DEVELOPING THE USE OF NIRS FACILITIES FOR ANALYSIS OF EUCALYPTUS WOOD AND CHARCOAL PROPERTIES

Mission report

2008 February 25 – March 8

Gilles Chaix

VALLOUREC AND MANNESMAN DO BRAZIL

CENTRE DE COOPERATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LE DEVELOPPEMENT – BIOS DEPARTMENT

APRIL 2008

DEVELOPING THE USE OF NEAR INFRARED SPECTROSCOPY (NIRS) FACILITIES FOR ANALYSIS OF EUCALYPTUS WOOD AND CHARCOAL PROPERTIES

Mission report

Gilles Chaix

The objective of this part of contract is to develop the use of Nirs for routine analysis of eucalyptus wood traits and charcoal properties in the framework of general tree breeding and charcoal production VMB research programs.

Problems and issues

Inclusion of wood quality traits in *Eucalyptus* selection and breeding strategies is becoming an important goal whatever the end uses are. Chemical, physical or anatomical characteristics affect both paper, sawn wood and charcoal yield and quality but the complex trait-trait relationships and the effect of growth are still poorly known. Choice of relevant characters for breeding, selection efficiency and genetic gain heavily rely to heritability values and magnitude of phenotypic variation within the breeding population as well as to genetic correlation between traits. Recent development of tools such as near infrared spectroscopy allow multi-trait assessment of large populations needed to provide genetic parameter estimations.

The general objectives of the current project entitled **DEVELOPING THE USE OF NEAR INFRARED SPECTROSCOPY (NIRS) FACILITIES FOR ROUTINE ANALYSIS OF EUCALYPTUS WOOD AND CHARCOAL PROPERTIES**, jointly implemented by Vallourec & Mannesman do Brazil (VMB) and Cirad are to strengthen the capacity of VMB in routinely using NIRS for effective inclusion of wood quality traits in Eucalyptus breeding and selection program for charcoal quality improvement used for steel making. The project focuses on capacity building and developing specific calibration equations for Eucalyptus woods and charcoal properties.

M. Nilson Guimares previously in charge of Nirs and genetic activities and trained by Cirad in 2006 was replaced by Leonardo Chagas and Leonardo Bhering since 5 months ago.

Objectives

1) to develop the use of Nirs facilities
2) to propose a routine analysis of eucalyptus wood and charcoal properties in the framework of general tree breeding research program
3) to validate calibration by studying the trait variability within the various samples representing the existing variability

Deliverables

1) Wood sampling strategy, collection and preservation in order to establish a reference collection for calibration
2) Advices on reference analysis methods (wet chemistry) in order to reduce laboratory error
3) Training for VMB scientist on the field of hardware maintenance, spectral acquisition, spectral data treatment, calibration and validation processes
4) Calibration equations for several wood and charcoal properties: wood density, total extractive content, lignin, cellulose content, fixed carbon, charcoal yield, ...

Contents

Tables and figures

Acknowledgements

The mission benefited from deep and rich discussions with the staff involved in Nirs activity, wood and charcoal properties and tree breeding program. VMB provided all the requested data and information for the implementation of the mission.

The consultant acknowledges all the people for their warm welcome and their large involvement in the success of the mission. In particular, deep, open and informative discussions were conducted with:

- Leonardo Chagas
- Helder Bolognani
- Leonardo Bhering

This report was prepared by the forestry department of Cirad for the account of VMB. It reflects Cirad judgment in light of the information available at the time of preparation of this report.

1 INTRODUCTION

Nirs tool has been recognized as a powerful analytical technique for rapid determination of various constituents in many agricultural and raw materials. The approach involves the acquisition of a reflectance spectrum after near-infrared radiation on ground or solid wood or charcoal samples. The resulting NIR spectral information is then calibrated against standard analyses obtained by conventional analytical techniques (standard method) using linear regression.

Near infrared spectroscopy is used more and more in several fields which chemical analyses (usually costly and time consuming) are needed:

- food (quality control, chemical analysis, ...)
- agriculture (proteins, sugars, ...)
- pharmaceutical (Control quality raw material and tablet products, ...)
- chemical industries (petroleum, polymers, ...)
- pulp and paper industries (first publication in 1988)

NIRS is a fast technique for qualitative and quantitative analyses of organic materials compared to chemical analysis. NIRS is an ecologically-friendly methodology (no chemicals products). It requires no sample preparation and it is less consuming in term of money and man-power, compared to chemical analysis after investment of apparatus. NIRS doesn't replace the reference analysis because there is a necessary calibration step but it decreases the number of analyses needed for routine and mass analysis.

As described in the Figure 1, the Nirs tool can be used at different steps, from the raw material characterization (chemical and/or physical wood properties) to the end product (fixed carbon, charcoal yield, ...).

