Excerpt from upcoming proposal pertaining to this experiment (not finished yet):

**Direct and indirect effects of rainfall on forb populations**

*Rationale*

In order to understand how species diversity will persist under future climate variability, I will first study the population dynamics of forbs with different life history strategies to climate extremes, namely changes in precipitation. In California annual grasslands, water is the primary limiting resource, with a significant amount of work showing strong correlations between grassland composition and productivity with variability in precipitation (Murphy 1970; Duncan and Woodmansee 1975; Pitt and Heady 1978; Young et al. 1981). Whether these responses are mediated by interactions with other species however remains to be understood. Some evidence supports a strong role for indirect effects of climate mediated by grass competition (Levine & Rees, Suttle), but other evidence supports a greater role for direct effects of climate (Levine & grad student experiment; Elmendorf & Harrison nestedness paper; recent PNAS paper, Pitt and Heady). By looking more closely at the forb population response across life history strategies, these seemingly disparate results can be understood.

*Question*

How do indirect and direct effects of changing climate, including altered patterns of extremes, affect native forb persistence and how does this vary by life-history strategy?

**H1**: The direct and indirect effects as well as their relative strength of climate extremes will vary among native forbs by life history strategy. Indirect effects of grass competition will be stronger than the direct effects of climate for stress tolerant forbs while the direct effects of climate will outweigh the indirect effects of grass competition for stress-avoiding forbs.

(Specifically, stress tolerant forbs will benefit from drought years because they are less affected by drought and more affected by grass competition. Fast-growing forb species, if they germinate, will not benefit from drought years because they are more affected by drought and less affected by grass competition.)

*Methods*

This experiment is taking place in an annual grassland at McLaughlin Natural Reserve. At the reserve, we have set up 10 rain-out shelters (3m x 3m) paired with 10 control plots, as well as 10 drought-alleviation plots also paired with 10 control plots. Treatment plots are paired with control plots to account to heterogeneity in community composition within the site. The rain-out shelters block out 100% of the rainfall and are 3mx3m. We installed them in early December 2015 and they remained on until mid-March 2016. The drought alleviation plots are equipped with sprinklers that are turned on during gaps of rainfall to mimic increased rainfall. (explain these treatments more). Within each experimental plot are two 30cm x 30cm subplots, one to monitor the effects of the treatments with grass competition, and one to eliminate grass competition. In October 2015, I seeded in 100 seeds from 6 species representative of a range of ecological strategies into each subplot (see Table 1). I visit the site approximately every 2 weeks throughout the growing season (November through June) and track germination, mortality, flower timing, and flower number within all 6 species with colored toothpicks. Along with the census, I also remove grasses from grass removal subplot as well as background species by hand during every visit. Due to the high proportion of germinators that occurred, I also thinned the plots to limit any forb-related density dependent effects. I will also sample a subset of flowering individuals from each species to measure per capita fecundity. Then, to monitor seed survival in the ungerminated portion, I buried seed bags filled with sand and 100 seeds of each species in September 2015. These will be harvested in April 2016 to seeds will be counted and tested for viability.

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| Species | Strategy |
| *Agoseris heterophylla* | Stress tolerator |
| *Clarkia gracilis* | Stress avoider |
| *Hemizonia congesta* | Stress tolerator |
| *Lasthenia californica* | Stress avoider |
| *Lupinus bicolor* | Stress avoider |
| *Plantago erecta* | Stress tolerator |

Table 1: List of species used for the experiment and their corresponding life-history strategy as determined by SLA, germination characteristics, and response to drought

*Analysis*

To test how forbs with different life strategies perform under different precipitation regimes, I will compare population growth rates across treatments within each forb strategy by parameterizing the annual plant model:

λ = *s*(1 – *g*) + *gF*

where *s* is the annual survival of seeds in the ungerminated portion of the population, *g* is the fraction of germinating seeds, and *F* is the number of seeds produced per individual. This model assumes no density dependence as well as constant mortality and germination rates across seed age. Since I am controlling density in my experimental plots to well below the average density per plot, density effects should be negligible. I will then compare population growth rates among treatments within each life-history strategy. I will use generalized linear models with (type of distribution?) with population growth rate as the response variable and watering treatment, life history strategy, and grass presence and the interactions between these as my predictor variables. I will also include a random variable to account for the nested structure of the grass treatment within the watering treatment.

