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| TerrAmaz : un partenariat inédit qui concilie lutte contre la déforestation  et développement en Amazonie | Land PortalDeSIRA: Development Smart Innovation through Research in Agriculture |  International Partnerships    **Protocol proposal for “Interactions between pest attacks and plant growth using a model approach applied to Robusta coffee in Uganda. Effects on production “**  **For the first year (2022/2023)**  ***DeSIRA ROBUST project***  **PhD Student: Under the supervision of:**  Houssem TRIKI Marc JAEGER  Fabienne RIBEYRE  Fabrice PINARD    Redactor: H. Triki, 2022, august, 16th |

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## Pests and diseases involved in the study:

BioAg1 = Black Twig Borer (BTB)

BioAg2 = Red Blister Disease (RBD)

BioAg3 = Coffee Leaf Rust (CLR)

BioAg4 = Coffee Berry Borer (CBB)

Contamination of the healthy coffee trees will be artificial.

Simulations of injuries will be used (pruning of young shoots, removal of leaves).

Dynamics of coffee leaf rust and coffee berry borer will be observed when available. Only to supply information to refine the models that are already at our disposal

## Coffee tree clone selection criteria

Homogeneity of trees history and age is requested. Preferably, choosing trees that have not suffered any serious attacks and were healthy the previous year.

## Clone 1:

Common among producers; Impacted by RBD.

## Clone 2:

NARO-Kituza Robusta clone, expected to be widely distributed; little impacted by RBD.

## Plot selection criteria

Similar conditions, except shading, close from each other and easily accessible.

Plot in good condition (maintained), in production.

No pruning of branches.

Possibility to schedule and control the harvest and the phytosanitary treatments.

Record the maintenance history of each plot.

**Note**: If any pest & disease is present on the category that it does not belong, it should be removed (by removing the attacked organs?).

**Healthy trees**:

For every category and condition, half of the healthy trees are protected from any exterior contamination to pest & disease. By covering it or constructing a small-scale greenhouse.

For the rest of the healthy trees, the selection should be based on finding the trees that are placed on the optimal condition that prevent them from stressing (which result in them being attractive to attacks)

## Design of experiment (DoE)

Criteria:

* Healthy, submitted to one or two attacks
* Clonal factor
* Shading

Most measurements are non-destructive

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Shade | Clone | | Criteria | Category Healthy | Category BTB | Category RBD | Category CLR \* | Category CBB \* | Category Sum \* | Category Destroy |
|  |  | | Sanitary status | Healthy | BioAg1 | BioAg2 | Bioag3 | Bioag4 | BioAg1 + BioAg2 or others | Healthy |
| No | C1 | | Tree number | 10 | 10 | 10 | 0 | 5 | 10 | 1 |
| C2 | | Tree number | 10 | 10 | 10 | 5 | 0 | 10 | 1 |
| Yes | C1 | | Tree number | 10 | 10 | 5 | 0 | 5 | 0 | 1 |
| C2 | | Tree number | 10 | 10 | 5 | 5 | 0 | 0 | 1 |
| Total | | 154 | | 40 | 40 | 30 | 10 | 10 | 20 | 4 |

\* Not to be done the first year

**Table 1:** distribution of trees by criteria and conditions for the duration of the experiments

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Shade | Clone | | Criteria | Category Healthy | Category BTB | Category RBD | Category simulation BTB | Category simulation RBD | Category Destroy |
|  |  | | Sanitary status | Healthy | BioAg1 | BioAg2 | Simul1 | Simul2 | Healthy |
| No | C1 | | Tree number | 5 | 5 | 5 | 0 | 5 | 1 |
| C2 | | Tree number | 5 | 5 | 5 | 5 | 0 | 1 |
| Yes | C1 | | Tree number | 5 | 5 | 5 | 0 | 5 | 1 |
| C2 | | Tree number | 5 | 5 | 5 | 5 | 0 | 1 |
| Total | | 84 | | 20 | 20 | 20 | 10 | 10 | 4 |

**Table 2:** distribution of trees by criteria and conditions for the first measurements

## Tree identification

Create a unique identifier for each tree and mark it on each (Method to be defined with Nacori). Locate the trees on the plot plan, by creating a template of trees localisation and labelling the various categories.

