Experimental Plans Summer 2018

# Leafhopper density effects on secondary metabolites by cultivar

## Rationale:

Preliminary data shows that some tea volatiles respond in a dose-dependent manner, others non-linearly, altering the quantity and blend of volatiles as leafhopper density increases. Leafhoppers are considered pests/bad for quality for green teas and beneficial for some oolong teas. Is this partly because tea plant genotypes are responding differently to leafhopper damage?

Also, plant responses to herbivores typically studied with 1 arbitrary density of herbivores and one genotype of plants, but studies that use a continuum of densities or multiple plant genotypes often find idiosyncratic responses.

## Experimental Design:

Choose two cultivars, one used for green tea (e.g. longjing #43), one used for oolong (e.g. Tie Guan Yin). Have Li Xin cut back plants to promote new growth.

Bag branches on plants such that about 20 young leaves are in each bag. Add leafhoppers in densities ranging from 0 - 1 per young leaf.

Monitor plants daily for signs of leafhopper damage. When highest density plants start to show severe damage, stop experiment

Remove bags, count remaining leafhoppers, collect volatiles with DCSE. Pick all young leaves in bag, scan, and lyophilize.

## Analysis:

GC-MS on DCSE samples, LC-MS on lyophilized leaves, ImageJ to get leaf damage from scans.

Use PLS regression to look for metabolites that best explain variation in leaf damage (or leafhopper density). Do the same for non-volatiles. I could do these analyses separately for the two cultivars to see if the same set of metabolites are impacted by herbivory. For key metabolites, I would compare the slope of concentration vs. leaf damage for the two cultivars.

# 2. Does leafhopper damage interact with processing to alter finished tea metabolites?

## Rationale:

Processing of oolong involves mechanically damaging leaves that are still alive in order to elicit metabolite production to improve tea aroma. Does leafhopper damage improve quality by increasing baseline volatile levels, by priming (that is, a greater increase during processing) or both? In a way, this is another way to ask: “why are leafhoppers good for oolong but bad for green tea?” since green tea is not mechanically damaged before kill-green.

## Experimental:

Use only oolong cultivar for this experiment. Bag a larger section or more plants so that final yield per sample is more than 20g dried leaves (I’ll do a pilot to figure out roughly how many young leaves this is).

Apply leafhoppers at 3 densities (0, 0.5 per young leaf, 1 per young leaf) and run experiment as described in #1, monitoring for damage daily.

Remove bags, do DCSE(?), scan random sample of leaves, microwave kill-green half of remaining leaves, “process” the other half.

Processing might be done by hand by agitating leaves for a certain amount of time, or I may be able to devise some way to do this by machine (placing leaves in labeled mesh bag inside of larger tumbler?).

## Analysis:

GC-MS on DCSE samples, environmental samples, and processed samples. PLS regression with each dataset to look for metabolites that best explain leaf damage. Univariate analysis of compounds known to contribute to oolong tea quality. Is “induction” by processing greater in leafhopper damaged leaves?

# Possible pitfalls for the summer:

***This will require a lot of leafhoppers.***

I will collect eggs and nymphs daily as soon as I arrive and attempt to build up a large lab colony before starting the experiment. Last year my density experiment only lasted 4 days before plants showed signs of damage, so I will stagger replicates in time to allow collection of more leafhoppers in between. I can even attempt to re-capture leafhoppers at the end of the experiment as I count them.

***Controlling herbivory***

I did my density experiment on small potted plants last year because of worries about systemic induction and using plants grown in the field that have already experienced herbivory. For example, if I only bag one branch to exclude leafhoppers, what happens if adjacent branches experience ambient levels of herbivory? In many woody species, systemic induction is localized to a branch, so I will attempt to put entire branches in a bag for my experiments to hopefully minimize the effects of systemic induction. Also, because my preliminary results show that density does matter, even if a plant has previously experienced leafhopper herbivory, adding more leafhoppers should still have an effect.