™).

## ***Data Analysis***

Data gathered in the interviews should be analyzed using a constant comparative approach that allows for the simultaneous collection and analysis of data (Esterberg, 2002; Miles and Huberman, 1994). In this method, data continues to be collected (specifically, interview data) until no new information is being revealed (the data saturation phase identified previously) (Creswell, 2003; Lincoln and Guba, 1985). At this point data collection is deemed to be complete.

Researchers unfamiliar with the coding and evaluation of qualitative data are encouraged to seek out training resources, in particular some of the work by Lyn Richards (Richards, 2005; Richards and Morse, 2007) and Miles and Huberman (1994). The primary data analysis process will include a combination of deductive coding, based on a coding tree generated from the interview questions, and inductive coding, based on free-coding and moving “up from the data” (Richards, 2005). Both tree-coding and free-coding will be based on emergent themes, thematic analysis, and pattern coding, following Miles and Huberman (1994). While each research area should work independently, regular meetings and workshops will ensure the collaboration necessary for a grounded theory approach to developing themes and a standardized coding mechanism. This process will allow for a standardized set of key codes that can be applied across all research areas, while also allowing for analysis of site-specific findings.

## **Constraints and Challenges**

The primary result from the interview process will be a set of detailed, in-depth explanations of land use and tropical dry forest management at each Research Area. The primary challenge of this component of the research is data management. Qualitative research generates very large data sets (Richards, 2005). It is the responsibility of researchers to ensure that data



–particularly working data– is appropriately backed up and is securely held during the research process. Researchers are encouraged to systematically provide Tropi-Dry Team Leaders with copies of the social science data so that it can be uploaded onto the Secretariat website to ensure that data remain securely stored and is not lost.

## **Data Reporting and Deliverables**

Fully coded interviews in a complete N-Vivo7 data file must be delivered to the Tropi-Dry secretariat by the end of year 2 (year 3 in some research areas). In addition, researchers must provide a comparative case study analysis of their two communities including the information provided by both Users and Influencers. This comparative analysis is expected to be a high-quality research paper suitable for publication in a top social sciences journal. The metadata requirements for interview data and case summaries are presented in Appendix III (p. 131).

## **CONCLUSION: FACILITATING THE SCIENCE-POLICY INTERFACE**

One of the primary goals of the Tropi-Dry project is to generate ecological and social science information that is both relevant and useful for decision-making. Through the combination of the qualitative and quantitative social science research methods described above, coupled with the complex sets of ecological and remote-sensing information generated in the other components of the project, this research endeavors to make the connection between science and policy more feasible.

The interactions between scientists, policymakers and the public are increasingly recognized as being messy and contentious (Garvin, 2001; Dunn, 1981). Over the past several decades, researchers have progressively focused on the need to make scientific information more relevant and more understandable to decision makers, policymakers (Pal, 1997; Salter, 1988) and the public (Roth *et al.*, 2004; Wynne, 1996; Wynne, 1995). Likewise, policymakers and scientists have recognized the importance of incorporating the public into the decision making process – especially decision making about the environment (Jasanoff and Wynne, 1998; Ravetz, 1996; Wildavsky, 1996). The Tropi-Dry project aims to bridge the gap between scientists, policymakers and the public via both the research process and its outcomes.

In the Tropi-Dry project, data collection – both ecological and social – relies on the participation of all three of these groups. While scientists take the clear lead in the collection of ecological and land cover data, both policymakers and local residents will participate in the collection of policy, historical, and local land management information. Through this participatory framework, the diverse experiences and expertise brought to the project by this array of participants will contribute as key components to the data collection process. Data analysis, data interpretation, and the implementation of policy change based on the data, all rely on interactions between the three parties in each Research Area. Since the very act of engaging in research engenders policy change and learning (Sabatier and Jenkins-Smith, 1993), it is anticipated that local residents, via the research process, will become active in the setting and re-setting of environmental management agendas (Kingdon, 1995). In this way, the outcomes of the research process will be more acceptable at the local level. Both the research process and the implementation of its outcomes are geared toward the incorporation of science into national and regional policy-making, as well as local practice.

Interactions between the three groups is based on three sets of 'interfaces.' The science-public interface occurs at the level of data collection in each of the Research Areas via relations between local residents and social scientists. The science-policy interface takes place in two ways: first, via key informant interviews to gather historical and policy data for each Research Area, and second, through the collaborations between ecological and remote sensing Tropi-Dry researchers and resident scientists working in each locale. The policy-public interface is traditionally the weakest link and the most difficult to maintain (Garvin, 2001; Stone, 1997). The key forum for this interface will be workshops organized in each country (*see information in Appendix III, p. 125-131*). These national workshops will bring policymakers and public/nongovernmental organizations together to discuss research goals and subsequent findings. With the Tropi-Dry researchers acting as intermediaries, the purpose of these workshops is to facilitate the incorporation of local knowledge into ecological and environmental policy development and implementation at regional/national levels. This form of public interaction and participation is increasingly recognized as a key way to bridge the gap between 'expert' and 'lay' knowledge (Catt and Murphy, 2003; Sinclair and Diduck, 2001; Arnstein, 1969) and facilitate the desired linkage between social and ecological processes in the management of Tropical Dry Forests.



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## APPENDIXES

# APPENDIX I

## PLOT NOMENCLATURE

### PLOT NOMENCLATURE

Each plot is assigned a unique four-character code that indicates country (first two letters), successional stage (letter) and replicate number (integer). For example, code MEI2 stands for Mexico- -intermediate successional stage-replicate 2. Latitude and longitude in decimal degrees are determined at the bottom left corner of each plot (0,0), with six decimal points.