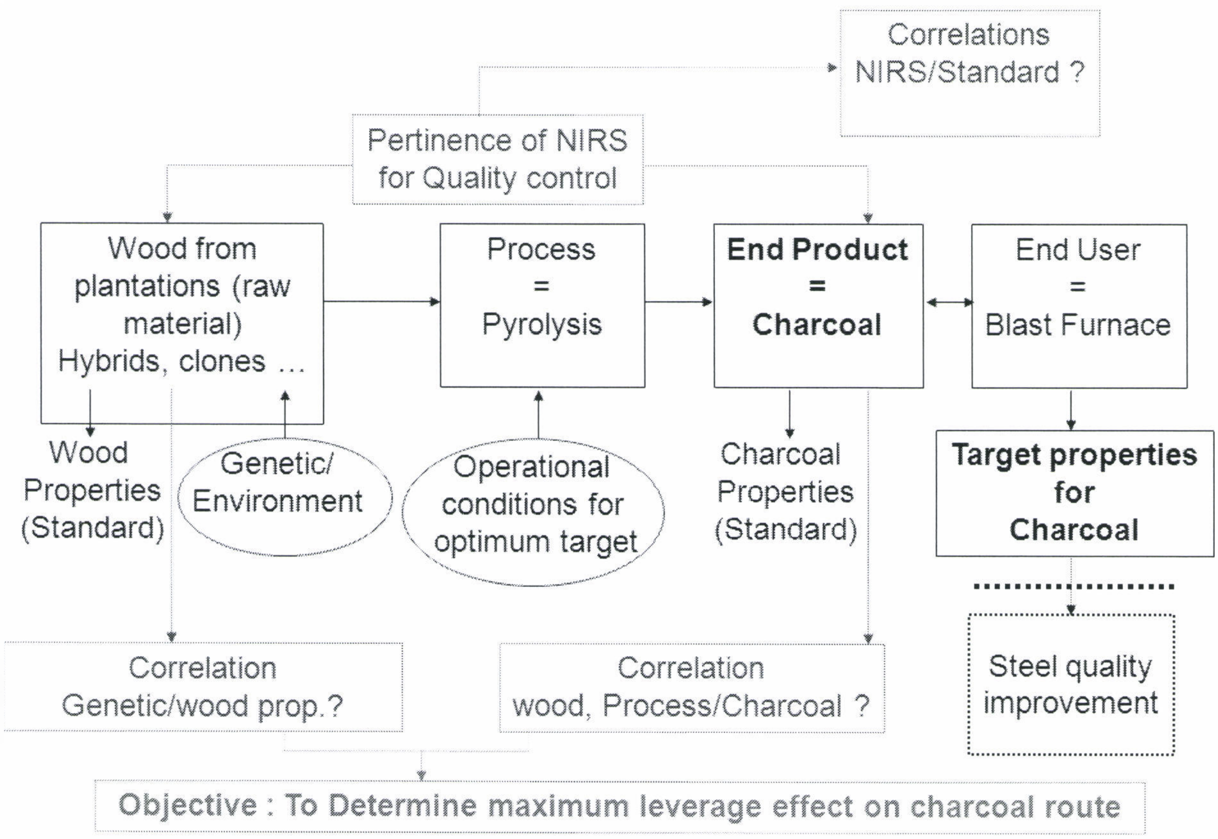

Figure 1 Nirs inputs during the charcoal production process

The calibration/prediction processes are based on a calibration set obtained from wood and charcoal chemical and physical analysis (reference values from laboratories) and Nirs spectral data. Theses two types of data were analyzed by multiple regressions to build a prediction equation. Then this equation is used for the prediction of new samples. The calibration/prediction processes are described by the Figure 2.

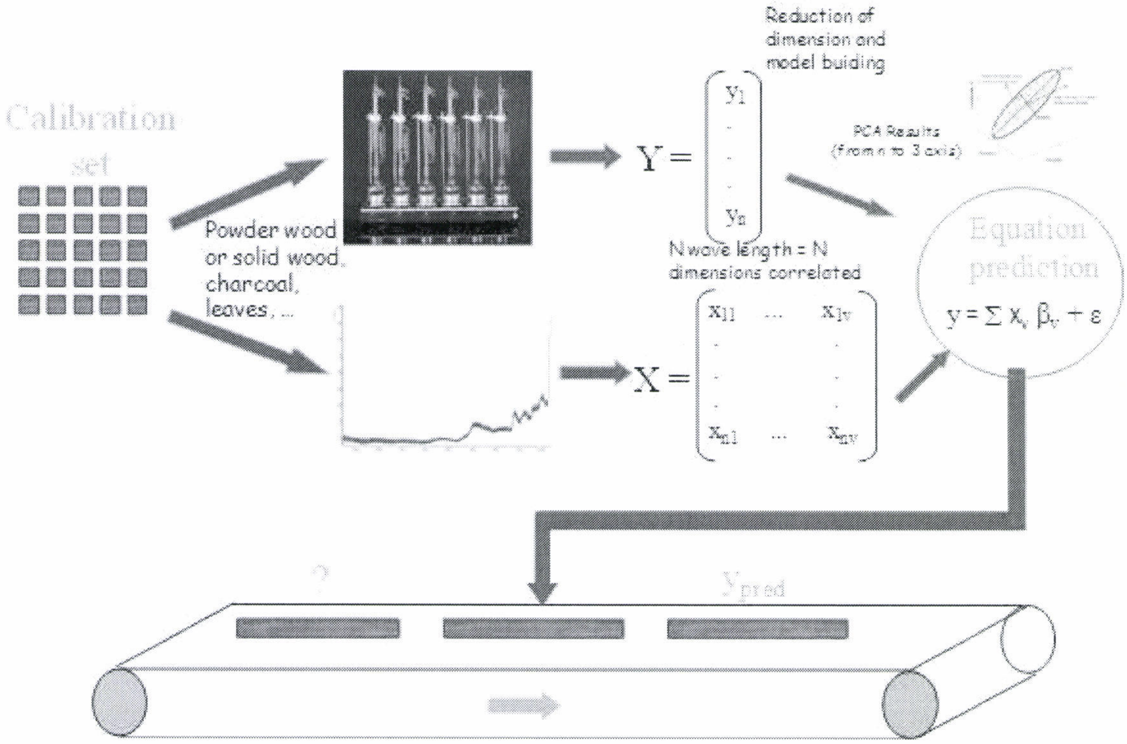

Figure 2 Calibration/prediction process

NIRS approach is based on reference values, and reduces the quantity of analyses. It requires mathematical process and a rigorous step by step control (from samples, reference value control to spectral analysis) and of course requires a training on wood and charcoal technologists. The samples have been analyzed as accurately and precisely as conventional techniques allow. Then standard methods and NIRS method will be compared in order to evaluate the potential of NIRS for each properties and each sample set depending on species, age, location, sylviculture, ... However Nirs tool doesn't replace the laboratory, the two are complementary and the investment in term of money and manpower capacity would be equal for both.

2 LIST OF PROJECT INVOLVING THE NIRS EQUIPMENT

Important point: to conduct efficiently the activities and to reach expected results, persons involved in wood and charcoal property analyses will be linked.

2.1 GENETIC BREEDING

Responsible: Helder Bolognani (VM)

Person involved: VM: Helder Bolognani, Leonardo Bhering, Leonardo Chagas

Cirad: Eric Mandroux, Jean Marc Gion, Philippe Vigneron, Gilles Chaix

UFLA: ?