Every studied branch should be marked in order to be monitored during the time of experiments and monitoring.

## Initial measurements

## Initial identification of developmental parameters of healthy coffee trees

Measurements will be taken two times per year (after each growth period) per clone and condition (shaded, unshaded) on 5 trees per category. Total number of trees = 5\*4\*2\*2 = 80 trees

**Note:** Development strategies can be different depending on the light conditions and climate.

* + 1. Plant architecture

**Trunk:**

Number of internodes in the trunk, position of dead or absent branches, number of leaves on the trunk).

**Branches:**

Description of 5 branches (leaves, fruits, flowers, internodes) always at the same distance from the top (2 opposite branches at 7 storeys (between nodes) from the top, 2 opposite branches at 9 storeys (between nodes) from the top; 1 branch at 15 storeys from the top). It will not be the same branches at the beginning and the end, but the same storey.

### Organ allometry parameters (destructive)

**Destructive:**

Destructive on 1 tree per clone and condition (Category Destroy). total number of trees = 4

20 leaves of varied sizes weight, length, width, and area (scan)

Weight, length, and diameter of 20 internodes

Diameter, volume, and weight of fruits of 20 glomerulus at 4 stages (small green, big green, yellow, red). (Compter les fruits en détails)

**On trees:**

On the selected branches, we need to take the following measurements:

- Length of internodes

- Internode diameter (the average of the two perpendicular diameters, almost to the tip)

- Leaf length (from tip to base)

- Leaf width (max)

With these parameters, we first apply descriptive statistics to calculate the mean and standard deviation for all the parameters.

We create a linear regression models for a number of samples, this will allow us to estimate the coefficient of the model. Then, the model will be tested and validated by using the rest of the samples. These models will be used to calculate the dry mass. One between the internode volume and its dry mass, the second from the product of leaves’ length, width and area to estimate the dry mass. Plus de detail

### Measurements related to plant functional parameter identification

On the plant to be destroyed:

Weigh all the branches with their leaves and fruits, weigh the leaves by Age. Those are requested to evaluate RUE, organ sink function and so on.

## Inoculation/introduction

The inoculation/introduction of the pests will be done according to the usual Nacori protocols, after the first measurement of the developmental parameters, preferably on a branch measured at architectural level.

Areas where pests have been inoculated into the tree are physically landmarked.

## Red blister

In case of the shaded trees; Since the shade is gradual and not complete, we test different levels of contaminations.

The contamination is done by spreading the fungus (spores) with a glove.

## Artificial damage

a planning of damage creation (cut on a young branch or leaf removal) on the tree will be done.

## Initial pest monitoring/inoculation

If contamination is artificial, trees will be chosen healthy

If artificial contamination is not possible: Identify the trees that may contain a pest or a disease. If so, measure the level of damage and contamination severity. If the tree is too much damaged and have a high level of contamination, it should be removed from the protocol.

On all trees:

* Choosing 4 branches (2 attacked and 2 healthy) at 2 different storeys:
* For the healthy branches: we favour taking them at the same storey as the attacked ones. In case the two branches of the same storey are attacked, we prefer a branch from the upper storey (n+1)
* Enumerate the number of branches that will be infected or damaged artificially.
* Enumerate the number of internodes.
* Record the number of attacked and healthy fruits, leaves and flowers.

## Continuous measurements

## Protection of the healthy trees

To be done with Nacori

## Developmental and functional parameters of coffee trees

Counting the new internodes, leaves and flowers/fruits.

Fellow the allometric protocol used in the initial measurements. This will allow us to expend the database and give us the possibility to fellow the dry mass on every growth cycle by using the calculated models.