Country	Co_code	Successional stage	SS_code	Replicate	Plot_code	Site	Latitude	Longitude
Mexico	ME	Pasture	P	1	MEP1	1		
Mexico	ME	Pasture	P	2	MEP2	1		
Mexico	ME	Pasture	P	3	MEP3	2		
Mexico	ME	Early	E	1	MEE1	3		
Mexico	ME	Early	E	2	MEE2	3		
Mexico	ME	Early	E	3	MEE3	2		
Mexico	ME	Intermediate	I	1	MEI1	1		
Mexico	ME	Intermediate	I	2	MEI2	4		
Mexico	ME	Intermediate	I	3	MEI3	4		
Mexico	ME	Late	L	1	MEL1	5		
Mexico	ME	Late	L	2	MEL2	1		
Mexico	ME	Late	L	3	MEL3	2		
Costa Rica	CR	Pasture	P	1	CRP1	1		
Costa Rica	CR	Pasture	P	2	CRP2	3		
Costa Rica	CR	Pasture	P	3	CRP3	4		
Costa Rica	CR	Early	E	1	CRE1	4		
Costa Rica	CR	Early	E	2	CRE2	1		
Costa Rica	CR	Early	E	3	CRE3	2		
Costa Rica	CR	Intermediate	I	1	CRI1	2		
Costa Rica	CR	Intermediate	I	2	CRI2	2		
Costa Rica	CR	Intermediate	I	3	CRI3	3		
Costa Rica	CR	Late	L	1	CRL1	5		
Costa Rica	CR	Late	L	2	CRL2	6		
Costa Rica	CR	Late	L	3	CRL3	6		
Venezuela	VE	Pasture	P	1	VEP1	1		
Venezuela	VE	Pasture	P	2	VEP2	1		
Venezuela	VE	Pasture	P	3	VEP3	2		
Venezuela	VE	Early	E	1	VEE1	2		
Venezuela	VE	Early	E	2	VEE2	2		
Venezuela	VE	Early	E	3	VEE3	4		
Venezuela	VE	Intermediate	I	1	VEI1	4		
Venezuela	VE	Intermediate	I	2	VEI2	3		
Venezuela	VE	Intermediate	I	3	VEI3	5		
Venezuela	VE	Late	L	1	VEL1	1		
Venezuela	VE	Late	L	2	VEL2	1		
Venezuela	VE	Late	L	3	VEL3	3		
Brazil	BR	Pasture	P	1	BRP1	2		
Brazil	BR	Pasture	P	2	BRP2	2		
Brazil	BR	Pasture	P	3	BRP3	1		
Brazil	BR	Early	E	1	BRE1	4		
Brazil	BR	Early	E	2	BRE2	4		
Brazil	BR	Early	E	3	BRE3	4		
Brazil	BR	Intermediate	I	1	BRI1	3		
Brazil	BR	Intermediate	I	2	BRI2	3		
Brazil	BR	Intermediate	I	3	BRI3	2		
Brazil	BR	Late	L	1	BRL1	1		
Brazil	BR	Late	L	2	BRL2	1		
Brazil	BR	Late	L	3	BRL3	5		

## DESCRIPTION OF SITES

Successional plots may be found at different geographical locations, or “sites.” Each site is given a descriptive name in reference to nearby towns or other geographical characteristics. Reference points close to the plots are included to facilitate location and navigation to the sites. In addition, mean annual temperature (°C, one decimal point) and total annual precipitation (in integers) will be recorded.

Country	Location	Name of site	Reference points	Mean annual temperature (°C)	Total precipitation (mm)
Mexico	Site 1				
Mexico	Site 2				
Mexico	Site 3				
Mexico	Site 4				
Mexico	Site 5				
Mexico	Site 6				
Costa Rica	Site 1				
Costa Rica	Site 2				
Costa Rica	Site 3				
Costa Rica	Site 4				
Costa Rica	Site 5				
Costa Rica	Site 6				
Venezuela	Site 1				
Venezuela	Site 2				
Venezuela	Site 3				
Venezuela	Site 4				
Venezuela	Site 5				
Venezuela	Site 6				
Brazil	Site 1				
Brazil	Site 2				
Brazil	Site 3				
Brazil	Site 4				
Brazil	Site 5				
Brazil	Site 6				



## APPENDIX II

### DATA ENTRY SHEETS

#### ECOLOGY COMPONENT

#### FLORISTIC COMPOSITION AND VEGETATION STRUCTURE

##### Floristic composition

This datasheet summarizes the species composition for each plot (without including other attributes about the species such as abundance, age structure, etc.). For practical purposes only, the fields in this datasheet are here displayed in rows, but in the actual database each of these fields should be placed in a column, with the different plots listed in rows.

Field	Datum 1	Datum 2, etc.
Country	VEN	
Collection date	14/05/2007	
Work year	1	
Plot code	VEP2	
Ind_code	VEP2001	
Family	Fabaceae	
Genus	Acacia	
Genus+species	<i>Acacia macracantha</i>	
Species code	ACMAC	
Life form	Tree	
Collector name	Ángel Fernández	
Coll_code	4341	
Collector number	443	
Phenological stage	Flower/Fruit	
Herbarium	VEN	
Identification specialist	Nelson Ramírez	
Comments	None	

**Country:** Country where the study is being conducted.

**Collection date:** Date the specimen was collected (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot codes are taken from datasheet I.A (plot nomenclature).

**Individual code:** Identification code of the specimen collected, comprised of plot code and specimen number.

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name.

**Species code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Life form:** Plant life form (tree, shrub, liana, etc.)

**Collector name:** Name of person who collected and prepared the specimen.

**Collector code:** A four-letter abbreviation of the full collector name.

**Collector number:** Personal numbering system used by the collector.

**Phenological stage:** Indicates if the species has flowers, fruits, both or none at the time of collection.

**Herbarium:** International ID code for the final destination of collected specimens.

**Identification specialist:** Name of the expert that identified the specimen if different than the collector.

**Comments:** Comments.

## Vegetation structure, complexity and dynamics

These datasheets are for recording data related to the vegetation structure, complexity and dynamics of each plot. All datasheets listed bellow are derived from two 'mother data sheets', one for woody vegetation and one for herbaceous vegetation, which contain all the information that will be obtained from each plot for vegetation analysis.

### Woody vegetation master data sheet

Field	Datum 1	Datum 2, etc.
Country	VEN	
Date	24/05/2007	
Work year	2	
Plot code	VEI1	
Ind_code	VEI1012	
Family	Fabaceae	
Genus	Acacia	
Genus+species	<i>Acacia macracantha</i>	
Species code	ACMAC	
Life form	T	
x-coordinate (m)	12.2	
y-coordinate (m)	5.8	
Number of trunks	2	
DBH1 (mm)	150	
DBH2 (mm)	340	
Estimated height (m)	12.4	
Height (m) "Ten tallest trees" per plot	12.4	
Status	P	
If D, cause	-	
Collection date	-	
Collector name	-	
Coll_code	-	
Collector number	-	
Phenological stage	-	
Herbarium	-	
Identification specialist	-	
Comment	-	

**Country:** Country where the study is being conducted.

**Date:** Date of data recording (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot codes are taken from datasheet I.A (plot nomenclature).

**Individual code:** Identification code of the specimen collected, comprised of plot code and specimen number.

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name.