2.1.1 CANDIDATE GENE SELECTION

- Eric Mandroux PhD
- SNP and lignin content variability

Needs: Calibration equation of lignin content for Eucalyptus hybrids based on 150 reference values from WC laboratory

Type of sample for calibration set: solid wood, powder, drill and grounded drill

Type of sample for prediction set: drill and grounded drill (non destructive sampling)

2.1.2 PROGENIES TRIALS AND CLONAL TESTS

- Different ages, locations
- Variability of phenotypic wood properties
- Heritability of wood properties
- Correlation among wood properties and growth
- Interaction genotype – environment
- Age-Age correlation of wood properties

Needs(1): Calibration equation on extractives, lignin content, wood density, and cellulose for each criteria: species, hybrids formula, age, site based on 150 reference values from WC laboratory

Type of sample for calibration set: solid wood, powder, drill and grounded drill

Type of sample for prediction set: drill and grounded drill, core (non destructive sampling)

2.1.3 SPECIES SURVEY TRIALS

- Variability of wood properties throughout species with high level of lignin and hybrid potential

See above (1)

2.1.4 First *E. camaldulensis* and *E. urophylla* provenance/progenies tests

- Estimation of wood properties by Nirs prediction
- Confirmation of value for current genitors and identification of new potential genitors based on wood properties values

See above [(1)]

2.2 Charcoal production estimation

Responsible: Fernando Fisher (UFLA)

Person involved: VM: Tulio Raad, Leonardo Chagas

UFLA: Fernando Fisher

Calibration for basic density

Calibration for Charcoal yield

Needs: Calibration equation on fixed carbon, charcoal yield on 200 reference values from laboratory

Type of sample for calibration and prediction sets: solid and grounded charcoal

2.3 Estimation of charcoal properties

Responsible: Alfredo Napoli (UFLA-VM-Cirad)

Person involved: VM: Tulio Raad, Leonardo Chagas

Cirad: Alfredo Napoli

Fixed carbon

Mechanical and physical properties

Relations between wood properties and charcoal properties

Needs: Calibration equation on fixed carbon, charcoal yield, physical resistance on 200 reference values from laboratory

Type of sample for calibration and prediction sets: solid and grounded charcoal

2.4 Estimation of mineral element in leaves

Responsible: Leonardo Chagas (VMB)

Person involved: VM: Leonardo Chagas, Raquel

Calibration for mineral element in leaves

Needs: Calibration equation on mineral element (indirectly) on 200 reference values from laboratory

Type of sample for calibration and prediction sets: dried leaves, grounded dried leaves

In conclusion, the number of reference analysis and Nirs prediction needed for whole projects will reach more than 1,000 and 10,000 per year respectively. Concerning the genetic requirement, the details will be identified in Vigneron report (2008). To obtain theses objectives, a crucial attention is expected for:

- a common identification of samples,
- a controlled conditions of storage sample collections used for calibration (because if WMB will change the Nirs equipment in the future, the best way will be to build new calibration based on the new Nirs data).
- a data base for reference values and Nirs data,
- a rigorous work plan for each year.

3 RELEVANT WOOD AND CHARCOAL QUALITY TRAITS FOR *EUCALYPTUS*

Nirs tool would be used for quantitative and qualitative properties. According to the objectives of VMB, the wood properties expected to be predicted by Nirs tool are:

- Basic density
- Extractives
- Lignin
- And cellulose contents

and charcoal properties expected to be predicted by Nirs tool are:

- Fixed carbon and volatiles matters
- Charcoal yield
- Mechanical properties

For quantitative traits, the priorities are extractives, lignin and basic density for wood and fixed carbon and charcoal yield for charcoal.

Concerning the mineral element predictions in the leaves we consider the hypothesis based on the link between mineral contents and organic molecule/physiological state. Nirs tool was developed for organic molecule predictions only but some physical properties depends on the organic contents (as basic density, shrinkage ...) and could be predicted by Nirs indirectly.

For qualitative traits, NIRS data and reference values (class properties, type of products) were predicted and identified by discriminate analysis (SIMCA[1] or PLS-DA[2] methods) but these approaches are not used by VMB yet.

---

[1] SIMCA: Simple Modelling of Class Analysis

[2] Partial Least Square – Discriminate Analysis

4 WORK PLAN FOR CALIBRATION / PREDICTION PROCESSES

The typical work plan for calibration / prediction processes presented in Figure 3 describes the VMB and Cirad work proposals from the breeding population to the equation prediction using. The proposed method is based on a set of 150 trees selected frm 300 trees for example according to the variability existing in the population. This variability is estimated on the Nir information analyzed by Principal Components Analysis, or on their predicted values obtained from the existing prediction equations. Properties are measured on the 150 samples, one part (100) is used for the prediction equation building and the other part (50) for the validation of prediction equation. During the prediction steps, some of these samples could appear as outliers, so their properties will be assessed and included in the calibration set.

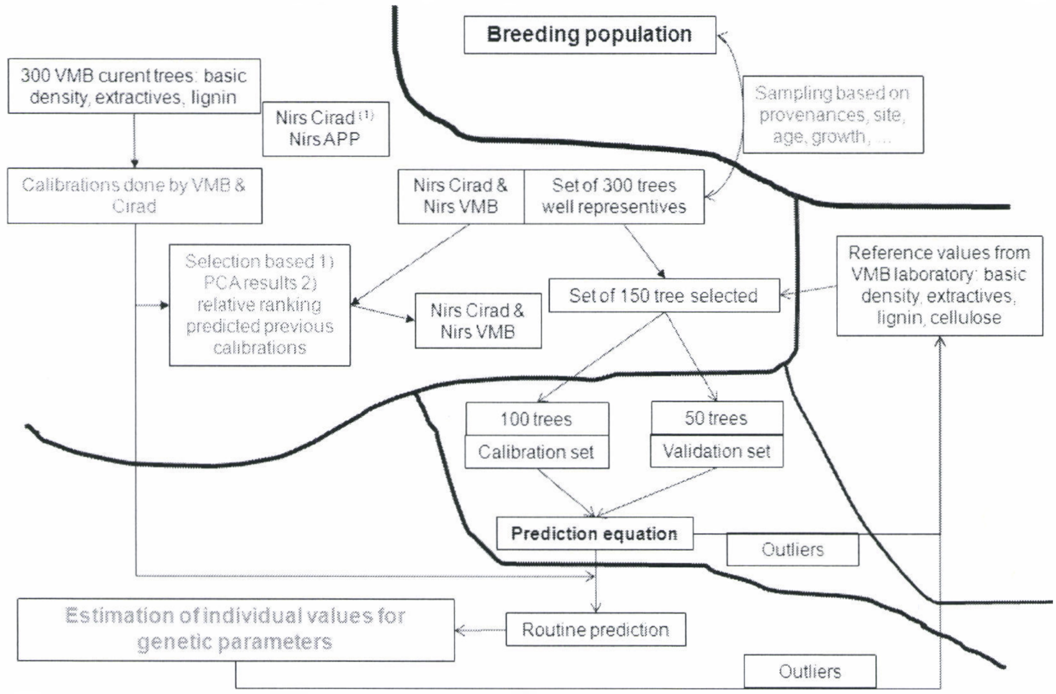

Figure 3 Work plan for calibration / prediction processes

5 SITUATION STATUS OBSERVED DURING THE MISSION

5.1 PREVIOUS WORKS

According to the Chaix mission report (2006), VMB selected trees to build calibrations. A first set of 279 trees (7 years old) were selected in accordance on these following levels of variability to the VMB mating design: specie levels for mother and father trees (*E. urophylla / E. camaldulensis* / Hybrids) from seedlings and clones, origins and well-distributed according to the provenances of *E. urophylla* and *E. camaldulensis*, diameter and height (based on index), region (3 farms). Trees were sampled according to the height (A: 1.8-2.3m height, B: +/- 25cm around the 50% of commercial volume height, C: +/- 25cm around the 75% of commercial volume height), distance from the pith (3 levels). The samples were divided in 4 parts: one for Cirad (solid forms), one for WC (needed ground operation), one part for VMB collection (solid forms) and the last one for pyrolysis process and charcoal analysis.

