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**Advice for future sap flow students!**

Paper that gives a good introduction to sap flow: Granier, A. (1987). Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. *Tree Physiology* 3: 309-320.

Paper that explains why we are interested in studying sap flow in wollastonite addition plots: Green, M.B., et al. (2013). Decreased water ﬂowing from a forest amended with calcium silicate. *Proceedings of the National Academy of Sciences* 110(15): 5999-6003.

Supplies: You will need ~**twelve, 12 V batteries** (we did fine with a few less at some points). We used 4 batteries per plot that lost charge slowly over several days (some lasted a week or more). We would check every two or three days with a **voltmeter** (you will need to be out to make sure the probes are all working every couple of days, so while you're there just check the batteries). When they started getting low we would swap in one or two fully charged batteries. We used **four chargers**. You will need **at least three Campbell data loggers** if you are measuring one site at a time: one for each plot (Ca and control plot), and one to keep in the lab for testing probes. You need a specific **USB cord** to connect the data loggers to your computer to view the measurements being taken (important for making sure the probes are working) and download data. Make sure your laptop is fully charged and holds battery life for three hours or more. In the field you will also need **battery cases** and **wires** to connect batteries to logger, **sensors** attached to **grey wire cables, drill, 1/8 in drill bits** (bring extra), **wire cutters, wire strippers,** extra **aluminum shields** and **heat sink** for sensors, **staple gun, staples, electrical tape, masking tape, tiny flathead screwdriver, flagging tape** for marking trees, **ruler, permanent marker, mylar covering, compass, scissors, large garbage bags** and **bungee cords** for protecting logger box and batteries. ( + other things you will need that are bolded throughout the rest of the document).

**Software** needed to download data from the datalogger can be found at <https://www.campbellsci.co.uk/pc200w> You also need a **driver** for the USB connection, but I can't seem to find it on the Campbell website. On my computer, the file name is PL2303\_Prolific\_Drivers, and the USB cord is called USB-RS232. Also, a **computer with relatively long battery life** is needed (at least 3 hours), so when you are setting up and troubleshooting in the field you don't have to worry too much about conserving power.

Things to do in the lab before setup in the field: Make sap flow probes and connect to **gray wire cables of varying lengths**. LONG cables (20+ m) were needed most and seemed to be more likely to not work, so make sure to have many. Scope out the site and estimate what length cables you will need (how far are the trees from the data logger cage including going up the tree and up to the cage). Make a lot more than you will need. Even though you will test them in the lab before you go out, some still won't work in the field, others will stop working after a couple days in the field, etc. For example, say you have 9 trees and 2 need long cables, 5 medium (10-15 m) and 2 short (5 m), make something like 6 long cables, 15 medium, and 4 short, since you can always use the medium cables for trees that are close to the cage.

We were shown how to make sap flow probes by Michele Pruyn and others who worked in her lab. The heating probe and reference probe are slightly different. There may be instructions in a sap flow document in the lab... I'm having a hard time remembering the exact procedure. Hopefully you can find that document and also talk to someone who knows how to make them. Maybe I would remember if I had the supplies.

Supplies needed to make probes: **steel medical needles, thermocouple wire** (two different types, not sure what they're called), **razors** to strip wire, **wood glue, soldering iron, aluminum sheaths**.

Before soldering the probes to the gray wire cables, you will hook up the probe to the gray wire just by twisting wires together, and then hook the gray wire up to the logger to test that the probe and wire are working together. If you see a reading on the computer of ~0.3-1, and can feel the heating probe heating up, everything is working, and the probe can be soldered to the gray wire, then wrapped carefully in aluminum foil (just the probe part) and wound up for storage.

Wiring: Wiring in the grey cable wires is as follows: white in H, green in L, red and black on the heating board. Where you put in red and black will depend on how many sets you are wiring in. The heating board is in sections of six terminals... if you have only one set of red and black wires, you will put them in positions 1 & 6 respectively. If two sets, first red in position 1, first black in 2, second red in 3, second black in 6. If three, first red 1, first black 2, second red 3, second black 4, third red 5, third black 6. It's very easy to get this wrong and it will mess up readings. You should do the wiring with your computer on and hooked up to the logger via the USB, so you can check whether you are getting readings that make sense as you wire them in (in the field or in the lab). If reading is negative, could just be that green and white wires are switched. If that's not the case, then check to make sure the red and black wires are in the right positions. If everything seems to wired correctly, but you're still not getting a reading, try switching the wires to a different volt that you know has worked in the past if you can. If all six positions are occupied by red and black wires, try moving to a new one and using only the red and black wires in question in positions 1 and 6, because occasionally the heating board can get overloaded if all six positions are filled. If you're still not getting a reading, the problem might be with the probes or the cable, or the connection between them. Check the to make sure the probes and cables are still connected correctly. A reading of 0 means that the heating probe is either not heating or the temperature difference is not being read correctly, so check to make sure everything is wired right and feel the heating board thing (not sure what it's called!) that corresponds with the section of terminals that you wired the red and black wires into. If that is heated, but there is still a reading of 0 then the problem is probably with the heating probe. NAN means the logger is not measuring anything, and that could be a problem with the logger, the cable, the sensors, or any combination! It may take a lot of troubleshooting to get everything hooked up correctly with accurate readings (~.3-1.0)

