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FruitRescue! - Apple phenotyping

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all suggestions are welcome !

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Disclaimer

This draft might change in the future, please notify us for any addition or modification you would like to share.

Abstract

The FruitRescue project aims to create a pipeline to predict fruit trees' genomic offset. To confirm the pipeline predictions, tree fitness parameters are measured in crop and wild tree orchards along a longitudinal gradient. Fitness is assessed through measurements of trunk diameter and the Nitrogen Balance Index (NBI), as well as monitoring phenological parameters such as bud break, flowering time, fruit set, and crop load.
This protocol is meant for Apple tree phenotyping.

Guidelines

Orchard Management

In each orchard, standard cultural management practices should be followed as typically implemented on the site. One exception is to be noted : to prevent influencing bitter pit, calcium spraying is not applied.
Each year, a summary of these treatments will be sent to Mathieu Brisson and Amandine Cornille.
During winter a pruning might be required between November and December.
In June, thinning by hand might be required. A maximum of 2 fruit per cluster should remain.

Data management

Throughout the years, you will collect a large amount of data. To ensure good quality and readability of the data when opening spreadsheets on your computer, it is crucial to be vigilant.
Here are some important guidelines concerning the data export step:

1. ***File Type***: Always check the file type and favor .tsv or .csv for single sheet documents.
2. ***Date Formatting***: Format the sheet's cells as text for dates in ISO 8601 (YYYY-MM-DD) format to facilitate reading across multiple computers and software.
3. ***Raw Data Backup***: Always make a copy of the raw data file before opening it.
4. ***Cell and Decimal Separators***: Check the cell and decimal separators before opening the file. For example, Dualex ".csv" output files contain data separated by semicolons, and opening the file carelessly could lead to the removal of the decimal separator.
5. ***Data Integrity***: It is better to reference the original file in another sheet rather than writing directly inside it.
6. ***Data Structure***: It is easier to understand the data when it is represented as a list rather than a map. Fill the given template with the data you have. If you have trouble filling it properly, leave it blank and contact the team who can provide you help.

After the data acquisition and eventual formatting, you will need to send it by mail to Mathieu Brisson, cc Amandine Cornille.

Materials

Caliper

Equipment	
S_Cal EVO Smart Caliper	NAME
Digital Caliper	TYPE
Sylvac	BRAND
810-1506	SKU
https://www.sylvac.ch/fr/produit/caliper-s_cal-evo-smart/	LINK
Max scale : 150mm, IP67, Bluetooth	SPECIFICATIONS

Dualex clamps

Equipment	
Dualex	NAME
Chlorophyll meter	TYPE
ForceA	BRAND
-	SKU
https://metos.global/en/dualex/	LINK

The optical chlorophyll meters used are the Dualex clamps (Cerovic. et al. 2012 ; https://metos.at/en/dualex/). For use, see: Youtube - Le Dualex - IFVSudOuest (https://youtu.be/Tfop94PvFDM.

Before start

Emails concerning the progress of the project should be sent to Mathieu Brisson cc Amandine Cornille.