The calibration set was selected by PCA analysis of NIRS data and by the properties predicted according to the Cirad previous calibration during the training of M. Nilson Guimares in 2006 (the values were not well predicted but the rank among the sample was supposed identified). A set of 90 wood samples were analyzed by VMB laboratory for wet chemistry (WC) data and a second set of 121 samples by IPEF laboratory for the same properties (extractives, soluble and insoluble lignin). Because the sample surface measured with glass tubing is quite small, 3 Nir spectrum were measured from 3 different aliquots for each sample and then averaged. Both VMB lab and IPEF lab WC analysis were not replicated and we didn't estimate the laboratory standard error on reference data.

In spite of the fact that the two laboratories used the same norms, the two sets of data couldn't be mixed probably of difference laboratory accuracies. For this reason, we had to build two independent calibrations.

5.2 COMMENTS ON THESE PREVIOUS WORKS

In spite of the time spend to calibrate these data, the result obtained with VMB and IPEF data are not acceptable for lignin prediction. The determination coefficient (R^2) between reference values and predicted values determined by the model we built is closed to 0.5 for lignin content. This low value expects the low accuracy of wet chemistry data (the two sets) for lignin. Relative to the huge quantity of samples, some mistakes are suspected on identification samples.

But R^2 for extractives reached 0.8 and we concluded to the possibility to predict theses properties. We suspected the low quality of reference value for lignin only.

A test based on several spectral acquisitions of the same sample showed some inaccurate spectrum and revealed a defect of the Nirs tool. The control we used showed instability in two parts of the spectral curves. One part is located in the lignin band absorbance which explains the low value of R^2 obtained for lignin contents and good values for extractives.

The Bruker maintenance of the apparatus is over, and VMB should ask to Bruker Company for a new control. The technician from Bruker will come to CAPEF for the maintenance equipment activity and for training the VMB users on routine control for the apparatus and OPUS uses. One day is sufficient.

In conclusion, this lignin calibration can't be used for future calibration selection set yet, and we need to confirmed or not that after the checking of the Bruker apparatus. The data produced by IPEF and VMB should be stored and will be used for new calibrations when Nirs apparatus will be checked. This previous work done by M. Nilson Guimares and Cirad can be considerer as ok due to the fact that we have obtained good resultants for the prediction of the extractive content. The calibration model achieves a high correlation coefficient (0.8) between reference values and predicted values.

Cirad has proposed to measure Nir spectrum data of the samples sent by VMB to Cirad before, and to verify the calibration results. If the results are acceptable, we'll conclude on the good accuracy of WC data. In this case, new calibrations could be conducted on Nir spectrum data from VMB Bruker and used for prediction and selection of new samples for next calibration set selections.

We spend time for a short training on OPUS software which managed the Nirs apparatus. With his previous knowledge M. Leonardo Chagas can use easily this software yet. The comparison of several spectral data preprocessing on quality of prediction equation is the major interest of OPUS. However, VMB bought Unscrambler software from CAMO as Cirad proposed and M. Leonardo Chagas knows very well this one.

6 Sample collection and preparation

Samples before analysis were prepared in accordance to NBR 14660 standards from ABNT based on TAPPI Test method T257cm-97.

VMB needs to establish systematic, simple and unique sample identification and build a data base (with Microsoft ACCESS software for example or proper data base software).

6.1 Sample collection for calibration samples

For the calibration set trees VMB needs to collect two disks at dbh, one for basic density measurement and one for WC and drill samples (Figure 4). Calibration will be built both on powder used for WC and drill samples collected on disk or tree before and after grounded step. WC laboratory requires only one part of the grounded disk.

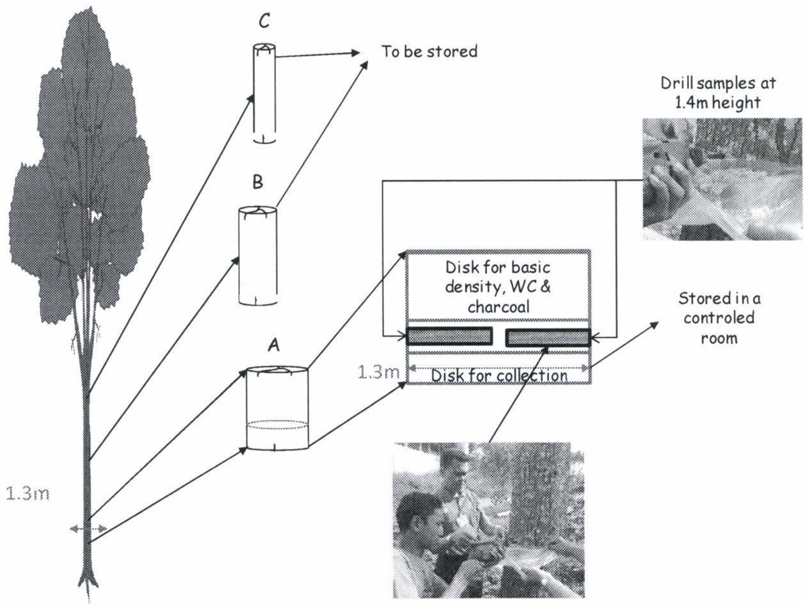

Figure 4 Sampling wood proposed for the calibration set

When trees for calibration need to be remain in the trials for further measurements, trees will be sampled by drills corer only. Drill sample quantities must be sufficient for WC laboratory (WC lab needs 15-20 grams of drills dried). But in this case basic density measure is impossible but will be predicted if VMB built a calibration equation based on drill samples before.

If VMB needs to reanalyze the samples we propose:

- To store in mill area solid samples after drying in plastic bag.
- To store in air-conditioned Nirs room powder samples (collection reference).