Setup in the field: the first things to do are to get the data logger hooked up to the batteries, and to have people start flagging/labeling the trees to be studied and drilling holes for the sensors (sensors/probes, same thing). Choose a side of the tree to insert probes (south facing, north facing, etc.) and be consistent with all trees. When picking a side, avoid any holes from previous years if using the same trees because they might interfere with sap flow. When inserting probes into the tree, make sure each one has an aluminum sheath. If you need to add an aluminum sheath to a probe, don't forget to dip it in some of the heat sink first (these are things you'll understand from making the probes). Other people can start laying out cables. Make sure to label the cables at the end near the logger with some masking tape so you know which tree they are coming from, so you can keep track of which trees are in which logger diffvolt position, and thus which data column when you download data (the data logger doesn't record anything about the trees). You can start wiring in cable wires once you know which cable will be coming from which tree, it doesn't matter if the probes are in the tree or not. You can decide if you want to do all the wiring with the logger box on the ground, or in the wire cage that elevates it off the ground. Make sure you feed the wires from the cables through the hole in the logger box, and not through the top where the lid will close. If you do it on the ground, the wires will have to be sort of folded under the logger box when you put the whole thing in the cage, but if you first put the box in the cage you can then feed the wires up through the mesh of the cage (this might not make sense now but hopefully will once you get there). When everything seems to be reading right, cover each probe with electrical tape, just to help ensure it won't fall out (stretch tape over the sensor and tape to the tree on either side), and then cover the probes with a mylar sheet, and staple to the tree. Double check that you are still getting readings that look good before you pack everything up, secure the logger box in the cage with bungee cords and cover that and the batteries with garbage bags.

Converting data: I have a word document from Michele Pruyn that details all the steps that must be followed in order to convert the raw data (which downloads as DAT files) to Excel, and then also to organize the data in Excel to a format that can be read in Baseliner software to convert temperature difference to sap flow, and then that is eventually converted back to a comma delimited DAT file (but save separate files through all steps of the conversion process). That document explains it pretty thoroughly. You will need to download **Baseliner software**. In Baseliner, you will import the DAT file for a particular plot, and choose to convert only the tree column headings (check "do not convert" for everything else). When it opens up, choose any tree from the drop down menu where it says "Top," and readings plotted against ordinal date and time should appear (there should be some characteristic fluctuations, peaking in the early morning, low during the afternoon). You can choose batt\_volt from the drop down menu for "Bottom" to make sure that any funky patterns aren't correlated with a dying battery. For each tree, choose a spot at the flattest, highest point of temperature difference, usually between 1:30- 3:00 AM, and drag from whatever that point is to the corresponding time (same decimals) of the next day (everything will be highlighted in red). This sets the baseline at that spot, which will be considered to be the point of lowest flow or "zeroing out" for that particular tree. You then press convert, and the red highlighting will jump to the next day, and you can keep clicking convert until all of the days you want to convert are converted with the baseline you set. Do this for each tree. Sometimes there won't be a characteristic diurnal pattern, though. If the data looks funky, you should first consider weather conditions for the days you were measuring. If there was a rainy day or two it affects sap flow, and all trees should show funky data around the same time. If there's just one or two trees that show a lot of noise and no obvious diurnal fluctuations, there was probably a problem with the probes/cables/logger/who knows. Also, some trees may have good readings for a few days, but then get funky. You will have to use some judgment to decide what is worth converting. Once everything that you want to convert is converted, "Save As" and choose to save only converted data, as well as to "include a header line" and "replace missing data with dots."

Analyzing data: Once data is converted you will open it in Excel and organize it in whatever way makes sense to you. You can then create graphs/average by species/whatever, to investigate any trends, but keeping in mind that with a small sample size of trees, looking at an average rather than plotting as individual trees can be deceiving. When I first plotted an average of my data by species, there seemed to be a clear trend, but it disappeared when I plotted by tree. Mark Green helped me construct "representative diurnal curves" using mean per hour in Excel. This plots the mean of all the data points within each hour for all the days collected for one particular tree, to give a representative "tree day" showing the average sap flow per hour over 24 hours. So for one tree, all the data points that have a time stamp between 12:00 am and 12:59 am (regardless of the day) will be averaged together. We then did the same thing in R, but used median per hour rather than mean to reduce noise in the data using a script written by Mark. Doing this for each tree seemed to be the most accurate way to visualize the data and make conclusions about any trends.

What I definitely would have done differently!!: 1. Convert the raw data to sap flow as soon as possible. There were times when I would make sure that the probes were all showing reasonable temperature difference readings out in the field, and everything looked fine, but then when I converted the data (in most cases a day or two after we had stopped measuring and taken down the sap flow stuff at the site) the fluctuations looked funky for some of the trees (didn't follow a characteristic diurnal pattern) and I couldn't use the data. I did it that way because I thought it would be easiest to convert all the data at once, rather than having to repeat the process every time I got new data. While that was probably the easiest way to do it, I also could have caught problems early on if I had converted data more often while sap flow was still being measured.

2. Work hard to get accurate temperature and precipitation data for all sites for the dates you are measuring. There is a temperature and humidity probe (long copper thing, sticks in the ground) that Michele Pruyn had used it in the past that can be hooked up to the logger, but I couldn't get it to work. Hubbard Brook and Bartlett had weather station/flux tower data, but not Jeffers Brook. This is an important covariate to be considered, so trying to get accurate temp/humidity data is important.

3. Take lots of notes when you're out in the field about exactly what you did, when you did it, why, etc. I was good about this at first but then not so good. Keep track of which cables and probes didn't work, so that when you get back to the lab you can test them again and figure out if the probe wasn't working or the cable, or if it might have been the logger. Take note of tree characteristics, even if they are qualitative assessments, things like crown size or the presence of more or less beech bark disease could be impacting sap flow, and you'll want to be able to look back on your notes and consider things like that if there is a lot of variation in sap flow between trees.

GOOD LUCK!!!! It's a lot to learn but it's not as bad as it sounds and it's so satisfying when you start to understand it!