Design and set up of a plant growth chamber for stable isotope labeling to investigate carbon attraction toward fruit sinks and plant reserves upon and after drought stress

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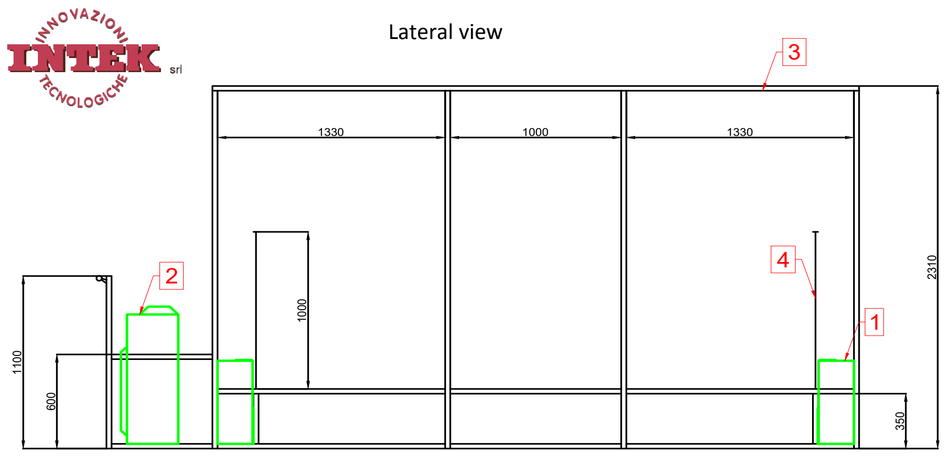
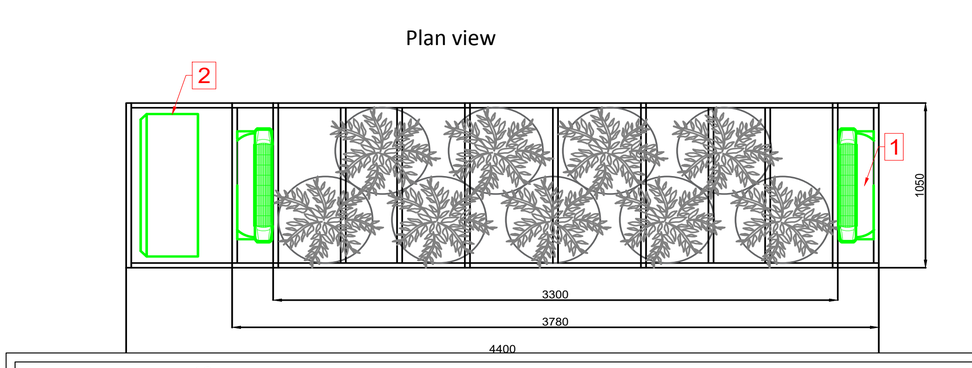
D. L. Patono[1], D. Said Pullicino[1], A. Firbus[1], A. Ferrandino[1], G. Gambino[2], L. Celi[1], Claudio Lovisolo[1,2]
[1]Dept. Agricultural, Forest and Food Sciences, University of Turin, Grugliasco, Italy
[2] Institute for Sustainable Plant Protection, National Research Council, Turin, Italy

Grapevine is a plant that adapts to periods of drought, which are correlated with berry quality.
In perennial plants, the root orchestrates the defense adaptations to drought, acting as a sink of the carbon (C) allocated during growth slowdowns, C which can floematically be released in the post-drought periods. In crops oriented to fruit production (and in this case in the grapevine) the root sink competes with fruits in receiving photosynthates during the growing season, and the competition increases with water stress.
In addition, the current climatic variations lead to the alternation of extreme events with periods of drought. The atmospheric CO_2 concentration, the triggering factor of the greenhouse effect, is constantly rising, and the C cycle in plants could partly mitigate the effect, but the high temperatures affect both photorespiration and C dynamics in plant and soil.

We aim to study C allocation kinetics in grapevine organs in a controlled water deficit system basing on studies of C allocation, trough pulse-chasing isotopic strategy. The isotope acts as a tracer of the floematic flows that are oriented towards different sinks during drought/rehydration cycles.
Grapevine fits well the subject of investigation, especially in relation to two well-known and deeply studied factors:

i) root: grapevines (*Vitis vinifera* L. cultivars) in commercial vineyards are grafted on different rootstocks, belonging to pure or hybrid genotypes of the genus *Vitis*. This confers to root systems a wide spectrum of vigour, water relations, and strategies of resistance/resilience to water stress.
ii) fruit: grape berries belong to hundreds of cultivars, spread all over the world. Both fresh consume (table grapes) and berry to win transformation (oenological viticulture) point to maximize fruit quality, driving in-field ripening dynamics toward accumulation onto berries (skin, pulp and seeds) of sugars and secondary metabolites. This to improve taste and flavor of either fresh berries or their derived wines, and to protect them from detrimental reactions happening during fruit conservation, oenological technologies, and wine ageing.