When destructive sampling needed to cut the trees, two supplementary disks must be collected along the bole for the intra-tree variability study (Figure 4). These disks will be stored as described above.

6.2 SAMPLE COLLECTION FOR PREDICTION SAMPLES

For the set tree prediction VMB needs drill samples grounded or not, this depends on the calibration results based on drill spectrum or drill grounded spectrum (Figure 5). The quantity will depend on the type of Nirs measurement required and on the representative sampling (the minimum is one drill radius till the pith of trunk).

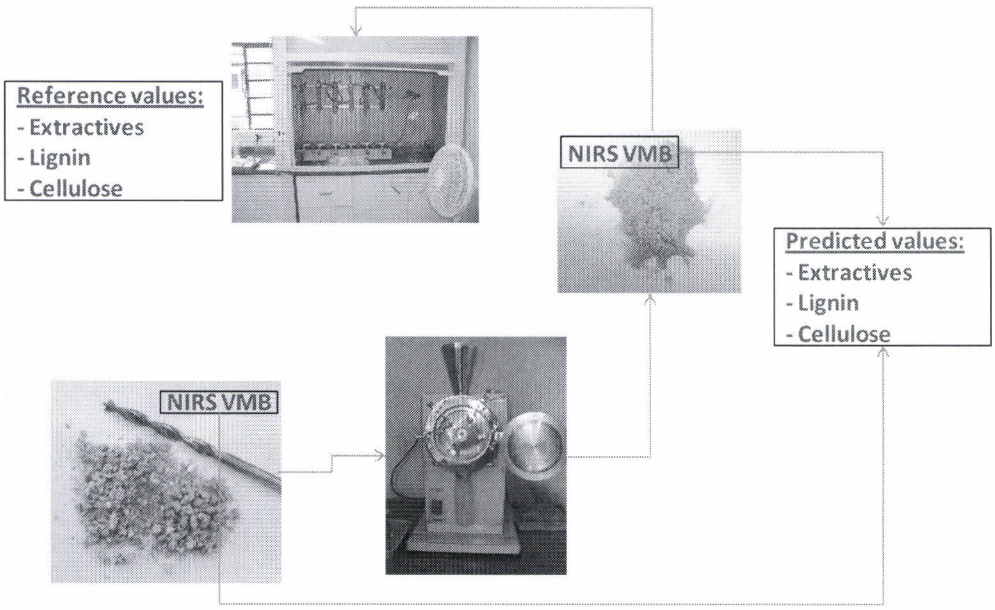

Figure 5 Prediction process and calibration process for non-destructive approach

6.3 GROUND OPERATION

From solid wood, the ground operation is conducted in two steps (Figure 6):

- First grounding from solid wood to 1st powder. During this step and even if loss are identified no t relevant, the technician involved in this process must verify to collect all the powder produced because most fine particles are more riche in lignin content and the powder collected after this first step could be non representative.

- Second grounding from the 1st powder to 2nd powder used by WC lab. Same control than above is needed.

For drill samples, the grounding operation is limited to the second.

The different steps which require Nirs spectrum acquisition are indicated in the Figure 5 and Figure 6. The objectives are:

- to build calibrations for each status wood,
- to compare the power of calibration and prediction from different type of wood status and choose the best one in term of prediction power and cost investment in time and in manpower,
- to compare the Nirs data between and among each status wood.

Then, we'll propose to measure Nirs only on the wood status selected.

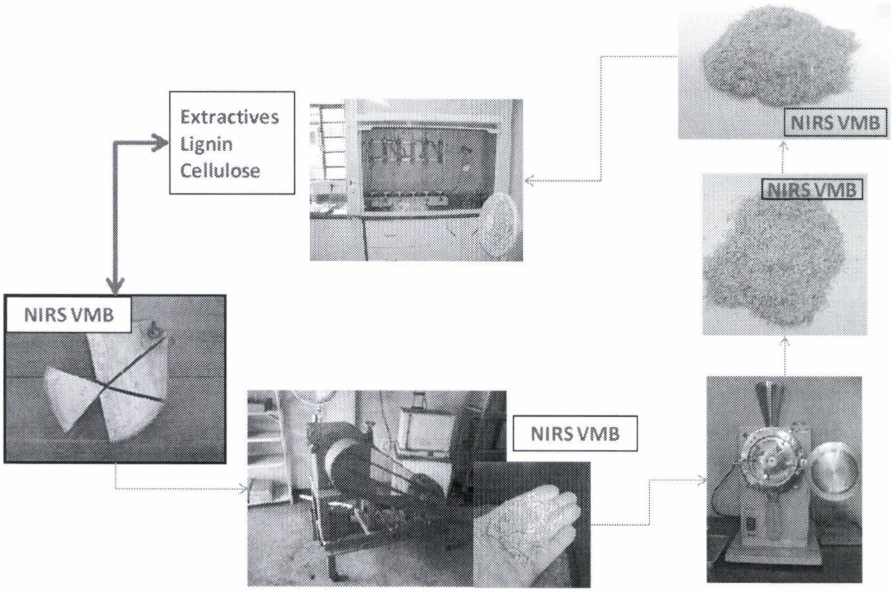

Figure 6 Description of different steps from solid wood to WC data

6.4 SPECIFIC OPERATION FOR CHARCOAL STUDIES RELIED TO NIRS

Specific steps for charcoal studies are described in the Figure 7. For charcoal properties, as fixed carbon, charcoal yield, Nirs spectrum will be acquired on charcoal powder.

Because mechanical properties are very interesting for steel making (resistance of charcoal under iron pressure in blast furnace), VMB will study the way to identify these properties fastly. Nirs equipment accuracy could be tested as well as acoustic methods developed in Cirad and would be applied further for on-line control process.