**Species code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Life form:** Plant life form (Tree: T, Shrub: S, Liana: L, Epiphyte: E, Climber: C, Herbs: H, Frútice: F).

**X-coordinate:** X coordinate for the specimen within the plot.

**Y-coordinate:** Y coordinate for the specimen within the plot.

**Number of trunks:** Number of trunks at breast height.

**DBH1:** Diameter at breast height in trunk 1 of the specimen.

**DBH2:** Diameter at breast height in trunk 2 of the specimen (if applicable).

**Estimated height:** Gross estimation of the specimen's height.

**Height "ten tallest trees per plot":** If this specimen is one of the ten tallest trees in the plot, its height must be estimated using a clinometer.

**Status:** A status field indicates whether it is the first time a specimen is labeled and measured (I), a new recruit (R), a previously measured individual (P) or an individual that died since the previous measurement (D).

**If D, cause:** If dead, the probable causes of death should be indicated: physical damage (P), animal damage (A), diseases (D), and/or other (O).

**Collection date:** If the specimen was collected, date the specimen was collected.

**Collector name:** If the specimen was collected, name of person who collected and prepared the specimen.

**Collector code:** A three letter abbreviation of the full collector name.

**Collector number:** Personal numbering system used by the collector.

**Phenological stage:** Indicates if the species has flowers, fruits, both or none at the time of collection.

**Herbarium:** International ID code for the final destination of collected specimens.

**Identification specialist:** Name of the expert that identified the specimen, if different than the collector.

**Comments:** Comments.

### *Woody vegetation species level data*

This datasheet is for recording information on the density, frequency and relative dominance of each species in each plot.

Field	Datum 1	Datum 2
Country	VEN	
Work year	2	
Plotcode	VEE1	
Family	Fabaceae	
Genus	Acacia	
Genus+species	<i>Acacia macracantha</i>	
Species code	ACMAC	
Density (ind/ha)	14	
Frequency	2	
Dominance (%)	34	
IVI	0.9	

**Country:** Country where the study is being conducted.

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot codes are taken from datasheet I.A (plot nomenclature).

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name..

**Species code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Density:** Density of the species within the plot.

**Frequency:** Frequency of occurrence of the species within the plot.

**Dominance:** Dominance of the specie in the plot (recorded as a percentage).

**IVI:** Importance value index

### Woody vegetation plot level data

This datasheet is for recording information on the vegetation structure in each plot, including maximum height, number of horizontal strata, stem density, number of species, and basal area.

Field	Data	Data
Country	VEN	
Work year	1	
Plot code	VEP2	
Height (m) "Ten tallest trees" per plot	Mean (SE)	
Stem density (stems/ha)	45	
Species number	24	
Basal area (m <sup>2</sup> /ha)	40	
CHCI	15.0	

**Country:** Country where the study is being conducted.

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot codes are taken from datasheet I.A (plot nomenclature).

**Height "ten tallest trees per plot":** Mean and SE estimates of the ten tallest trees in the plot.

**Stem density:** Number of stems per hectare.

**Number of species:** Number of species within the plot.

**Basal area:** Basal area covered in the plot in m<sup>2</sup>/ha.

**CHCI:** Holdridge complexity index.

### Herbaceous vegetation master data sheet

Field	Data	Data
Country	VEN	
Date	24/05/2007	
Work year	1	
Plot code	VEI1	
Ind_code	VEI1012	
Family	Poaceae	
Genus	Brachiaria	
Genus+species	<i>Brachiaria decumbens</i>	
Species code	BRDEC	
Life form	Herb	
Cover area (%)	45.2	
Collection date	-	
Collector name	-	
Coll_code	-	
Collector number	-	
Phenological stage	-	
Herbarium	-	
Identification specialist	-	
Comments	-	

**Country:** Country where the study is being conducted.

**Date:** Date of data recording (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot codes are taken from datasheet I.A (plot nomenclature).

**Individual code:** Identification code of the specimen collected, composed of plot code and specimen number. In the case of many herbaceous species belonging to the Poaceae family, an "individual" is considered to be a cluster of tightly clumped plants.

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name.

**Species code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Life form:** Plant life form (herb, vine, etc.)

**Cover area:** Area covered by the species in the subplots (recorded as a percentage).

**Collection date:** If the specimen was collected, date the specimen was collected.

**Collector name:** If the specimen was collected, name of person who collected and prepared the specimen.

**Collector code:** A three letter abbreviation of the full collector name.

**Collector number:** Personal numbering system used by the collector.

**Phenological stage:** Indicates if the species has flowers, fruits, both or none at the time of collection.

**Herbarium:** International ID code for the final destination of collected specimens.

**Identification specialist:** Name of the expert that identified the specimen, if different than the collector.

**Comments:** Comments.

### *Herbaceous vegetation species level data*

Field	Data	Data
Country	VEN	
Work year	1	
Plot code	VEL1	
Family	Poaceae	
Genus	Brachiaria	
Genus+species	<i>Brachiaria decumbens</i>	
Species code	BRDEC	
Density (ind/ha)	1200	
Frequency	200	
Dominance (%)	40	
IVI	3.1	

**Country:** Country where the study is being conducted.

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot codes are taken from datasheet I.A (plot nomenclature).

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name..

**Species code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Density:** Estimated density of the species in the subplots.

**Frequency:** Estimated frequency of the species in the subplots.

**Dominance:** Estimated dominance of the species (in percentage) in the subplots.

**IVI:** Importance value index.

## **SOIL, MYCORRHIZAE AND RHIZOBIUM CHARACTERIZATION**

Physical-chemical characterization of soil samples, mycorrhizae activity and diversity will be examined in each country in year 1 and year 4, two times per year (once during the dry season and once during the rainy season). Values reported per cell correspond to average values of 4 samples.

### **Soil, mycorrhizae and litter biomass**

Country:	VEN	VEN	VEN
Work year:	1	1	1
Season:	Dry	Dry	Dry
Plot ID:	VEP1	VEP1	VEP1
Attention: Mean Values of 4-Samples	Layer A	Layer B	Layer C
	0-10 cm	10-20 cm	20-40 cm
Organic Carbon			
% Sand			
% Clay			
% Silt			
N Concentration (g/Kg)			
P Concentration (g/Kg)			
C/N Ratio			
Ca Concentration (cmol/Kg)			
Mg Concentration (cmol/Kg)			

Al Concentration (cmol/Kg)			
Na Concentration (cmol/Kg)			
S Concentration (cmol/Kg)			
K Concentration (cmol/Kg)			
Si Concentration (cmol/Kg)			
Base saturation (%)			
pH			
Al saturation (%)			
H+			
CEC			
Soil density			
Water holding capacity (mm)			
Organic Matter			
Mycorrhizae Activity			
Mycorrhizae Diversity			
Litter biomass			

## VEGETATIVE AND REPRODUCTIVE PHENOLOGY

### Plot level phenology

Plot level phenological data considers the phenological status of all marked individuals within the phenology transects in each plot, independently of the number of individuals found per species.