Design of the ^{13}C labelling chamber
[1] Fan coil unit Sabiana type mod. Futura FSC, tot coil power 2,15 kW.
[2] Refrigeration unit Carrier type mod. 30 AWH 006, tot coil power 4,73 kW
[3] Polycarbonate panels anti-UV treated, thickness 6mm.
[4] Deflector made of polycarbonate anti-UV treated, thickness 6 mm.
System design and installation made with INTEK Innovazioni Tecnologiche srl

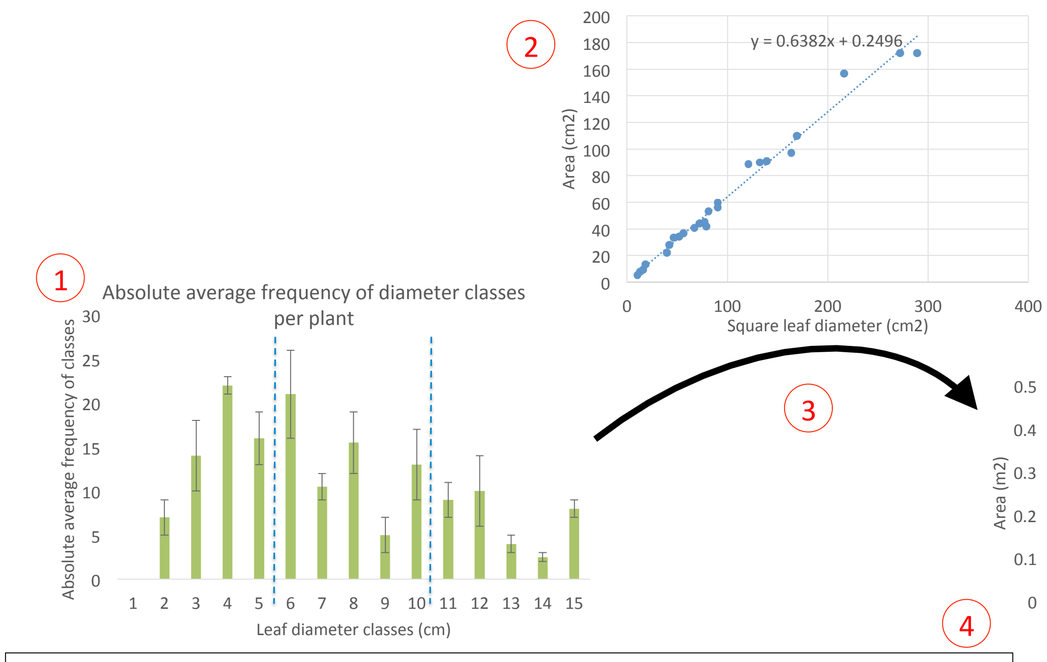
Open questions:
- How are neosynthesized sugars translocated at different rates depending on the hydration status of the plant?
- Are these sugars during stress periods redistributed following different ratios toward different sinks?
- Is quality of the berry related to variations in delivery dynamics of sugars during water stress?
- Is rate of respiration of sugars different depending on whether or not water stress is present?

Experimental set up
Three treatments x three replicates (irrigated controls IRR, drought stressed plants WS, and re-hydrated recovering plants REC) simultaneously in the ^{13}C labelling chamber (= 9 plants in the chamber).Three plants with optimum hydration act as a control for both the water stress line and the recovery line.

All plants during the translocation time (period following the pulse of ^{13}C) are all exposed to the same environmental conditions, minimizing environmental (solar radiation, temperature, air humidity) influence on C translocation rates.

At the end of the ^{13}C pulse: leaf sampling to know the amount of $^{C}13$ assimilated by the square meter of leaf surface.
Time kinetics: Sampling leaves, berries, roots and soil at subsequent time points to understand deliveries of C toward sinks.
Once a maximum of ^{13}C has reached the various districts, by repeating sampling it is possible an estimation of ^{13}C leaving the district (respiration /re-translocation).

Results of preliminary investigation for setting air fluxes into the growth chamber for stable



(1) The longest diameter of every leaf per plant was measured and reported in this graph. The dotted lines represent following grouping of diameter.
(2) Regression area/square diameter for leaves of grapevine cv Barbera.
(3) By combining absolute average frequencies of diameter classes with relative leaf area it is possible to calculate total leaf area of the plant.
(4) The total area of the three groups of diameter were counted separately as representing different leaf development stages, corresponding to different photosynthetic activity.
(5) Ambient PPFD can be tuned artificially with LED lamps to allow appropriate light intensity inside the chamber.
(6) Net photosynthesis at different light intensity of the three groups were measured to allow prediction of photosynthetic rate inside the chamber.
(7) By combining total leaf area of grops with light intinsity trend of the chamber and photosynthetic rates of groups is possible to predict total CO_2 assimilation per plant, **in order to design experimental pulses.**

Photosynthetic assimilation, stomatal regulation and respiration are checked in the various phases to size $^{13}CO_2$ enrichment flows in the chamber. Maximum assimilation ranges from 6 to 13 µmol m^{-2} s^{-1}, reduced possibly by stomatal control 3 o 4 times at the end of the drought period. Light responses are evaluated to optimizing chamber illumination. A model optimizing light and temperature is possible for plants in the various stages of the experiment.
In the same sinks taken for isotopic analysis, the expression of genes involved in carbohydrate transport will be investigated. Genes encoding proteins that regulate the delivery of sucrose to the sinks and which catalyze the hydrolysis of the sucrose discharged to trigger respiration or C storage will be analyzed.

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