Minimum Cuticular Conductance (g_{MIN}) Protocol & R-Script

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Set Up:

Unroll a strip of boxing tape so that it spans a length wide enough for several leaves to be placed edge-to-edge and not over-lap. Fasten each end of tape between two laboratory stands, shelves, etc. Make sure the tape is elevated high enough to prevent leaves from touching the lab bench once they are adhered to the tape. Place a fan, preferably rotating, in front the the tape such that the entire length of tape receives wind. Obtain a thermometer, relative humidity sensor, and a timer.

Leaf Area:

1. For a given species, select at least 5 healthy leaves.
2. Label each leaf 1-5 in sharpie on the adaxial base of the leaf base next to the midvein.
3. Place leaves in scanner bed, scan, and calculate leaf area (see Leaf Morphology Protocol)

3.1. Record leaf area on gmin data sheet.

4. After scanning place leaves into a plastic bag and exhale into the bag.

Seal Petiole:

5. Turn on the hot-plate.
6. Place the tin can on the hot-plate until red wax has been melted.
7. Carefully dip the petiole into the hot wax, removing the leaf from the wax immediately after it is coated.
8. Place leaves with wax on lab table until wax hardens, then place leaves back into a plastic bag.

Note: Make sure that the entire *base* of the petiole, not necessarily the entire petiole, is covered in wax. The goal is to prevent water from evaporating out of the wound left after the leaf is detached from the stem. Sometimes the petiole may need to be dunked twice in wax to ensure an appropriate seal.

Leaf Mass:

9. Randomly remove a leaf from the bag, weigh it, and record its mass in the appropriate section of the data sheet
10. After weighing a leaf, place it into another plastic bag for weighed leaves.

Drying Leaves to Calculate g_{min}:

11. Dump out leaves from the plastic bag onto the lab bench, start a timer for 10 minutes, and record the time (24-hr time to the closet second).
12. Randomly place leaves on the boxing tape with the tops of the leaves adhered to the tape, and leaf tips facing down. Usually, very little of a leaf's lamina has to touch the tape in order for it to stick. Here, the idea is to maximize the area of leaf that will be exposed to wind.
    12.1. Make sure the edges of the leaves do not overlap.

Note: Since different areas of the tape may receive different wind velocities, it is important that leaves are placed randomly along the tape.

13. Once leaves are all hung-up and drying, record the temperature and relative humidity at the lab bench.
14. Once the timer goes off (after 10 minutes), carefully remove the leaves from the tape and place them into a plastic bag.
15. Take the leaves to the scale and weigh the leaves randomly
    Note: Take care to record the leaf mass in the appropriate places.
16. After a leaf is weighed, place it into a separate plastic bag for “weighed leaves”
17. Return to step 11 and repeat every 10 minutes for 2-2.5 hrs.

Data entry and R code:

18. Enter data into an excel sheet, and make sure the column names are formatted like so:

accession	gen	spe	family	leaf	time	temp	rh	mass	area

19. Time, temperature, and rh should not be empty for any row

```
gmin<-read.csv("/Users/timothyperez/Dropbox/CH1/Data/gmin/gmin.csv", header=T)
gmin$genspe=paste(gmin$genus, gmin$species)
gmin$alt=15

#here is the function for calculating gmin, adapted from:
http://prometheuswiki.org/?page=Minimum%20epidermal%20conductance%20(gmin,%20a.k.a
.%20cuticular%20conductance)

Gmin=function(area, alt, time, mass, temp, RH){
es=(0.611)*exp((17.502*temp)/(temp+240.97)) #saturation vapor pressure Kpa (eq. 3.8, Jones
p. 41)
ea=es*(RH/100) #vapor pressure of air (eq. 3.11, Jones p. 41)
pa=101.3*exp(-alt/8200)#atmospheric pressure
mfVpd=(1-(RH/100))*(es/pa) #fraction vapor pressure deficit
gmin.mmol=-((coef(lm(mass~time))[[2]]/60)/mean(na.omit(mfVpd)))*(1000/18/((
area*2)/10000))
return(gmin.mmol)
}

#Note 3: parameters must be entered into the function as follows: leaf area (cm^2), altitude (m),
#time (minutes in decimal form), leaf mass (g), air temperature (˚C), relative humidity (%).

gmin$gf=paste(gmin$genspe, gmin$leaf)
us=unique(gmin$gf)
gdf=list()
for(i in 1:length(us)){
  df1=subset(gmin, gmin$gf==us[i])
   gdf[[i]]=data.frame(genus=as.character(unique(df1$genus)),
    species=as.character(unique(df1$species)),
    leaf=unique(df1$leaf),
```

```
    gmin.mmol=Gmin(df1$area, 15, df1$time, df1$mass, mean(na.omit(df1$temp)),
mean(na.omit(df1$rh))))
}
gmindat=as.data.frame(do.call("rbind", gdf))
```

References:

H. G. Jones, *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology* (Cambridge University Press, New York, USA, ed. 3, 2014).

Sack and Scoffoni. 2011. PrometheusWiki. Minimum epidermal conductance (g_{min} a.k.a. minimum cuticular conductance). http://prometheuswiki.org/?page=Minimum%20epidermal%20conductance%20(gmin,%20a.k.a.%20cuticular%20conductance)