Field		Data	Data
Country		VEN	
Date		26/09/2007	
Work year		3	
Plot code		VEE3	
Ind_code		VEE3024	
Family		Moraceae	
Genus		Cecropia	
Genus+species		Cecropia obtusifolia	
Species code		CEOBT	
Life form		Tree	
month 1	Green leaves	V	
	Dead leaves	II	
	Floral buds	V	
	Flowers	I	
	Unripe fruit	I	
	Ripe fruit	I	

**Country:** Country where the study is being conducted.

**Date:** Date the data is recorded (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot codes are taken from datasheet I.A (plot nomenclature).

**Ind code:** Individual code, composed of plot code and individual number.

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name..

**Species code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Life form:** Plant life form (tree, shrub, liana, etc.)

**Month:** Month in which phenological data is being recorded.

**Phenophase categories:** Green leaves, dead leaves, floral buds, flowers, unripe fruit and ripe fruit. The Roman numerals refer to the following percentages: I (0%), II (1-25%), III (25-50%), IV (50-75%) and V (75-100%).

## Species level phenology (optional)

Species level phenological data considers the phenological status of a particular species in a given successional stage. At least ten different individuals per species must be marked for this study.

Field	Data	Data
Country	VEN	
Date	12/11/2007	
Work year	2	
Plot code	VEP1	
Ind_code	VEP1005	
month 1	Green leaves	IV
	Dead leaves	I
	Floral buds	III
	Flowers	II
	Unripe fruit	II
	Ripe fruit	I

**Country:** Country where the study is being conducted.

**Date:** Date the data is recorded (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot codes are taken from datasheet I.A (plot nomenclature).

**Ind code:** Individual code, composed of plot code and individual number.

**Month:** Month in which phenological data is being recorded.

**Phenophase categories:** Green leaves, dead leaves, floral buds, flowers, unripe fruit and ripe fruit. The Roman numerals refer to the following percentages: I (0%), II (1-25%), III (25-50%), IV (50-75%) and V (75-100%).

## Leaf Area Index (LAI)

LAI will be estimated using an LAI-2000 for early, intermediate and late successional stages. The number of sampling points for which this variable will be estimated varies among successional stage: 55 points for early stage, 28 for intermediate stage, and 8 points for late stage.

Field	Data	Data
Country	VEN	
Date	30/06/2008	
Work year	2	
Plot code	VEL2	
Point 1, 1	1.3	
Point 1, 2	1.4	
Point 1, 3	1.8	
Point 1, 4	0.9	
Point 1, 5	1.6	
Point 1, 6	1.5	
Point 1, 7	1.5	
Point 1, 8	1.7	

**Country:** Country where the study is being conducted.

**Date:** Date the data is recorded (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot codes are taken from datasheet I.A (plot nomenclature).

**Point i, j:** The estimated LAI value for each point (from 1-55 for early stage, 1-28 for intermediate stage and 1-8 for late stage plots).



## Floral biology attributes

Field	Data	Data
Country	VEN	
Family	Bombacaceae	
Genus	Pachira	
Genus+species	<i>Pachira quinata</i>	
Species code	PAQUI	
Organization	Inflorescence	
Position	Ext branch	
No. flowers/inflo	3 (0.6)	
Odor	Fragrant	
Color	White	
Sexuality	Hermaphrodite	
External length (mm)	130 mm	
Internal length (mm)	45 mm	
External corolla diameter (mm)	125 mm	
Internal floral tube diameter (mm)	10 mm	
Number stamens	102 (5.6)	
Number ovules	160 (5)	
Pollination syndrome	Chiropter/Sphing	
Floral visitors	Bat/sphingid	
Seed dispersal syndrome	Anemochory	

**Country:** Country where the study is being conducted.

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name.

**Species code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Organization:** Solitary or inflorescence.

**Position:** Cauliflory, internal branches, external branches, penduliflory.

**No. flowers/inflo:** Mean number of flowers per inflorescence ( $\pm 1$  SE)

**Odor:** Sweet, musky, fragrant, etc.

**Color:** Describe color (pink, red, light green, purple, violet, blue, etc.)

**Sexuality:** Hermaphrodite, monoecious, dioecious, androdioecious, etc.

**External length:** External floral length in mm.

**Internal length:** Internal floral length (floral tube) in mm.

**External corolla diameter:** External corolla diameter in mm.

**Internal floral tube diameter:** Internal corolla diameter in mm.

**Number stamens:** Number of stamens.

**Number ovules:** Number of ovules.

**Pollination syndrome:** Inferred based on floral traits and published information (anemophily, hydrophily, melittophily, chiropterophily, psychophily, myophily, ornitophily, phalaenophily, sphingophily, cantharophily, etc.)

**Floral visitors:** General categories of floral visitors (large bee, small bee, wasp, hummingbird, passerine, bat, fly, hawkmoth, etc.)

**Seed dispersal syndrome:** Inferred based on fruit traits and published information (anemochory, herpetochory, myrmecochory, ornitochory, etc.)

## LITTERFAL

### LAI calibration and litterfall

Field	Species	Data	Data
Country: VEN			
Date: 25/08/2008			
Work year: 3			
Plot code: VEE2			
PAI: 45,8			
Tray 1 litterfall dry weight (g)	Sp 1	23	
	Sp 2	45	
	Sp 3	24	
	Sp 4	34	
	Sp 5	11	
	Sp n	34	
Tray 2 litterfall dry weight (g)	Sp 1	34	
	Sp 2	22	
	Sp 3	12	
	Sp 4	5	
	Sp 5	45	
	Sp n	12	

**Country:** Country where the study is being conducted.

**Date:** Date the data is recorded (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot codes are taken from datasheet I.A (plot nomenclature).

**PAI:** Plant area index

**Tray x litterfall dry weight:** Dry weight of litterfall per species per tray.

## Specific leaf area per species

This measurement is taken for each species per successional plot.