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-
Amandine Cornille
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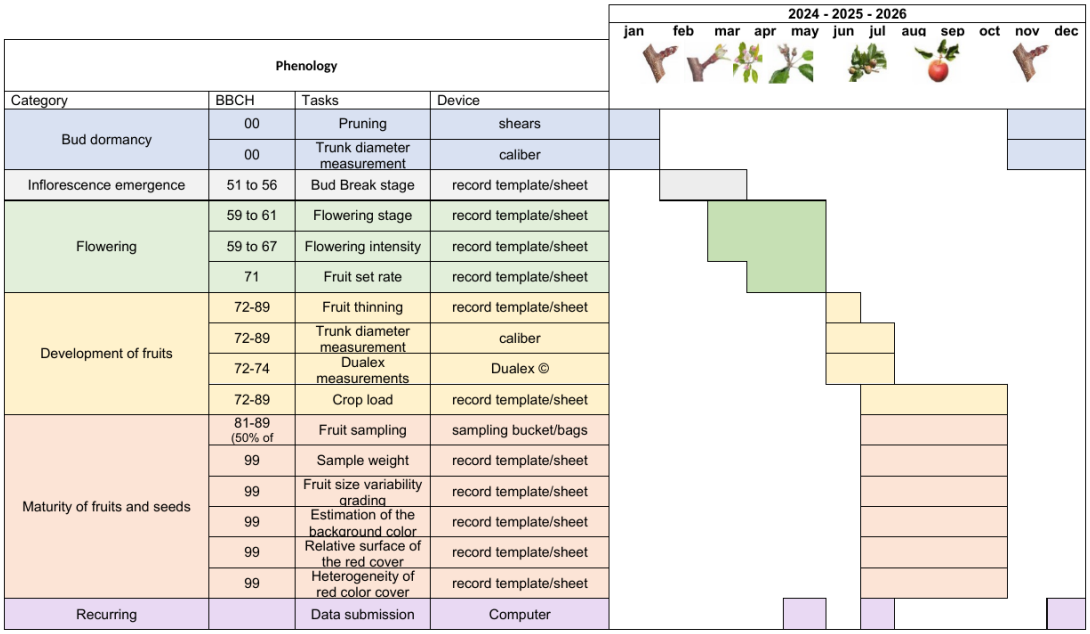


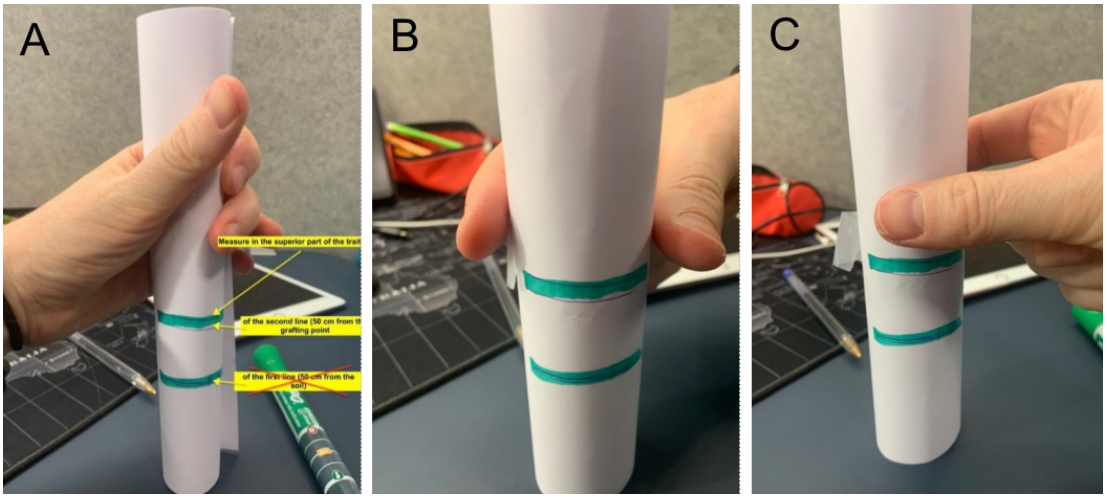
Phenology			
Category	BBCH	Tasks	Device
Bud dormancy	00	Pruning	shears
	00	Trunk diameter measurement	caliber
Inflorescence emergence	51 to 56	Bud Break stage	record template/sheet
Flowering	59 to 61	Flowering stage	record template/sheet
	59 to 67	Flowering intensity	record template/sheet
	71	Fruit set rate	record template/sheet
Development of fruits	72-89	Fruit thinning	record template/sheet
	72-89	Trunk diameter measurement	caliber
	72-74	Dualex measurements	Dualex ©
	72-89	Crop load	record template/sheet
Maturity of fruits and seeds	81-89 (50% of	Fruit sampling	sampling bucket/bags
	99	Sample weight	record template/sheet
	99	Fruit size variability grading	record template/sheet
	99	Estimation of the background color	record template/sheet
	99	Relative surface of the red cover	record template/sheet
	99	Heterogeneity of red color cover	record template/sheet
Recurring		Data submission	Computer

Trunk diameter

1 The trunk diameter is an indicator of the tree vigor (Waring 1987). Genetic information as well as environmental cues including abiotic and biotic stresses can modify the source-sink relationships and therefore modify the trunk growth during the year. Tree adaptation to its location can thus be assessed through this measurement.

