Nov 22, 2019

# Flavonol, Anthocyanin, and Chlorophyll Indices using a Force-A Dualiex Scientific+

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1 Works for me dx.doi.org/10.17504/protocols.io.u6yezfw

TERRA Reference Phenotyping Platform

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ABSTRACT

This protocols describes the use of a hand-held sensor to estimate polyphenols and cholorphyl content of leaves based on absorbance and transmitance of specific wavelenghts of visible and near infrared light.

The instrument does not directly measure these chemical concentrations, but measures reflectance and absorbance indices that have been demonstrated to relate to leaf level concentrations.

We use it to measure Chlorophyll, anthocyanin, and a nitrogen balance index.

Chlorophyll Concentration (µg/cm$^2$): The instrument is factory calibrated to measure leaf chlorophyll concentration.

$$\mathrm{Chl} = \frac{\mathrm{NIR}-\mathrm{Red}}{\mathrm{Red}}$$

Flavanol Index (Flv)

$$\mathrm{Flv} = \log \frac{\text{NIR fluo excited red}}{\text{NIR fluo excited UV-A}}$$

Anthocyanin Index

$$\mathrm{Anth} = \log \frac{\text{NIR fluo excited red}}{\text{NIR fluo excited green}}$$

Nitrogen Balance Index (NBI): a relative measure of nitrogen status

$$\mathrm{NBI} = \frac{\text{Flavanol Index}}{\text{Flavonol Index}}$$

Note that hte instrument documentation does not provide specific wavelenghts, only approximate wavelengths in this diagram (from Dualex Brochure.pdf):



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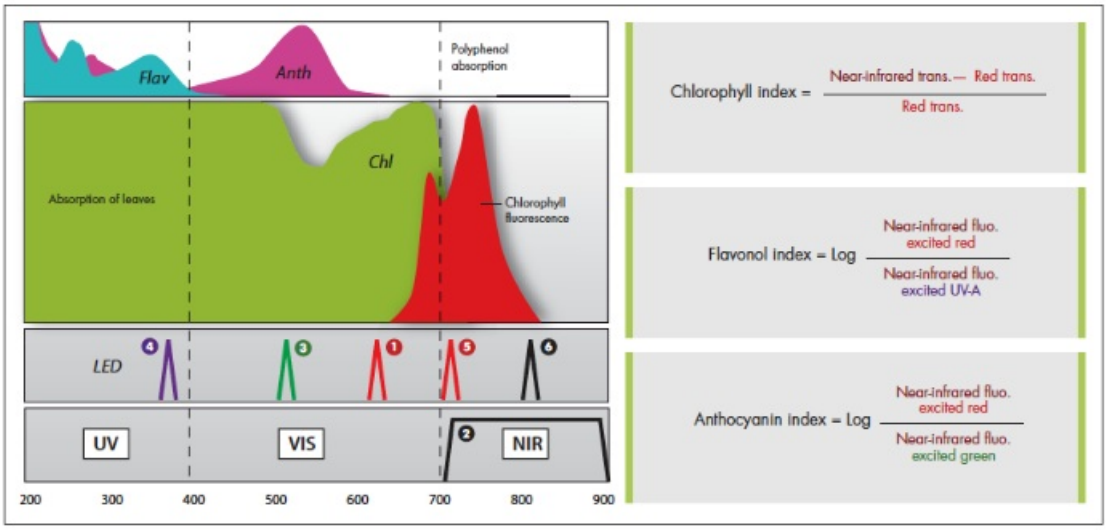
## POLYPHENOLS measurement

Near-infrared chlorophyll fluorescence ❷ is measured under a first reference excitation light ❶ not absorbed by polyphenols. It is compared to a second sampling light specific to a particular type of polyphenols (e.g. green ❸ for anthocyanins or UV-A ❹ for flavonols). Only a fraction of this light reaches the chlorophyll in the mesophyll and can generate near-infrared fluorescence.

## CHLOROPHYLL measurement

The leaf chlorophyll content can rapidly and accurately be assessed from light transmission. A first wavelength very close to the red ❺ quantifies the chlorophyll and a second in the near-infrared ❻ can take into account the effects of leaf structure.




THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

References Cerovic, Z.G., Masdoumier, G., Ben Ghozlen, N., Latouche, G. (2012) A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. Physiologia Plantarum, ISSN 0031-9317.