Country:	VEN		
Plot ID:	VEL2		
Species 1	Leaf area (cm <sup>2</sup> )	Dry weight (g)	SLA (specific leaf area)
Leaf 1	56	2.6	
Leaf 2	57	2.3	
Leaf 3	67	2.8	
Leaf 4	45	1.5	
Leaf 5	55	1.7	
Leaf 6	67	2.5	
Leaf 7	60	1.8	
Leaf 8	87	3.4	
Leaf 9	46	1.5	
Leaf 10	46	1.7	
Leaf 11	76	3.0	
Leaf 12	67	2.6	
Leaf 13	60	1.7	
Leaf 14	67	2.6	
Leaf 15	65	2.7	
Mean	61.40	2.29	
SD	11.48	0.60	
SE	2.96	0.15	

**Country:** Country where the study is being conducted.

**Plot ID:** Plot identification is taken from datasheet I.A (plot nomenclature).

**Leaf area:** Measured with a LI-COR 3000 using fresh material.

**Dry weight:** Weight of each leaf dried separately.

**Specific leaf area (SLA):** Calculated for each species.

**Mean:** Calculated mean of the values for all the leaves from species *x*.

**SD:** Standard deviation.

**SE:** Standard error.

## Litterfall at the site level

Dry weight litterfall estimates based on litterfall samples obtained from trays placed in each plot. Measurements should be taken on a monthly basis.

Field	Data	Data
Country	VEN	
Date	25/07/2008	
Work year	3	
Plot code	VEL3	
Tray 1 dry weight (g)	120	
Tray 2	34	
Tray 3	56	
Tray 4	67	
Tray 5	80	
Tray 6	23	
Tray 7	122	
Tray 8	150	

**Country:** Country where the study is being conducted.

**Date:** Date the data is recorded (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot code is taken from datasheet I.A (plot nomenclature). (*Note: in the plot nomenclature datasheet, late/mature successional stage plots are indicated with an "L", not an "M"*)

**Tray x dry weight:** Dry weight of litterfall trapped in each tray.

## BIOTIC INTERACTIONS

### Seed dispersal by bats

Field	Datum 1	Datum 2, etc.
Country	VEN	
Work year	2	
Date	15/11/2007	
Season	MRS	
Successional stage	I	
Plot code	VEI3	
Time	2230	
Ind_code	VEI3011	
Recapture	R	
Family	Phyllostomidae	
Genus	Vampyrops	
Genus+species	<i>Vampyrops helleri</i>	
Species code	VAHEL	
Guild	F	
Gender	M	
Reprod. condition	R	
Age	A	
Forearm length (mm)	38.5	
Weight (g)	18.8	
Fecal sample	Y	
Seed Sp 1	<i>Cecropia obtusifolia</i>	
No. seeds Sp 1	16	
Seed Sp 2	-	
No. seeds Sp 2	-	
Seed Sp 3	-	
No. seeds Sp 3	-	

**Country:** Country where the study is being conducted.

**Work year:** Project year number (1,2,3,4 or 5).

**Date:** Date the data is recorded (dd/mm/yyyy).

**Season:** Capture season (BRS= beginning of rainy season; MRS= middle of rainy season; BDS= beginning of dry season; MDS= middle of dry season).

**Successional stage:** Successional stage (P= pasture; E= early; I= intermediate; L= late or mature).

**Plot code:** Plot code is taken from datasheet I.A (plot nomenclature).

**Time:** Time of capture.

**Ind\_code:** Specimen ID code, composed of plot code and individual number.

**Recapture:** NR: not recaptured; R: recaptured.

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name..

**Species code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Guild:** C= carnivore; F= frugivore; GI= gleaning insectivore; AI: air insectivore; N= nectarivore; O= omnivore; S= sanguivore.

**Gender:** M= male; F= female.

**Reprod. condition:** Reproductive condition (for males and females: R= reproductive, NR= non reproductive; for females: P= pregnant, L= lactating, PL= post-lactating).

**Age:** J= juvenile, S= subadult, A= adult.

**Forearm length:** Forearm length in mm.

**Weight:** Weight in g.

**Fecal sample:** Y= yes or N= no

**Seed Sp n:** Indicate the plant species *n* present in the fecal sample.

**No. seeds Sp n:** Indicate number of seeds of Sp *n* found in the fecal sample.

## Seed dispersal by birds

Field	Data	Data
Country	VEN	
Work year	2	
Date	15/11/2007	
Season	MDS	
Successional stage	I	
Plot code	VEI2	
Time	0830	
Ind_code	VEI3015	
Recapture	NR	
Family	Mimidae	
Genus	Mimus	
Genus+species	<i>Mimus gilvus</i>	
Species code	MIGIL	
Guild	F/I	
Gender	M	
Reprod. condition	NR	
Age	J	
Molt condition	Y	
Weight (g)	22.5	
Fecal sample	Y	
Seed Sp 1	<i>Cereus repandus</i>	
No. seeds Sp 1	23	
Seed Sp 2	-	
No. seeds Sp 2	-	
Seed Sp 3	-	
No. seeds Sp 3	-	

**Country:** Country where the study is being conducted.

**Work year:** Project year number (1,2,3,4 or 5).

**Date:** Date the data is recorded (dd/mm/yyyy).

**Season:** Capture season (BRS= beginning of rainy season; MRS= middle of rainy season; BDS= beginning of dry season; MDS= middle of dry season).

**Successional stage:** Successional stage (P= pasture; E= early; I= intermediate; L= late or mature).

**Plot\_code:** Plot code is taken from datasheet I.A (plot nomenclature).

**Time:** Time of capture.

**Ind\_code:** Specimen ID code, composed of plot code and individual specimen number.

**Recapture:** NR: not recaptured; R: recaptured.

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name.

**Species\_code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Guild:** C= carnivore; F= frugivore; G= granivore; I= insectivore; N= nectarivore; O= omnivore.

**Gender:** M= male; F= female.

**Reprod. condition:** R= reproductive, NR= non reproductive.

**Age:** (J= juvenile, A= adult)

**Molt condition:** describe degree of molting.

**Weight:** Weight in g

**Fecal samples:** Y= yes or N= no

**Seed Sp n:** Indicate the plant species *n* present in the fecal sample.

**No. seeds Sp n:** Indicate number of seeds of Sp *n* found in the fecal sample.

## Identification of bird species based on point count surveys

Field	Data	Data
Country		
Work year		
Date		
Season		
Succes. Stage		
Plot code		
Time		
Ind_code		
Family		
Genus		
Genus+species		
Species code		
Guild		
Gender		
Molt condition		

**Country:** Country where the study is being conducted.

**Work year:** Project year number (1,2,3,4 or 5).

**Date:** Date the data is recorded (dd/mm/yyyy).

**Season:** Capture season (BRS= beginning of rainy season; MRS= middle of rainy season; BDS= beginning of dry season; MDS= middle of dry season).