1.1 For grafted trees, a white mark is placed 50cm above the grafting point with marking spray. For ungrafted trees, the mark is placed 50cm from the ground.

1.2 After cold hardening, during winter, and 1 month after flowering during summer using a digital caliper, measure the trunk diameter on the uppermost part of the white mark :
make a first measurement then a second at 90°.



Measurement of the trunk diameter.
(a) measure of the diameter above the grafting point (grafted trees might have two marks).
(b) We measure the trunk at a given angle (fingers acting as the caliper) then we measure 90° from the first measurement (c).

Dualex

2 1 month after the end flowering, in general from the end of May to the end of July. The Dualex measures Chlorophyll and Flavonoid content and enables the calculation of the Nitrogen Balance Index (NBI). These elements are used to assess the tree photosynthetic activity and nitrogen nutrition (Cerovic et al. 2012).

2.1 From the base to the top of the tree, the leaves are randomly chosen among those most exposed to light. The dualex measurements are done on the upper side of the leaves. 10

measurements should be done. When trees don't have enough leaves, take notes of the trees with not enough leaves.

2.2 There are some points of vigilence when taking measurements with the Dualex :

- ID your Dualex if you use more than one.
- Write down Line and Number of each tree associated with the group given by the Dualex (and eventually Dualex ID).
- Write down the reason of a missing measurement (not enough leaves, tree death...)
- Always look at the Dualex screen after each measurements to check on the integrity of the data (no star (*) should appear on the screen).

Do not open a Dualex ".csv" file without having a copy : opening it without the right settings can remove the decimal separator of the data. Do not attempt to modify the files : use a proper script or macro to summarize the results and use a secondary file to describe the Date, Group, Dualex ID, tree position and tree ID.

Phenology

3 **Bud break / Bud burst**

After cold hardening, buds become dormant. Entering in endodormancy, buds are in a stage of physiological inhibition of bursting (Lang, 1987). Only heat accumulation can lift this stage of growth inhibition, even in favorable conditions, switching the buds to their ecodormant stage. During the ecodormancy, buds are physiologically capable of sensing their environment but the bud break is repressed until conditions are favorable (Fadon et al. 2020). The lifting of the ecodormancy encompassing the bud swelling and burst stage is therefore a fitness trait or an indicator of local adaptation (Anderson et al. 2012)

3.1 For apple trees, the burst usually occurs in february. Measure trees at stage 07/53 of the BBCH scale corresponding to stage of bud burst (Bagglioni B/C stages).
Head twice a week to the orchard and record the stage of all trees.

4 **Flowering and fruit set**

Flowering is a critical step for reproduction success. Its timing and its intensity has long term effect on multiple life history trait (Pallas et al. 2018). Genetic, physiological and environmental factors are thus controlling this complex set of traits that can have a long term effect on the survival and reproduction of trees . Flowering Time intertwined in this complex set of characters is therefore a critical indicator of tree fitness in regard of climate change (Anderson et al. 2012).

4.1 Flowering Time

From march to may, apple trees start flowering. Measure trees at stage 59 to 61 of the BBCH scale (Bagglioni E2/F1) when 10% of the flower have bloomed.
Head twice a week to the orchard and record the stage of all trees.

