**Goal:** to understand how trade-offs in WUE/RGR that affect indirect and direct responses of climate and grass competition

**Overview:** Most measurements will be taken on individuals grown in the greenhouse under optimal conditions but, in order to ground these measurements in reality and to ensure that the ranking of these measurements is consistent in my study species, each trait will be taken on a smaller subset of individuals in the field from naturally occurring plants across the landscape chosen at random on my next visit to the field station by placing transects through natural populations of each species, and tagging 10 random individuals. I will do sequential leaf area estimates on 5 of them during each census (once a month) for RGR (though having ten tagged of similarly sized individuals from the beginning for insurance in case of mortality), then I will collect these specimens for SLA/WUE/%N

**Tools:**

* Toothpicks
* String
* Twisty ties
* Flagging
* Drill
* Measuring stick
* Data sheet

**Traits**

1. Water use efficiency
2. Relative growth rate
   1. Greenhouse based: destructive sampling AND nondestructive sampling
   2. Field based: nondestructive sampling, which may vary by species and include height and or canopy but always includes total leaf area
      1. Counted total leaf number and estimated total leaf area on each plant. We estimated total leaf area by measuring the length and width of representative small and large leaves on each plant and then counting the total number of leaves in each size class.
         1. So, take picture of representative small and large leaves!
   3. Species based measurements for nondestructive sampling:
      1. PLER
         1. Total leaf area
      2. CLGR
         1. Height and total leaf area
      3. HECO
         1. Height, canopy, and total leaf area
      4. CETR
         1. Height, total leaf area
      5. CAPA
         1. Height, canopy, total leaf area
      6. LACA
         1. Height and total leaf area
      7. AGHE
         1. Total leaf area
3. Seed mass and seed shape (these two traits together are better predictors of seed longevity than seed mass alone – Thompson 1993, Funes et al. 1999)
   1. Count out 100 seeds and weigh, for 10 replicates per species (already done)
   2. Seed shape: see Thompson et al. 1993 and Funes et al. 1999)
4. Leaf nitrogen content
5. Ratio of maximum electron transport (Jmax) to maximum carboxylation (VCmax) calculated from assimilation (A) vs. internal CO2 concentration (Ci) (see Huxman 2008)
   1. Will accompany Jenny in the greenhouse for LiCor training
   2. Will borrow Jenny’s LiCor (or, alternatively, contact Matt Gilbert about different sized chambers, and/or Annie Schmidt if need a LiCor)
   3. Easier to do on greenhouse plants, can manipulate potted plant to get as much of the plant in the chamber as possible
      1. Greenhouse pots can be small BUT in order to use the LiCor the size of the chamber needs to be smaller than the area of the pot, otherwise might need to cut away part of the pot in order to get enough of the plant into the chamber
   4. Most of this work will take place in the greenhouse, however for each species, we will take a couple in field measurements on individuals outside of my experiment

**Methods** (adapted from Angert et al. 2007, Angert et al. 2009, Huxman et al. 2008, and Angert et al. 2014)

1. **Greenhouse initiation** (Dec 2016 - Jan 2017)
   1. Germinate seeds on 1.5% agar in Petri dishes OR in vermiculite in growth chamber
      1. Just to be safe sow 200 seeds per species, looking for ~100 individuals per 7 species
      2. Note: because species germinate after different lengths of time, sow species in reverse order
   2. Transplant seedlings to conical pots containing mixture of 55% potting soil, 30% sand, 15% vermiculite and transfer to greenhouse
   3. Pots should be watered daily
2. **Throughout growing season**: every two weeks, 2 randomly chosen individuals will be chosen for RGR measurements
   1. Greenhouse
      1. Non-destructive: total leaf area of the plant will be calculated by taking photos and analyzing them in ImageJ, this will be to link RGR measurements in the greenhouse with nondestructive field based measurements
      2. Destructive: the 2 individuals chosen in the greenhouse will be harvested, cut at the soil level and separated into root, leaf, stem, and reproductive (flower, bud, fruit) biomass
         1. Biomass will be dried and weighed
   2. Field
      1. Nondestructive: In field during every census visit, (~ once a month) total leaf area/height of 2 individuals per species on previously chosen individuals will be taken by taking photos and analyzing them in imageJ
3. Prior to reproduction, 10 greenhouse individuals and 2 field individuals per species:
   1. **LiCor**
      1. Near midday on a consistently sunny day, under standard conditions, the LiCor will be used to measure A and Ci
   2. **SLA**
      1. The individual will then be harvested, total leaf area will be measured by taking an image of each plant on a flatbed scanner and process image to estimate leaf area then drying leaves to 60C and weighing biomass
   3. **WUE** & **%N**
      1. Biomass from SLA will be ground and analyzed for both carbon isotope discrimination and %N