**Successional stage:** Successional stage (P= pasture; E= early; I= intermediate; L= late or mature).

**Plot code:** Plot code is taken from datasheet I.1 (plot nomenclature).

**Time:** Time of capture.

**Ind\_code:** Specimen ID code, composed of plot code and individual specimen number.

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name.

**Species code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Guild:** C= carnivore; F= frugivore; G= granivore; I= insectivore; N= nectarivore; O= omnivore.

**Gender:** M= male; F= female.

**Molt condition:** describe degree of molting. (if possible).

## List of plant species dispersed by bats and birds

Field	Data	Data
Country	VEN	
Succes. stage	VEI2	
Plant family	Moraceae	
Plant genus	Ficus	
Plant genus+species	<i>Ficus mathewsii</i>	
Dispersal agent	Bat	
Dispersal agent family	Phyllostomidae	
Dispersal agent genus	Artibeus	
Dispersal agent genus+species	<i>Artibeus jamaicensis</i>	
Dispersal agent Rel. Import.	0.8	

**Country:** Country where the study is being conducted.

**Successional stage:** Successional stage (P= pasture; E= early; I= intermediate; L= late or mature).

**Plant family:** Family of the plant dispersed.

**Plant genus:** Genus of the plant dispersed.

**Plant genus+species:** Full taxonomic binomial or species name of the plant dispersed.

**Dispersal agent:** Seed dispersal category (bat or bird).

**Dispersal agent family:** Family of the dispersal agent.

**Dispersal agent genus:** Genus of the dispersal agent.

**Dispersal agent genus+species:** Full taxonomic binomial or species name of the dispersal agent.

**Dispersal agent Rel. Import.:** Relative importance index of the dispersal agent for this plant species = # specimens of dispersal species X carrying seeds of this plant species/Total number of specimens of all species carrying seeds of this plant species.

## Herbivory general pattern

Field	Data	Data
Country	VEN	
Date	15/08/2008	
Work year	3	
Plot code	VEL1	
Ind_code	VEL1001	
Family	Mimosaceae	
Genus	Pithecellobium	
Genus+species	<i>Pithecellobium saman</i>	
Species code	PISAM	
DBH (mm)	1200	
Plant level	Top	
Leaf ID	1	
Leaf total area (cm <sup>2</sup> )	12	
Folivory damage leaf area (cm <sup>2</sup> )	2	
No. of galls Morpho 1	3	
No. of galls Morpho 2	5	
No. of galls Morpho 3	-	
Gall damage leaf area (cm <sup>2</sup> )	2.3	
No. of leaf miners Morpho 1	1	
No. of leaf miners Morpho 2	-	
No. of leaf miners Morpho 3	-	
Insect mining damage leaf area (cm <sup>2</sup> )	1.8	

**Country:** Country where the study is being conducted.

**Date:** Date the data is recorded (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot code is taken from datasheet I.A (plot nomenclature).

**Ind\_code:** Specimen ID code, composed of plot code and individual specimen number.

**Family:** Taxonomic family.

**Genus:** Taxonomic genus.

**Genus+species:** Full taxonomic binomial or species name..

**Species\_code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**DBH:** Diameter at breast height in trunk of the specimen.

**Plant level:** Top= top, Int= intermediate, Bot= bottom.

**Leaf ID:** Leaf identification number.

**Leaf total area:** Leaf total area in cm<sup>2</sup>.

**Folivory damage leaf area:** Area of leaf damaged by folivory in cm<sup>2</sup>.

**No. of galls Morpho 1:** Number of galls of morpho 1 in the leaf.

**No. of galls Morpho 2:** Number of galls of morpho 2 in the leaf.

**No. of galls Morpho 3:** Number of galls of morpho 3 in the leaf.

**Gall damage leaf area:** Area of leaf damaged by insect galls in cm<sup>2</sup>.

**No. of leaf miners Morpho 1:** Number of leaf miners of morpho 1 in the leaf.

**No. of leaf miners Morpho 2:** Number of leaf miners of morpho 2 in the leaf.

**No. of leaf miners Morpho 3:** Number of leaf miners of morpho 3 in the leaf.

**Insect mining damage leaf area:** Area of leaf damaged by insect mining in cm<sup>2</sup>.



## Canopy herbivore insects

Field	Data	Data
Country	VEN	
Date	23/09/2008	
Work year	3	
Plot code	VEE1	
Plot position	C	
Emergent tree genus+species	<i>Phitecellobium saman</i>	
Host tree ID	C1	
Host tree genus+species	<i>Spondiix bombim</i>	
Host tree species code	SPBOM	
Herbivore insect ID	VEE1C1001	
Insect order	Hemiptera	
Insect family	Aphididae	
Insect genus	Cerataphis	
Insect genus+species	<i>Cerataphis fransseni</i>	
Number collected	4	
Herbivore type	Sap-sucker	

**Country:** Country where the study is being conducted.

**Date:** Date the data is recorded (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot\_code:** Plot code is taken from datasheet I.A (plot nomenclature).

**Plot position:** Position of the plant within the plot (five possible positions: A= left bottom corner; B= right bottom corner; C= center of plot; D= left top corner; E= right top corner).

**Emergent tree genus+species:** Taxonomic identification.

**Host tree ID:** Host tree identification code based on plot position. For example, if in position C, possible IDs are C1, C2, C3 or C4.

**Host tree genus+species:** Full taxonomic binomial or species name of the host tree.

**Host tree species code:** A five-letter acronym of the species name (first two letters of the genus, first three letters of the species).

**Herbivore insect ID:** Identification code for collected insects, composed of plot code, host tree ID and insect specimen number.