4.2 Flowering intensity

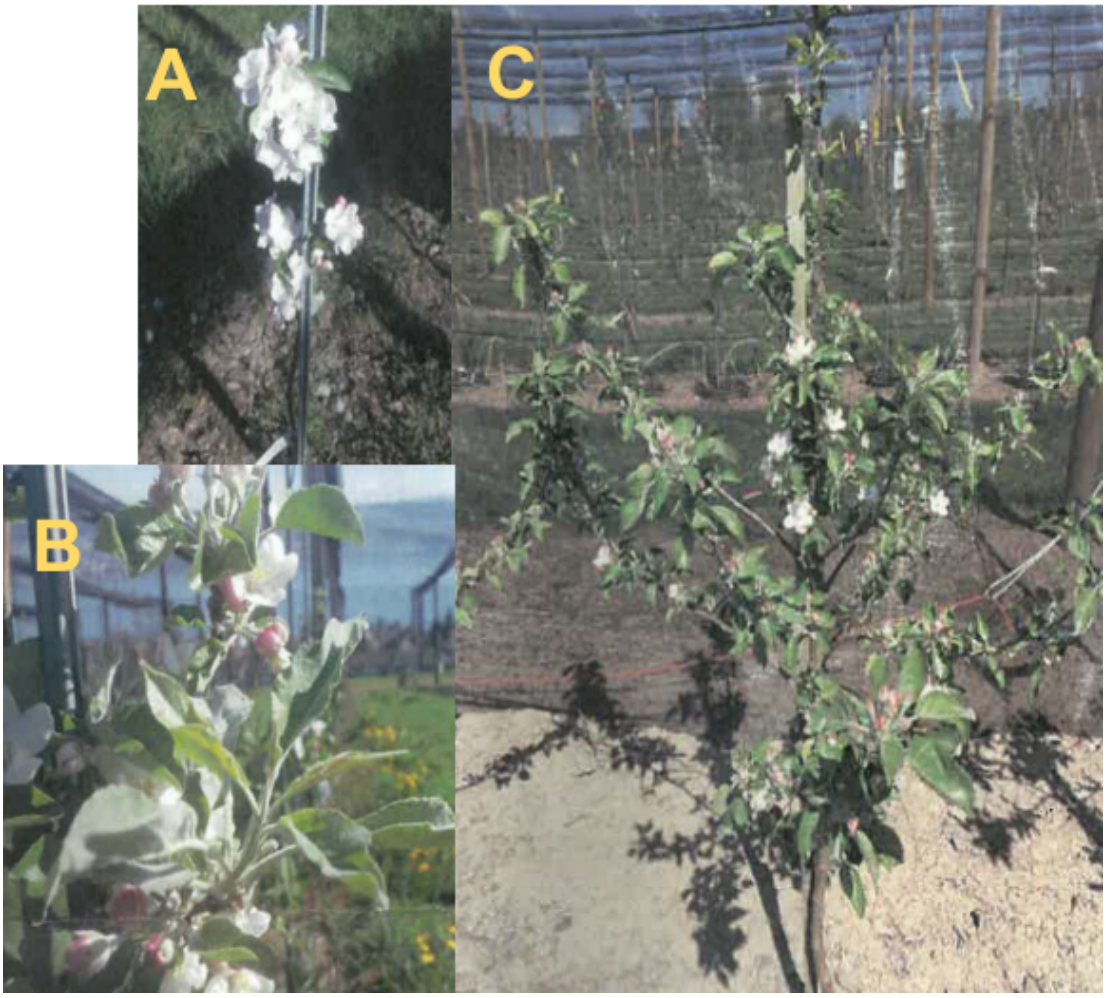
Flowering intensity or floribundity will be recorded as the number of bloomed flowers over the maximum number of possible flowers (%). The maximum number of possible flowers is set when all the buds have flowered. The trees are graded when they reach 50% of Flowering and it

can be then re-evaluated at 100%. The most important thing is not to look at the flower number but to see if the flower distribution on the tree is according to the number of buds.

1- 0%
3- 25%
5- 50%
7- 75%
9- 100%

Examples:

- A young apple tree sprouts 4 new leaves. Each sprout then produces a flower; the intensity would be 100%.
- Another tree also grows 4 sprouts but only half of the tree's top flowers; the intensity will be 2 or 12,5%.
- If the top of this same tree flowers entirely, then the intensity is 5 or 50%.
- A very small tree with a single sprout that sees its top part entirely flowering is 9 or 100%.