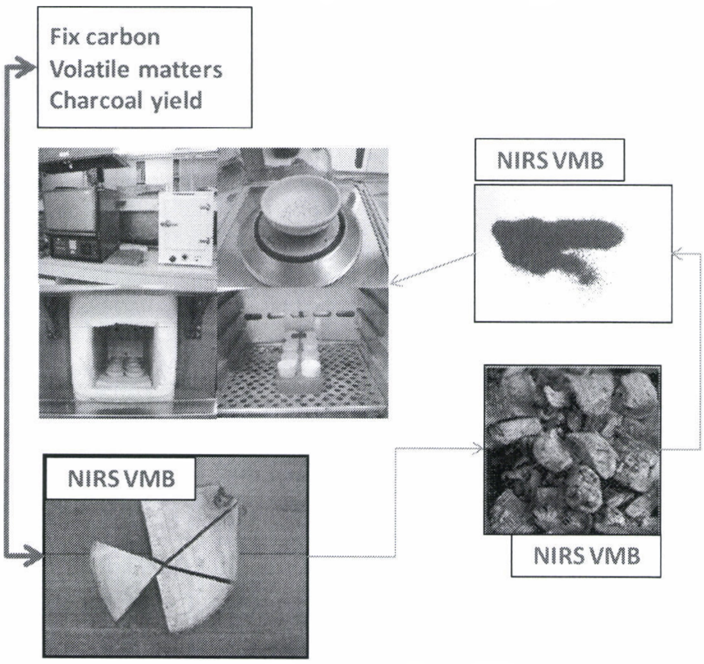

Figure 7 Different steps for charcoal studies

6.5 GLOBAL ROUTE FROM SOLID WOOD TO CHARCOAL PROPERTIES

According to the VMB R & D objectives and the necessary connection between the two laboratories expected for efficient results, all samples analyzed by the two laboratories should be measured by Nirs tool from solid wood to charcoal status (Figure 8). Then it would be possible to link wood properties (WC data, basic density) and charcoal ones according to pyrolysis process, quality of raw material. The goal would be to build charcoal properties prediction based on Nirs spectrum information of wood selection.

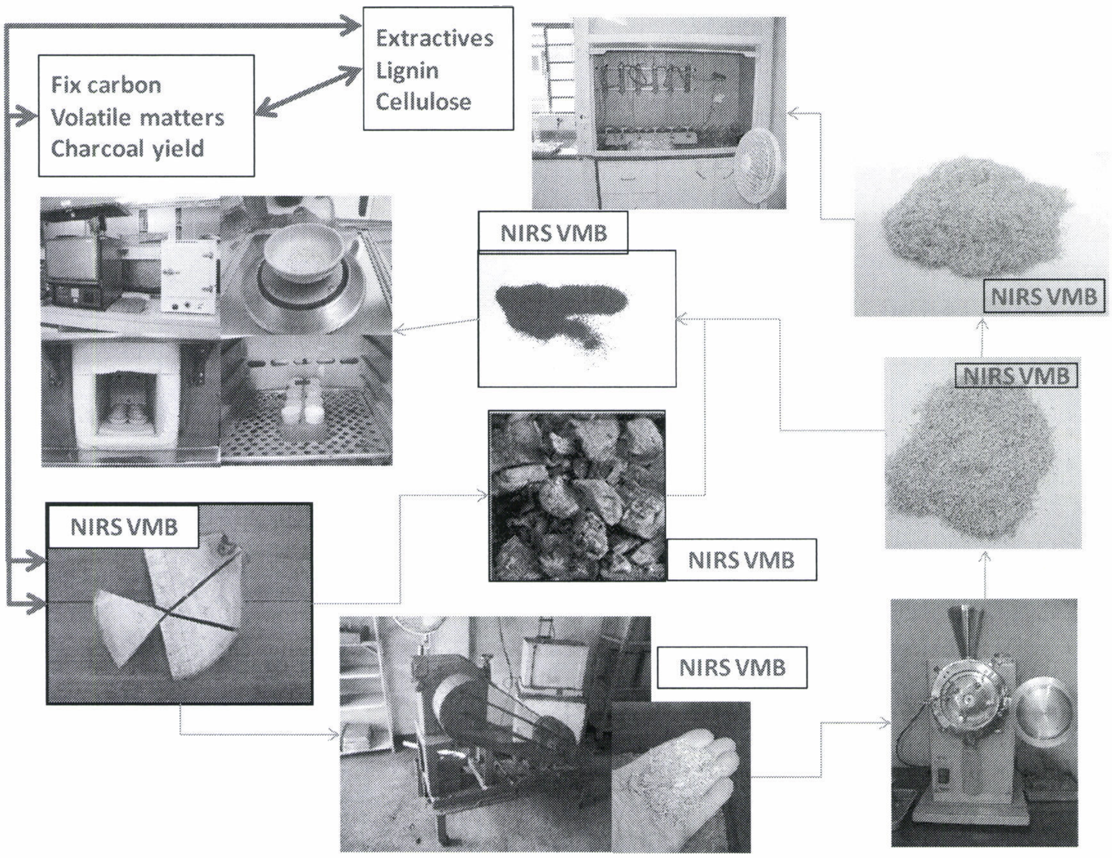

Figure 8 Complete route proposed for Nirs activity from solid wood to charcoal

7 ACCURACY OF REFERENCE ANALYSIS

VMB has decided to invest in proper WC laboratory. It's a very good decision to use only VMB lab and to invest new equipment in order to improve the quality of the method. The most important is to maintain the same method and its quality in term of laboratory standard error.

7.1 MANPOWER AND EQUIPEMENT

During the mission, we received 40 WC data from VMB WC laboratory. In spite of few numbers of samples for Nirs spectrum analysis, we have tested the laboratory accuracy based on these new data.

The current error is estimated to 0.3% which confirms the good efficiency of M. Helder, and we encourage him to maintain this quality of work. But he shares his time between WC lab, mineral lab and Nirs lab. VMB needs one permanent person in the WC lab to maintain the same data accuracy and to reach the genetic breeding program required. This technician should be under the control of M. Helder and Leonardo Chagas.

The laboratory needs supplementary material to increase his capacity according to the number of projects involving Nirs tool and WC reference values needed: balance (bought by VMB), Soxlet (bought by VMB), ...

7.2 CHECKING PROPERTIES ANALYSIS

7.2.1 WOOD CHEMICAL PROPERTIES

The wood samples are conditioned according to NBR 14660 standards from ABNT (Associação Brasileira de Normas técnicas).

The determination of wood extractives content is carried out in accordance with NBR 14853 standards (ethanol-toluene dichloromethane) from ABNT based on Tappi T204 cm-97 standards. Six grams of wood powder for WC are extracted per samples.

Insoluble lignin was determined following the standard NBR 7989 procedure based on TAPPI method T222 om-98. 0.3 gram of wood powder are required for each analysis. In this method, the polysaccharides (cellulose and polyoses) are hydrolyzed by sulfuric acid solution. Acid-insoluble lignin is filtered off, dried and weighed.

Two repetitions are required per sample, a third one is analyzed if difference between the two values is above 3 %.

Cellulose contents will be determined according to NBR 14080 methods based on TAPPI Test method T223cm-84.

7.2.2 BASIC DENSITY

The determination of basic density is carried out in accordance with NBR 11941 standards from ABNT.

