Carbon isotope descrimination - part I - trapline - v0.1

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Abstract

Protocol for collecting gas for measurement of carbon isotope descrimination during photosynthesis.

See the following references for more background:
H. Griffiths (1993) Photosynthesis and Production in a Changing Environment pp 181-1927
Farquhar et al. (1989) Annu. Rev. Plant Physiol. Plant Mol. Bioi. 1989. 40:503-37

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Protocol

Setting up IRGA

Step 1.

Set the following environmental variables in the LICOR LI-6800

CO2S	400 ppm
Txchg	25 °C
H2OR	10-14 mbar (aim for Tleaf 25 °C)
PAR	2000 (100% Red)
Flow	250 ml min^{-1}
Fan speed	14500 rpm
deltaP	0.1 kPa

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Control temperature on block (Txchg), not leaf otherwise photosynthetic rate will start to oscillate due to constant adjustment of stomata.

TLeaf should ideally be within +/- 0.03oC of 25oC as measurement is temperature sensitive. 25oC is the value that C3 photosynthetic calculations are paremeterized for. You can manipulate with humidity or Txchg on reference

Preparing plant for measurement

Step 2.

- Clamp leaf in cuvette, under a bright ambient light.
- Adjust H2OR so that Tleaf is around 25 °C. This will need adjusting when leaf is reaching stability in the cuvette.

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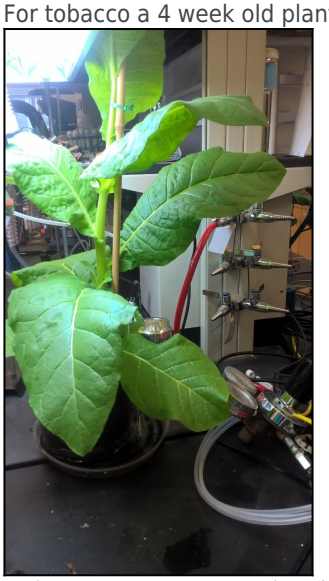
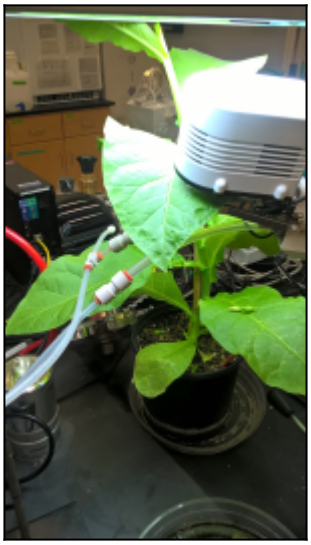
For tobacco a 4 week old plant prior to flowering is in the ideal state (health example below).



Make sure not to stress the plant by introducing tension when clamping.

Illuminate from above (at least 200µE), this makes sure the surrounding leaf tissue is photosynthesising to reduce chance of diffusion of metabolites influencing measurements, this is more important for homobaric leaves such as tobacco.

It helps to let the plant acclimate in the lab for ~24h prior to measurement.



Wait with measurements and CO_2 trapping until complete steady state has been reached.

- Assimilation rate (A) and steady state fluorescence do not change over time (10 min window)
- Stomatal conductance (gs) does not change over time (10 min window)

This can take ~2h depending on the plant. Check regularly (~every 15 mins after the first half hour)

Note that mesophyll conductance can be altered by a whole range of factors (cell size, leaf expansion, age of leaf etc etc) so care should be taken in performing comparisons and should try to keep to plants at a consistent developmental stage and growth conditions.

Step 3.

When plant has reached a steady state switch inlet gas to 2% oxygen. Use a flow controller to moderate gas use, setting flow to 6.

Change the constant value in LICOR

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2% O2 is used to suppress photorespiration simplifying downstream calculations of carbon flux. Note this will change assimilation and photosynthesis values which will take time to stabilize again, you may witness oscillations in A and F which will dampen down over time.

When there is no flow the value should be ~0.5 or less

If the cylinder is running low order a replacement, always keep a backup cylinder to avoid running out of gas

Setting up prepline

Step 4.

Turn on cold finger

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This will be used to cool down acetone to below -70oC as required for sublimination of frozen CO2

There is only an ON/OFF setting.

Step 5.

Place cold finger in dewar of acetone and wait to cool down (>-70oC), it should turn milky white

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Cover the top of the dewar with foli to prevent ice forming and falling into the acetone. Do not rest the finger on the bottom of the dewar otherwise an ice lump forms and it will stick

Step 6.

Check temperature of acetone is below -70oC

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Step 7.

Fill two dewars 3/4 full with LN2

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These will be used to condense gases for sample collection. This can be done whilst waiting for the acetone to cool down

Step 8.

Open glass valves and wait until vacuum gauge reads around 5.5 mbar on each prep line. Adjust by opening/closing pump valves if required.