*Expected Results*

Overall, if I find that both forbs types do better in wet years than in dry years, however the presence of grasses alters this response for some forbs but not others, this would support my hypothesis that the direct and indirect effects climate on forbs varies by life history strategy. Specifically, if I find that population growth rates for stress tolerant forbs are higher in the water addition treatment than in the drought treatment without grasses, but in the presence of grasses, population growth rates are higher in the drought treatment than in the water addition treatment, this will indicate a significant interaction between treatment and grass presence. This would support my hypothesis that the indirect effect of grass presence for these stress tolerant forbs outweighs the direct effect of a favorable year. Similarly, if I find no significant interaction between grass presence and watering treatment for stress avoiding forbs, this will indicate that the direct effect of climate is stronger than the grass-mediated effect.

Protocol

1. My plots are a subset of a larger experiment looking at the effects of drought on the plant community; all of the plots I am using are marked with a pink flag. The center of the plot is where community cover is being assessed however there are manipulations occurring around the border of each plot so pay attention to where you step.Macintosh HD:Users:Marina:Library:Containers:com.apple.mail:Data:Library:Mail Downloads:E15473B1-A0F7-4A6A-B368-E7F6116A034C:plot layout.pdf
2. Experimental treatments are applied in pairs so that next to every treatment plot is a paired control plot:
   1. All red flags: watering control plot
   2. 3 red + 1 blue flag: watered plot
   3. White flags: rain-out shelter or shelter control plots
3. Plots are numbered with a steel tag on the lower right rebar, when you arrive at a plot, check this number to ensure you are in the correct plot
4. In each plot I have two subplots, one with grass competition tagged with orange nails (subplot A) and one without grass competition tagged with purple nails (subplot B)
5. Upon arriving at a plot and after checking the plot number
   1. Remove grasses from plot B
   2. Remove background species (anything growing that is not one of the focal species) from BOTH plots
   3. For each species
      1. Look at number of expected individuals for each species
      2. Check for mortality by counting off toothpicks and seeing if there is a living individual of that species near the toothpick; if there isn’t, remove the toothpick and record total number that died under “n.died”
      3. Check for germination by looking for any new individuals that do not have a toothpick next to them, record this number in the “n.germ” column
      4. Pull all new germinants for every species BUT LACA & LUBI; record how many were pulled in the “n.pull” column
         1. If new LACA or LUBI – do not pull, instead, mark them with the correct toothpick color
         2. Note: this means that n.germ & n.pull should be the same # for all species/plots except LACA and LUBI (which should have a 0 in the n.pull column)
      5. Record flowering
         1. Flo.A – number of individuals with buds
         2. Flo.B – number of individuals with open flowers
         3. Flo.C – number of individuals setting seed
      6. Record flower number (n.flow)
         1. For 5 random individuals, record the number of flowers per individual
6. Most toothpicks are plastic and are easily discoverable however I ran out and had to use hand dyed toothpicks for some (CLGR, PLER, and HECO). Their color should still be distinguishable however be careful. If they look different, deduce what color they should be based on the individual they are near and place a new toothpick next to the individual if necessary.
7. Some of the numbers MAY be off by one or two – this is fine. However the majority of them are correct so if you are counting wildly different numbers, look around the edges of the plot, move other plants gently out of the way, or try looking at the plot from a slightly different angle to try to find all the individuals.
8. PLER and LACA look slightly similar – PLER is fuzzier and greyish green while LACA is spikier and bright yellow/green. PLER’s leaves all come from a basal central axis while LACA has leaves growing along the main central stem.

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| **Species** | **Toothpick color** |
| Agoseris heterophylla (AGHE) | White |
| Clarkia gracilis (CLGR) | Pink/red |
| Hemizonia congesta (HECO) | Orange |
| Lasthenia californica (LACA) | Green |
| Lupinus bicolor (LUBI) | Blue |
| Plantago erecta (PLER) | Yellow |