Basic density is measured by difference between anhydrous weight and saturated weight, and volume displacement based on disk or one part of the disk.

7.2.3 CHARCOAL PROPERTIES

We didn't check methodologies used by VMB lab but we recommend these main points:

- To use 2 repetitions per sample, three or more if difference is more to % (to be determined).
- To estimate Standard Error of Laboratory (SEL) for each methodology and each technician (fixed carbon, charcoal yield, ...).
- To establish methodology for the control (reference samples tested every month, exchange data with other laboratories) according to the control of WC analysis.

7.3 IDENTIFYING THE WET CHEMICAL AND PHYSICAL LABORATORY CAPACITIES FOR WOOD

After the recruitment of new technician and the acquisition of a soxlhet, the maximal annual capacity we expect is 1,000 for wet chemistry analyses (25 trees x 4 week x 10 months).

The VMB laboratory capacities should be sufficient for the routine analysis. For the genetic analysis which need data on several hundred or several thousand of samples, these capacities are far sufficient to provide the data to breeders in due time.

Nirs tool allows that. The maximal annual capacity we expect is 10,000 predictions (100 x 10 days/month x 10 months, including travel for drill collection).

	Samples per week today	Samples per day/week today in few month
Sample collection		100 drills / day / men
Extractives and lignin	9	25/day
Basic density	200	200

7.4 STANDARD ERRORS OF LABORATORY

Our main recommendations concern the following controls which influence greatly the calibration quality:

- Standard error of laboratory for each method and each technician (extractives, lignins, basic density, cellulose, fixed carbon, carbon yield)
- Methodology for the control (reference samples tested each month, reference sample set exchanged with other laboratories)
- To maintain the twice analysis per sample

The precision of prediction equations heavily rely on quality of trait assessments both for wood properties and charcoal properties.

Quality of assessment depends on the precision of the apparatus used and on the repeatability. In order to estimate the repeatability (i.e. the consistency of the result over several assessments of the same trait with the same sample) we propose to implement the following controls.

- Daily controls

Maintain the double analysis for each sample. If the difference is higher than the error previously defined, the sample will be analyzed a third time.

- Estimation of SEL

The methodology is presented in the Figure 9.

- Select two samples with different values of WC data and prepare a large quantity of wood powder for two hundred analyses minimum.
- Repeat the WC analysis ten times for each sample (for all WC traits) using routinely methods.

- Monthly controls

These two previous samples will be analyzed each month. If the value excesses the SEL a second analysis will be necessary to verify the accuracy of the lab and the technician.

- Inter-lab controls

VMB will try to organize a checking process by a sample set exchanges with other laboratories in Brazil.

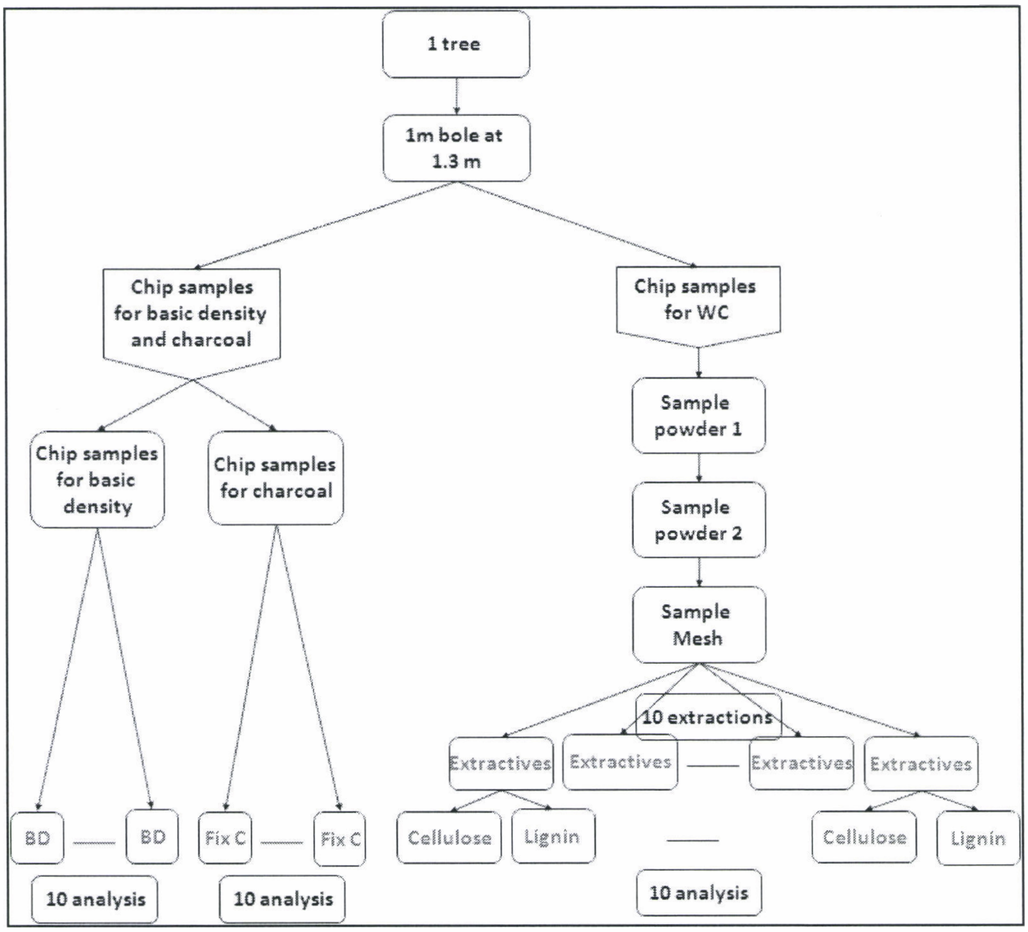

Figure 9 Methodology proposed for standard error of laboratory estimation

8 NIRS MEASUREMENT AND STATUS OF EQUIPMENT

For the spectrophotometer efficiency stability, sample conservation, repeatability, calibration and prediction quality, it's compulsory to maintain the wood samples at constant temperature and humidity conditions. The goal is to maintain the humidity of the wood sample at the same level during NIRS and prediction process because water absorbance bands affect the wood specific absorbance. VMB dedicates the Nirs room for NIRS activity and long term storage of calibration sets collection which will be used as permanent control. The samples to be predicted will be stored in the same room at least one week before spectral acquisition in order to stabilize water content. During the mission, the humidity conditions were not acceptable both for the samples status and the apparatus standardization but the equipment was out of order. Now this problem is solved.