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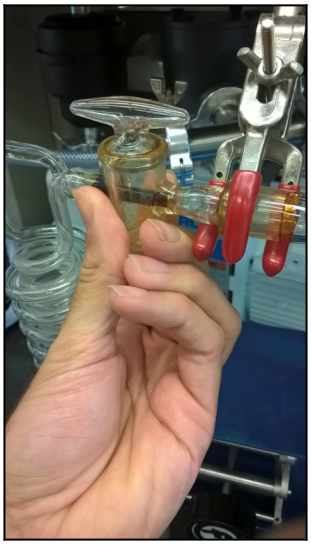

Provide support from below when opening/closing valve. Apply downward pressure while twisting to minimize the chance of introducing leaks (this is the most common source in the system).

Adjust the vacuum pressure by opening the valve to the pump (red arrow). This ensures that approximately the same amount of gas is collected for reference and sample and ensures consistency between measurements

Step 9.

Check that both collection tubes are open

Step 10.

Check if leaf is still in stable state (both A and gs)

Start Measurement

Step 11.

Match LI-6800 manually

Check settings

- automatch OFF
- Fluorescence OFF

Step 12.

Immerse both traps in LN2 (2 dewars)

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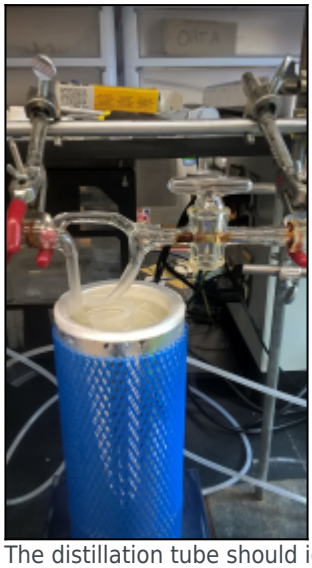

The distillation tube should ideally be covered close to the top

Step 13.

Set timer and start logging every minute min for 5 min (LI-6800, autoprogram)

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Step 14.
Take fluorescence measurement (Fm'Fs)

Step 15.
Close the glass valves, watch the vacuum drop to low 10-2 or high 10-3E.

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Twist perpendicular to the flow direction otherwise there can be leaks, check the pressure gauge

Step 16.
Close the pump valves.

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Step 17.

For each prep line (one after the other) make sure the collection tubes are open.

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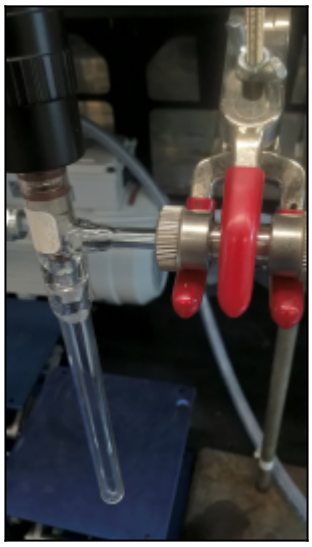

Step 18.

In a single motion remove distillation tube from LN2 and submerge in cold acetone to subliminate gases

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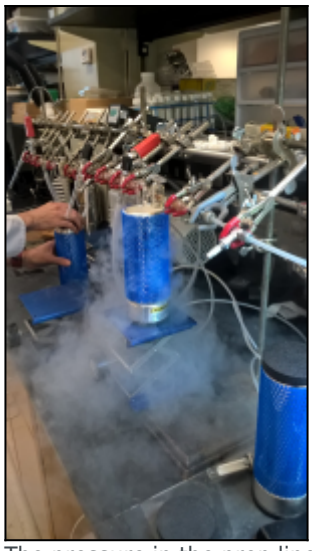

The pressure in the prep line should rise as gas is released

Step 19.

Quickly submerge the collection tube in LN2 to condense the released gas

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The pressure should fall again as gas is drawn down into the collection tube. By following this procedure it is possible to collect gases and remove the water vapour from samples which would interfere with measurements

Collection tube should be dipped in LN2, covering at least the bottom 2cm

Step 20.

Close collection tube

Step 21.

Remove acetone and liquid N dewars

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When the vacuum stabilizes all gas has been condensed again, this is the point when you can close tube and remove from LN2

Collect drips of acetone from distillation tube in beaker

Step 22.

Remove collection tube from prep line and leave to thaw in rack

Put new (empty and open) collection tube in place and open pump valve

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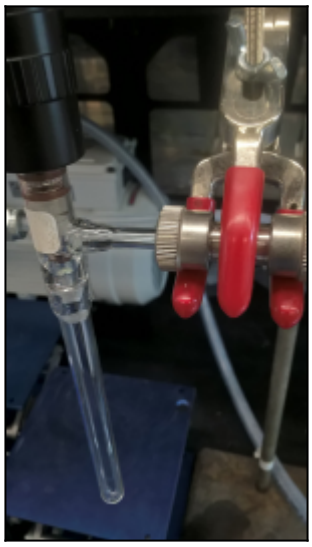

Opening the pump valve cleans out the prep line and residual air in the new collection tube

Step 23.

Close low O2 cylinder and adjust constant value in LICOR