**Insect order:** Taxonomic order.

**Insect family:** Taxonomic family.

**Insect genus:** Taxonomic genus.

**Insect genus+species:** Full taxonomic binomial or species name.

**Number collected:** Number of specimens collected.

**Herbivore type:** Sap-sucker or chewer.

## Canopy branch herbivory

Field	Data	Data
Country	VEN	
Date	14/11/2008	
Work year	3	
Plot code	VEL3	
Plot position	A	
Emergent tree genus+species	<i>Ficus trigonata</i>	
Host tree ID	A1	
Host tree genus+species	<i>Tabebuia chrysantha</i>	
Host tree species code	TACHR	
Branch ID	1	
Branch length (cm)	34	
No. leaves	4	
Leaf ID	2	
No. galls per leaf	2	
No. insect miners per leaf	3	
Leaf area lost by folivory (cm <sup>2</sup> )	4	

**Country:** Country where the study is being conducted.

**Date:** Date the data is recorded (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot code:** Plot code is taken from datasheet I.A (plot nomenclature).

**Plot position:** Position of the plant within the plot (five possible positions: A= left bottom corner; B= right bottom corner; C= center of plot; D= left top corner; E= right top corner).

**Emergent tree genus+species:** Full taxonomic binomial or species name..

**Host tree ID:** Host tree identification code based on plot position. For example, if in position C, possible IDs are C1, C2, C3 or C4.

**Host tree genus+species:** Full taxonomic binomial or species name of host tree.

**Host tree species code:** A five letter acronym of the species.

**Branch ID:** Identification code for each branch.

**Branch length (cm):** Length of each branch.

**No. leaves:** Number of leaves per branch.

**Leaf ID:** Leaf identification number.

**No. galls per leaf:** Number of galls per leaf.

**No. insect miners per leaf:** Number of insect miners per leaf.

**Leaf area lost by folivory:** Area of leaf damaged by folivory in cm<sup>2</sup>.

## Understory herbivore insects

Field	Data	Data
Country	VEN	
Date	23/09/2008	
Work year	3	
Plot code	VEE1	
Plot position	B	
Host tree ID	B1	
Host tree Genus+species	<i>Psycotria viridis</i>	
Host tree species code	PSVIR	
Herbivore insect ID	VEE1B1008	
Insect order	Lepidoptera	
Insect family	Nymphalidae	
Insect genus	Heliconius	
Insect genus+species	<i>Heliconius charithonius</i>	
Number collected	2	
Herbivore type	Chewer	

**Country:** Country where the study is being conducted.

**Date:** Date the data is recorded (dd/mm/yyyy).

**Work year:** Project year number (1,2,3,4 or 5).

**Plot\_code:** Plot code is taken from datasheet I.A (plot nomenclature).

**Plot position:** Position of the plant within the plot (five possible positions: A= left bottom corner; B= right bottom corner; C= center of plot; D= left top corner; E= right top corner).

**Host tree ID:** Host tree identification code based on plot position. For example, if in position C, possible IDs are C1, C2, C3 or C4.

**Host tree Genus+species:** Full taxonomic binomial or species name of host tree.

**Host tree species code:** A five-letter acronym of the species

**Herbivore insect ID:** Identification code for collected insects, composed of plot code, host tree ID and insect specimen number.

**Insect order:** Taxonomic order.

**Insect family:** Taxonomic family.

**Insect genus:** Taxonomic genus.

**Insect genus+species:** Full taxonomic binomial or species name..

**Number collected:** Number of specimens collected.

**Herbivore type:** Sap-sucker or chewer.

# APPENDIX III

## DATA ENTRY SHEETS

### SOCIAL COMPONENT

#### Statistical indicators worksheet

	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	Total	Year of Data	Data Source
<b>1.Economic indicators</b>								
1.a.Economic activity by sector (%)								
Primary								
Secondary								
Tertiary								
Quaternary								
1.b. Economically-active population (%)								
Primary								
Secondary								
Tertiary								
Quaternary								
<b>2. Population Indicators</b>								
2.a. Total Population (#)								
2.b. Population growth rate								
2.c. Population Density								
2.d. Age (#)								
# ≤14								
# 15-59								
# ≥60								
2.e. Age (% of Total)								
% ≤14								
% 15-59								
% ≥60								
2.f. Sex (#)								
# ≤14 Female								
# 15-59 Female								
# ≥60 Female								
# ≤14 Male								
# 15-59 Male								
# ≥60 Male								
2.g. Sex (% of Total)								
% ≤14 Female								
% 15-59 Female								
% ≥60 Female								
% ≤14 Male								
% 15-59 Male								
% ≥60 Male								
2.h. Education (Average years of schooling)								
2.i. Income (Average individual income for persons age 15-59)								

	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	Total	Year of Data	Data Source
<b>3. Land Use Indicators</b>								
3.a. Basic statistical unit reported								
Name of unit (e.g. ejido, municipality, census tract)								
Size of unit (area extent in hectares)								
3.b. Urban/Rural								
% designated Urban								
% designated Rural								
% Other								
3.c. Land Use								
i. Agriculture								
1. # hectares								
2. % total land used for agriculture								
3. Primary crop								
Other (secondary) crops								
ii. Ranching								
1. # hectares total allocated to ranching								
2. Head of cattle per hectare								
3. % of land area allocated to ranching								
iii. Forestry								
1. # hectares total allocated to forestry								
2. % of land area by type of forestry activity (if available)								
iv. Fisheries Activity (Yes/No)								
v. Other important type of land use								

## User Interview Instrument

### USER INTERVIEW INSTRUMENT FOR TROPIDRY

COMMUNITY NAME: .....

#### General Information

• Sex:	F <input type="checkbox"/>	M <input type="checkbox"/>			
• Age:	18-29 <input type="checkbox"/>	30-39 <input type="checkbox"/>	40-49 <input type="checkbox"/>	50-59 <input type="checkbox"/>	> 60 <input type="checkbox"/>
• Length of residence	..... years. Where from .....				
• Occupation	.....				
• Role in the community	.....				
• Education level	.....Years of education .....				
• Family size	.....				
Family composition	.....				

#### Social History

1. What is the history of this community/area?
2. How long have people lived here/has this settlement been here?
  - 1.1 Why and how was it developed?
  - 1.2 Who first settled here and when?
3. What is the social organization of this area?
  - 1.1 Who is in charge? Who makes decisions and how?
  - 1.2 What are the local institutions?
  - 1.3 What do you think have been the most important historical events that have influenced the way people use the land?

#### Knowledge of Natural History

4. What was the natural environment like here in the past?
  - 1.1 Climate
  - 1.2 Flora and Fauna
  - 1.3 Water
5. Have you noticed changes in the natural environment?
6. Who/what has been responsible for those changes?
  - 1.1 Motives for changes?
  - 1.2 Incentives for changes?