8.1 NIRS MEASUREMENT

VMB uses glass tubing (Figure 10) because the quantity of wood powder is small. These glass tubing came from Bruker probably. The bottom of these tubes is obviously not flat and not regular. The best products are FIOLAX®-klar from Schott[3]. This point is crucial because the quality of glass tubing could include variability in the spectrum data. Their proper absorbance would be different depending on glass quality and glass width.

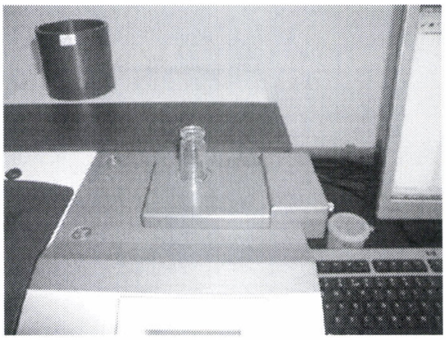

Figure 10 Glass tubing used for wood powder spectral measurement

First VMB needs to verify the origin of these glass tubing. Secondly VMB needs to verify the variability of these glass tubing by Nirs measurement of the same wood powder set (10 samples) in different glass tubing (10) choosing randomly and by using the PCA to verify the independence of the glass tubing effect.

We propose a checking method for the Bruker apparatus. We propose to exchange samples and WC data with Cirad to compare the results obtained. First we need to define a control sample set (10 samples) and we'll compare the NIRS spectral. VMB will send the list of IPEF samples (119) and reference values to Cirad. Cirad will try to calibrate in Montpellier the set of "IPEF samples" selected in the 300 solid wood samples sent by VMB to Cirad in 2006, VMB sent all wood powder for WC analysis to IPEF and IPEF didn't return them.

[3] http://www.us.schott.com/pharmaceutical_packaging/english/download/us_product_lines.pdf

Very Important points:

- **All samples analyzed by laboratories (wood, charcoal, leaves, ...) should be measured by Nirs tool (the data must be store to be use further). The time needed for Nirs spectral acquisition is short and it would be interesting to return and explore database well implemented.**

- **One part (sufficient for Nirs measurement) of all samples measured by Nirs tool should be stored as reference collection in controlled conditions (to be able to measure the samples again).**

8.2 STATUTS OF NIRS EQUIPEMENT

We observed variability on several spectrum for the same sample with rotate cup and with glass tubing. The cause is due the humidity variability in the Nirs lab or in the Nirs apparatus. During the mission, we try to stabilize the relative humidity in the room and change the dessicator inside the apparatus but the problem was not resolved. We propose to ask a checking by the Bruker technician because it seems to an internal problem of the apparatus.

This spectral artifact is observed on Nirs spectrum of previous calibration. The bad results obtained on lignin calibration based on IPEF WC data and VMB data are due probably from that.

The Bruker control of the apparatus is over, and VMB should ask to Bruker Company for a new control. In the same time, the Bruker technician could train M. Leonardo Chagas for the routine control of the apparatus. One day is sufficient.

VMB needs to maintain on line 24 on 24 hours the Bruker apparatus and air dried conditioned in the Nirs room.

8.3 NOTATION AND NUMBERING SYSTEM

The sample identification is a crucial point.

Three files will be compiled from all the current data files concerning the NIRA activities:

- One sample identification file with Species, location, trials ...
- One chemical and physical file where each laboratory analysis data will be referenced
- One spectral file with details on spectral acquisition.

We recommended the methodology and the forms as described in annexes.

9 TRAINING NEEDS

For the future we identified some points which could be improved by VMB staff training:

- Wavelength selection (improve model, necessary for nirs "on line" protocol)
- Discriminate analysis
- Charcoal properties calibration
- Nirs "On line" protocol (fixed carbon for charcoal produced by CAPEF, humidity of wood before pyrolysis, ...)
- Error of laboratory estimation (incertitude estimation) by control at each step (balance control, ...)
- Routine control of Bruker spectrometer

These training needs could be organized by Gilles Chaix (backup missions and training in France) and Cirad partners (for on line protocol) except the last point (Bruker from Brazil).

At this moment all efforts will be concentrated on the analysis and development of calibration curves. During the progress of these works, specific courses will be discussed.

10 MATERIAL NEEDS

We identified several types of investment in term of specific equipment for NIRS activity. The 2 first are the most urgent and the last ones will be expected in the near future to replace or to develop new activity.

Urgent equipment needed and purchased:

- Soxlets (2 x 6) is the most urgent needs to increase the lab capacity according to the genetic breeding objectives.
- Rotate cup 50 mm from Bruker to improve the capacity of measurement and improve the calibration accuracy (the variability of glass tubing could include skew).

Equipment to be ordered:

- Ground apparatus (from solid sample to first powder – granulometry 4mm) as Retsh SM200[4] for a better preparation of sample and good representative samples from different status.
- Bruker optic fiber for calibration based on distant measures (according to the on line project).

[4] http://www.retsch.com/products/milling/cutting-mills/sm-2000/

Annex 1 Sample identification file proposed

Numerous sample	Species	Trials	Provenance	Descendance	Bloc	Repetition	Location	Soil	Age	Feeling date	Sampe collected	Date	Assessor
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
18													
19													
20													
21													
22													
23													

Species
1: Eucalyptus urophylla
2: Eucalyptus camaldulensis
3: Eucalyptus hybrids
4:
5:
6:

Soils
1: mineral soil
2: pet land
3:
4:

Sample collected
1: wood disk
2: wood drill
3: wood core
4: leaves
5:
6:

Annex 2 Example of chemical and physical file proposed

Numerous sample	Numerous analysis	Extractives	Date	Assessor	Cellulose	Date	Assessor	Lignin	Date	Assessor	BD Disk	Date	Assessor
1	1												
1	2												
1	3												
2	4												
2	5												
2	6												
2	7												
3	8												
1	9												
1	10												
1	11												
1	12												
4	13												

Charcoal yield	Date	Assessor	fixed carbon	Date	Assessor	xxxxx	Date	Assessor

Annex 3 Spectral file proposed

Numerous sample	Numerous analysis	Numerous spectra	Type sample	Face for solid wood	Assessor	Date spectra acquisition	Number scan	Accurancy (nm)
1	2	1						
1	2	2						
1	3	3						
2	3	4						
3	3	5						
4	5	6						
5	2	7						
6	5	8						
7	6	9						
		10						
		11						
		12						
		13						

Type sample
1: wood solid
2: wood drill
3: wood grounded drill
4: wood core
5:
6:

Face
1: radial
2: axial
3: tangential