## Harvest monitoring

Weight the fruits from the monitored branches individually. In order to compare the weight of fruits coming from a healthy tree and the weight of the fruits coming from infected ones.

## Pest monitoring

In both cases of pest infections, the monitoring is to be done every 3-4 weeks.

### Within the RBD infected trees

The following features should be counted and on the 4 \* 5 coffee trees of Category RBD

**On coffee trees:**

* Number of healthy and infected branches.

Each infected branch should be marked

**On every infected branch:**

* Storey (from top of the tree)
* For each glomerulus of the affected branch
  + Affected fruits and unaffected fruits and their growth status (small green, large green, yellow, red).

the identification protocol is specific to the status of fruits, e.g., on green fruits, attacks are identified by a brown spot with a potential purple halo; and on red fruits attacks are identified by a very dark, or even black, area, which may be covered by a layer of silver spores.

* + Looking for lesions on small berry (which is a sign of an early contamination)
  + Affected leaves. On the infected leaves, the length and width of:
    - The leave.
    - The spread of the fungus.

(Dark brown circular lesion on the upper surface of the leaves).

* + Record whether the branch is affected by anthracnose.

**On one healthy branch per tree, in the same level as an infected branch:**

* Storey (from top of the tree)
* For each glomerulus of the branch
  + Number of fruits and their growth status (small green, large green, yellow, red).
  + Number of leaves.

**To look for:**

* Usually, the fungus stays on fallen leaves, and this can be a way for it to spread. However, due to the fact that on the field we only noticed contamination on the fruits. And a proper observation should be done on the propagation process.

In case of the shaded trees:

The RBD does not have enough light to develop, this is why we should take only a small quantity of trees and use them to validate this observation.

### Within the BTB infected trees

The following features should be counted and on the 4 \* 5 coffee trees of Category BTB

**On coffee trees:**

* Number of healthy and attacked branches

**On every affected branch:**

* Storey (from top of the tree)
* For each node of the affected branch
  + Number of green leaves and the leaves that turn yellow.
  + number affected fruits, healthy fruits
* Record whether the fungus becomes pathogenic
* Looking for any sign of dieback

**On one healthy branch per tree in the same level as an infected branch:**

* Storey (from top of the tree) For each node of the affected branch
  + Number of green leaves.
  + Number of healthy fruits.

### Within the simulated damages trees

The following features should be counted and on the 2\*2 \* 5 coffee trees of Category Simul1 and simul 2

**On coffee trees:**

* Number of healthy and damaged branches

**On one branch per tree in the same level as a damaged** **branch:**

* Storey (from top of the tree) For each node of the affected branch
  + Number of green leaves.
  + Number of healthy fruits.
* Looking for any sign of dieback

## Pest monitoring precautions

Monitoring various trees for different pest and diseases can lead to unwanted spreading by manipulation. To avoid this, the technician needs to fellow a pre-prepared course and work on one pest by day. At the end of each monitoring, the tools are sanitized and cleaned.

## Protocol for securing raw data and data processing

In the course of our work, the data collected in the field should be digitised according to the formats of the different protocols. Photography is desired and necessary for each case that presents interpretation difficulties. All the information must be stored with clear and accessible referencing, for future use and possible recalculations.

Raw data should be stored in a specific directory in files named by measure and day. They should not be modified. A copy of the raw file will allow the verify the data. They should be sent by email after every measurement to supervisors.

## Requested material

* Flat scan and test pattern (sheet and surface allometry)
* Bluetooth electronic callipers (diameters / thickness of organs) (Houssem Buy 2 units) amazon
* Precision scale (organ weighing)
* Digitizer (3D) for tree-top acquisition Houssem
* Information capture and storage tools (mobile phone or tablet, and laptop)

**Notes:**

The fruits are put in 3 categories:

- Small fruits

- Large fruits

- Harvested fruits (red)