Further information on Flowering Intensity :
A. Small tree intensively blossoming : FI=9
B. This tree looks like it is intensively blossoming but a cluster in the foreground bears no flowers. This position has to be counted. FI=7
C. At first sight this tree doesn't seem to be intensively flowering but almost all clusters are bearing flowers : FI=8

4.3 The fruit set rate

When pollination is successful, flowers will develop into swollen, immature and very small green fruits but when unsuccessful, the flowers that did not receive enough compatible pollen will not develop into a fruit and look like small green stars.

The number of fruits are counted 2 to 3 weeks after flowering (=mean fruit number by clusters after abscission). This fruit set is the quantitative measurement of the proportion of flowers developing into fruits.

This will allow to calculate the percentage of fruit set. This is the percentage of flowers that become fruit.

% fruit set per tree = (# fruit / # Flowering buds)* 100

4.4 Crop Load

Environmental and Internal cues can also lead to fruit drop after pollination, diminishing the total fruit set in the weeks following pollination and establishing the tree's crop load. Estimation of the crop load is done after the June fruit drop but before hand thinning. It is expressed in percent of present fruits compared to the maximum possible number of fruits. The tree express its maximum possible number of fruits when bearing fruits on each buds. We use the following scale :

1 = 0% of fruit set meaning 0 fruits on the majority of the tree's cluster
2 = 1 fruit per cluster for most of them
3 = 25% of fruit set meaning most cluster bear 2 fruits
4 = 40% mix of 2-3 fruits per cluster but a majority of 2 fruits per cluster
5 = 50% of fruit set
6 = 60% 3 and 4 fruits per cluster but a majority of 4 fruits per cluster.
7 = 75% of fruit set 3, 4 et 5 fruits per cluster
8 = 4-5 fruits per cluster depending on the tree
9 = 100% of fruit set, all the fruits have developed on all cluster

Fruits

5 Fruits play a crucial role in the maturation, protection and the dispersal of the seeds and thus the effective reproduction of the mother plant. Fruit pigmentation can be a protection against stresses (Espley and Jaakola 2023) or a signal perceived by animals feeding on them in order to disseminate the seeds (Steyn, 2008). Furthermore the fruit is also a major sink thus reflecting resources allocation and overall tree fitness.

5.1 Fruit sample weight

When apples are ripen 15 fruits are sampled. The fruits sample weight is measured with a weighting scale. The weight is written in grams.

5.2 Fruit sample size

The size of the fruits is determined by measuring the width and the height of the fruit.

5.3 Fruit sample size variability among the sample.

0- The sample is more homogeneous (more than 3-4 fruits seem to have a similar size)
1- The sample is more heterogeneous

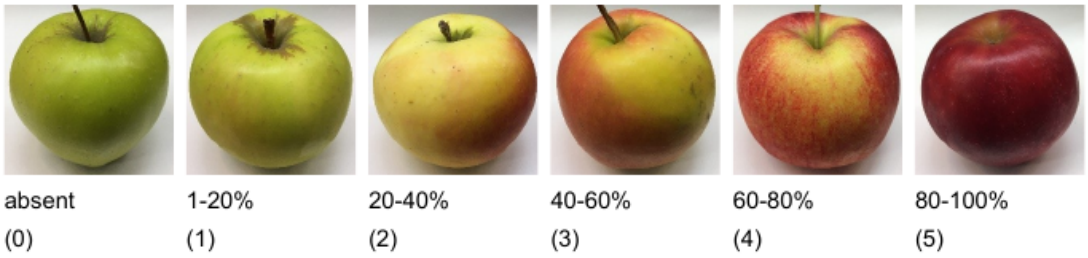
5.4 Fruit background color

The fruit background color is estimated by using a scale from 0 to 2 on average by tree.

Scale for the measure of background color.

5.5 Relative surface of red color cover on the background

Estimation of the % of surface red color above the background color, given by a scale from 0 to 5, mean by tree.



Scale for the measure of the relative surface of red color cover.Scale for the measure of the relative surface of red color cover.

5.6 Heterogeneity of the red color cover

Grade the color diversity intensity among the fruit sample either homogeneous (0) or heterogeneous (1).

Protocol references

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