Dualex Brochure.pdf

Dualex_Manual.pdf

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Dualex Scientific | View | |

MATERIALS TEXT

Dualex Scientific Brochure:

Brochure-DUALEX®-SCIENTIFIC-NEW (1).pdf



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BEFORE STARTING

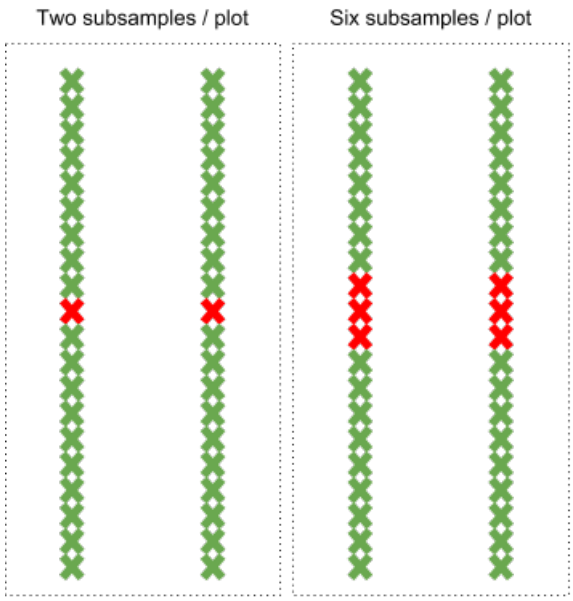
Make sure that the instrument battery is fully charged.

## Take measurements

1 For each two row plot:

For two subsamples per plot: Identify the 1-3 replicate plants per row to measure. For a standard 3 m row, this would be the plant closest to 1.5m from the end.

For six subsamples / plot: Identify the center plant and two adjacent plants in each of the two rows




## Identify the youngest fully expanded leaf on each plant

2 On each plant, the youngest, fully expanded leaf should be measured. To determine if a leaf is fully expanded, observe that the leaf is attached at the ligule; a leaf that is not fully expanded will still be partially wrapped around the stem.



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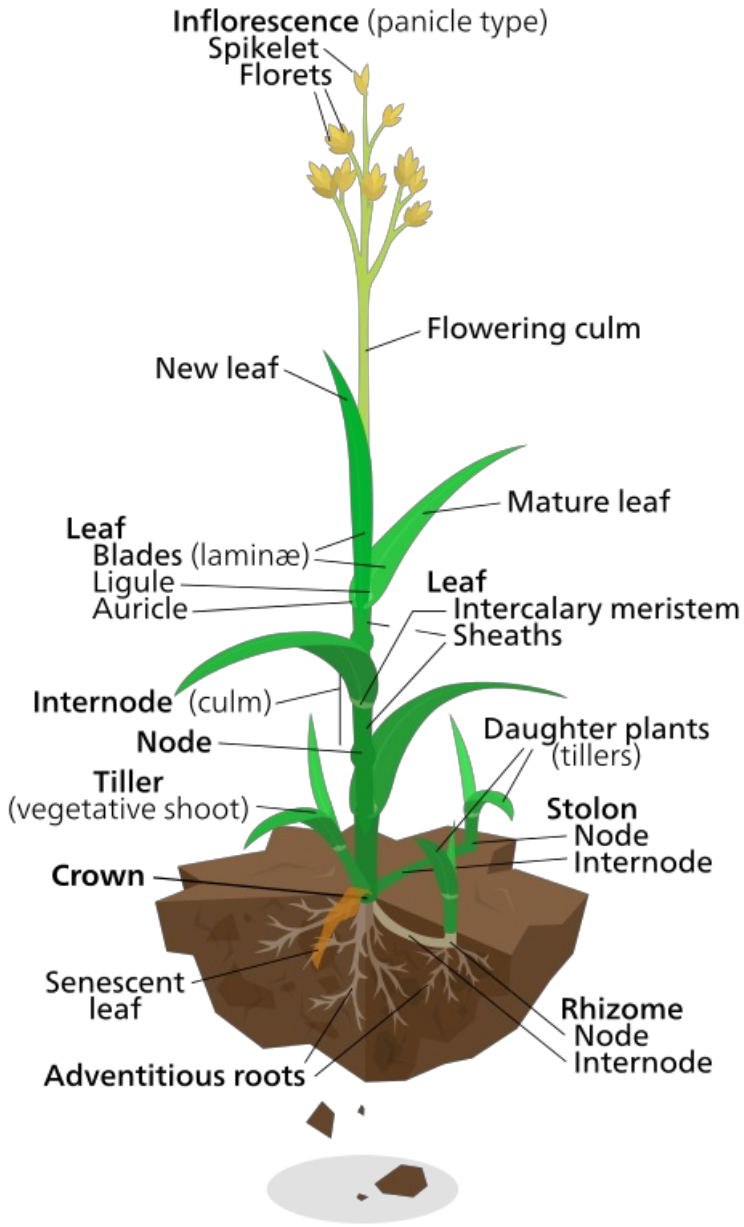


Image from Wikipedia CC-By 3.0 Kelvin Ma



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## Identify a sunlit, horizontal location on each leaf to measure

3 The measurement should be taken on a part of the leaf that is
- sunlit
- approximately horizontal to the ground
- approximately halfway between the leaf rib and edge
- free of disease or herbivory.


## Attach Dualex and measure

4

DUALEX SCIENTIFIC+™ Chlorophyll and Polyphenol-Meter
Handheld Sensor
**Dualex E-B90-DUALEX4**

- Clip Dualex sensor to leaf
- Click "OK"
- Instrument will collect measurements

## Record plot name and measurement number

5

## Format and upload data

6 See Data Entry documentation for BETYdb

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