## Environmental Perceptions

7. What do you think of the natural environment?
  - 1.1 Does it have value? How and why?
  - 1.2 Does the dry forest have value? How and Why?
  - 1.3 What part(s) of the dry forest are the most important? Why?
8. What do you think is the relationship between society and nature?
9. What do the people here think about nature?
10. What beliefs or views do people here hold about the relationship between humans and the natural environment?
11. Do you think that the natural environment provides benefits to humans and their well-being? If so, how?
12. Over time, what do you think your community has gained from the natural environment?
  - 1.1 Benefits
  - 1.2 Environmental services
13. Have efforts been made here to protect the natural environment?
  - 1.1 Have those been related to the benefits provided by the environment?
  - 1.2 Who has been responsible for this protection?
14. What do you think the natural environment will be like here in the future?
15. What would you like it to be like?

## Land Use History and Public Policies

16. Who does the land belong to?
17. What are the current ways in which the land is used here?
18. How has land use here changed over time?
  - 18.1 When were changes made?
  - 18.2 How were they made?
  - 18.3 Who made the decisions about changing the land use?
  - 18.4 Why were those choices made? (motives/incentives)
  - 18.5 What have been the consequences of those changes?
19. What actions/programs/projects have influenced land use here?
  - 1.1 Environmental
  - 1.2 Agricultural
  - 1.3 Forestry
  - 1.4 Water
  - 1.5 Tourism
  - 1.6 Fishing
  - 1.7 Other
20. Who has benefited most from policy changes?
21. Has there been disagreement over land use in the past?
22. What do you think will happen in the future regarding land use? (Who will be involved/when/why)
23. Do you know about the local park/protected area?
  - 1.1 Who benefits from it?
  - 1.2 Does the park/protected area play any part in your life?
  - 1.3 What do you think of it?



## **Influencer Interview Instrument**

### **General Information**

#### **Social History**

1. What is the history of this community/area?

- Organization .....
- Occupation/Job .....
- How long have you been in this position? .....
- What is your involvement with this area? .....

2. How long have people lived here/has this settlement been here?

2.1 Why and how was it developed?

2.2 Who first settled here and when?

3. What is the social organization of this area?

3.1. Who is in charge? Who makes decisions and how?

3.2. What are the local institutions?

3.3. What do you think have been the most important historical events that have influenced the way people use the land?

#### **Knowledge of Natural History**

4. What was the natural environment like here in the past?

4.1. Climate

4.2. Flora and Fauna

4.3. Water

5. Have you noticed changes in the natural environment?

6. Who/what has been responsible for those changes?

6.1. Motives for changes?

6.2. Incentives for changes?

#### **Environmental Perceptions of Local Residents**

7. What do people here think of the natural environment?

7.1 Is it valuable to them? How and why?

7.2 Is the dry forest valuable to them? How and why?

8. What do you think people here see as the relationship between society and nature?

9. What do the people here think about nature?

10. What beliefs or views do people here hold about the relationship between humans and the natural environment?

11. Do people here think that the natural environment provides benefits to humans and their well-being? If so, how?

12. Over time, what do people here think they have gained from the natural environment? (benefits, environmental services)

13. What do the people here think the natural environment will be like in the future?

## **Environmental Perceptions of the Respondent**

14. What do you think of the natural environment?
- 14.1 Does it have value? How and why?
- 14.2 Does the dry forest have value? How and Why?
15. What do you think is the relationship between society and nature?
16. Do you yourself think that the natural environment provides benefits to humans and their well-being? If so, how?
17. Over time, what do you think this community has gained from the natural environment? (benefits, environmental services)
18. Have efforts been made here to protect the natural environment?
- 18.1 Have those efforts been related to the benefits the environment provides?
- 18.2 Who has been responsible for this protection?
19. What do you think the natural environment will be like here in the future?
20. What would you like it to be like?

## **Land Use History and Public Policies**

21. Who does the land belong to?
22. What are the current ways in which the land is used here?
23. How has land use here changed over time?
- 23.1 When were changes made?
- 23.2 How were they made?
- 23.3 Who made the decisions about changing the land use?
- 23.4 Why were those choices made? (motives/incentives)
- 23.5 What have been the consequences of those changes?
24. What actions/programs/projects have influenced land use here? (environmental, agricultural, forestry, water, tourism, fishing and other)
25. Who has benefited most from policy changes?
26. Has there been disagreement over land use in the past?
27. What do you think will happen in the future regarding land use? (Who will be involved/when/why)
28. Do you know about the local park/protected area?
- 28.1 Who benefits from it?
- 28.2 Does the park/protected area play any part in your life?
- 28.3 What do you think of it?

## **Policy Issues**

29. What factors or drivers have most influenced, and continue to influence, the development of policies related to land use?
30. How are land use policies implemented?
31. Are there NGOs or other groups in this area that influence decisions about land use?
32. How do those groups influence land use?
33. What drivers influenced the development and implementation of the nearby park/protected area?

## **Proposed Metadata Requirements**

### **1. POLICY SUMMARIES**

- o Author(s) of document
- o Affiliation of author(s)
- o Contact address for obtaining an original copy of this document
- o Production date (year, month)
- o Abstract (English / Spanish / Portuguese)
- o Country
- o Region (if necessary)

### **2. STATISTICAL DATA**

- o Author/compiler of the data
- o Affiliation
- o Contact address
- o Date supplied to Tropi-Dry
- o Country
- o Region (if necessary)
- o Geo-coordinates of the subject area
- o Summary of the data

### **3. INTERVIEWS (for each interview conducted)**

- o Name of interviewer
- o Affiliation and contact information for interviewer
- o Date of interview
- o Location of interview
- o Category of respondent: User / Influencer
- o Age of respondent: 18-29 / 30-39 / 40-49 / 50-59 / ≥60
- o Sex of respondent: F / M
- o Summary
- o Research site
- o Contact (name / location)

### **4. CASE SUMMARIES**

- o Name of primary researcher
- o Contact information
- o Author(s) (name and affiliation of each)
- o Research date (start / finish)
- o Country
- o Region
- o Community
- o Abstract/summary

[illegible]

NOTES

Handwriting practice lines consisting of 40 horizontal dotted lines.

## This image shows a full page of white paper with horizontal dotted lines. The lines are evenly spaced and run across the width of the page, providing a guide for handwriting or typing. There are no margins, text, or other markings on the page.





## NOTES

A series of horizontal dotted lines for taking notes.



**Ediciones IVIC**

