Phenological sensitivity as a mediator of plant interactions

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Motivation and Dissertation Framework

Phenology, the timing of annual life cycle events, allows organisms to synchronize important life history transitions with optimum environmental conditions (Forrest & Miller-Rushing, 2010). Phenology is an important mediator of ecosystem processes (Cleland *et al.*, 2007; Piao *et al.*, 2007) and species interactions (Leverett, 2017; Yang & Rudolf, 2010), and plays a major role in determining species' range limits (Chuine & Beaubien, 2001). Pronounced shifts in phenology observed across a broad range of taxa have been reported, with plant phenology shifting by 3-5 days on average per decade (Menzel *et al.*, 2006; Parmesan & Yohe, 2003; Root *et al.*, 2003). Phenological sensitivity to climate change, defined as the shift in phenological event date per degree of temperature change, varies among taxa, and there is evidence that within given communities species' phenologies are shifting at different rates (Cleland *et al.*, 2012; Ovaskainen *et al.*, 2013). These differential sensitivities are likely to have far reaching effects on community interactions, but our ability to predict these second order effects remain limited.

In my proposed dissertation, I seek to advance our understanding of how phenology influences species interactions under changing climate conditions. Sitting at the nexus of traditional community ecology and global change biology, my proposed dissertation will explore 1] how differences in sensitivity to environmental conditions can produce significantly different phenological patterns and 2] how differential phenological responses alter community interactions.

In **Chapter I**, I use experimental manipulations and statistical modeling to evaluate the current evolutionary hypotheses regarding flower-leaf phenological sequences in temperate woody plant species, and investigate differential phenological sensitivity between flower and leaf buds as a potential mechanism for this variability.

In **Chapter II**, I turn my attention to seed germination phenology, where I probe the effect of variable stratification periods and incubation temperature on germination of a large suite of temperate, herbaceous plants, to determine interspecific differences in responses to changing climate, and evaluate the likelihood of germination rank changes under future climate scenarios.

In **Chapter III**, I expand the scope of my inquiry in chapter II, compiling a global database of experimentally manipulated germination trials, and use meta-analysis techniques to better understand the reaction norm of germination phenology in response to environmental manipulations.

In **Chapter IV**, I perform a direct test of the impact of phenological shifts on community interactions, by using alternate climatic treatment to manipulate the germination phenologies of two species growing in a pairwise competition experiment.

Background

In my disseration, I focus on the phenological transition between dormancy and growth, a time in which new growth is both most suseptable to mortality, and has potential to contribute signifantly to the overall fitness of the organism (Rathcke & Lacey, 1985). This phenological transition takes place in both seeds and buds of perennial plants and is mediated by a complex combination of environmental factors, evolutionary tradeoffs, and physiological processes. These factors, along with an understanding of the statistical frame-

works that are used to model spring phenology,are critical background to my dissertation work and I discuss each one briefly below.

Eco-physiology and Evolution of Spring Phenology: Plants sense and interpret a complex set of environmental cues that signal the changing of the seasons and can respond by fine-tuning their phenological transitions accordingly (Vitasse *et al.*, 2013). For plants growing in temperate regions, it is widely accepted that phenological transitions are responses to the interaction of exogenous environmental conditions, temperature and photoperiod (Forrest & Miller-Rushing, 2010), with endogenous cues like circadian clocks (Visser *et al.*, 2010). Temperature and light are also considered to be the main drivers of seed germination (Finch-Savage & Leubner-Metzger, 2006). In the temperate zone, both seeds and buds generally cycle through a state of dormancy, a temporary state in which metabolic activity is minimized, preventing organism growth, development or activity. Dormancy allows organisms to persist by conserving energy during periods that are unfavorable for biological functioning, which in the temperate zone, is the cold months of winter. There is a complicated taxonomy of dormancy in seeds (Baskin & Baskin, 2004), and the most common dormancy class in the temperate regions of the world is physiological dormancy (PD), in which a physiological inhibition mechanism is present in the embryo and prevents radical emergence (Finch-Savage & Leubner-Metzger, 2006). While globally, there are several climactic factors that contribute to dormancy break, in our region, this transition from dormancy to growth tends to be mediated by exposure to a prolonged period of cool temperatures (chilling or stratification), followed by warm (forcing or incubation) temperatures. It should be noted that the first visible sign that dormancy is broken is germination or budburst, so as such, it is difficult, and even controversial, to mark where dormancy ends and growth begins (Bewley, 1997; Long *et al.*, 2015).

It has been suggested that the major selective forces dictating spring phenology are shared by both spring germinating seeds and buds. The adaptive benefits of early phenological transition, an extended growing season, predator avoidance, and reduced competition for resources are balanced by the higher risk of mortality due to biotic and abiotic factors, resulting in stabalizing selection (Rathcke & Lacey, 1985). For flower buds, the availability of pollinators may be an additional selection factor. As such, the phenological optimum evolves in relation to other functional traits and life history tradeoffs.

While the selective forces, environmental conditions and conceptual framework for the transition from dormancy to growth may be quite similar for seeds and buds, there is evidence that the physiological mechanisms regulating these processes are quite different. In buds, short day conditions and the end of the season induce dormancy by isolating meristematic cell interactions through heavy deposits of callose in the plasmodesmata(Rinne *et al.*, 2011; Sager & Lee, 2014). Winter chilling degrades the callose, reengaging cellular integration for the transition to growth under suitable conditions.

In seeds, callose has been shown to play a secondary role in domrancy by maintaining a physical, seed coat imposed doramancy (Leubner-Metzger, 2003), but it the hormone abscisic acid (ABA), that is generally thought to initiate and maintain dormancy. (Baskin & Baskin, 2014; Fenner, 2000). It has been hypothesized that the balance of ABA and the growth promoter gibberelic acid (GA_3) acting at differnt parts of a seeds "life" are the primary regulators of dormancy and germination in seeds (Kucera *et al.*, 2005; Leubner-Metzger, 2003). Environmental treatments such as cold or warm stratification and afterrippening, have been shown to contribute to the degradation of ABA, and an upregualtion and increased sensitivity to (GA_3), as well as ethylene and brassinosteroids, which promotes germination (Kucera *et al.*, 2005).

Model of Spring Phenology: Many models have been developed to use temperature to predict phenology in woody plants (Chuine *et al.*, 2002), while seed science most commonly uses a single model, the thermaltime or cardinal temperature model, or its variant the hydrothermaltime model which includes water potential as a parameter (Bradford, 2002).The thermaltime model of germination describe the relationship between time, temperature and germination fraction at sub-optimal temperatures.

$\theta_T(g) = (T - T_b)t_g$

where $\theta_T(g)$ is thermal time to gemination, T is the treatment temperature, T_b is the base temperature below which germination rate is 0 and t_g is the time to a given germination fraction. If not observed experimentally, T_b can be inferred through statistical methods by regressing the reciprocal of germination time against a given germination fraction and determining the x-intercept (Pritchard *et al.*, 1999). If supra-optimal temperature are included in the experiment, which would be indicated by a reduction in germination rate at higher temperatures, a constant K_t is added to the equation to modify the thermal time parameters

by the following equation:

$\theta_T = (k_T(T - T_o))(T - T_b)t_g$

where T_o is the optimum temperature.

While this model is widely toted for its biological accuracy in predicting the germination phenology of non-dormant seeds (Bradford, 2005), the standard thermaltime model is less effective for phenological predictions with dormant species (Batlla & Benech-Arnold, 2015).

Several attempts have been made to modify this modeling framework to better incorporate a dormancy module. Conceptually, dormancy break treatments reduce T_b or(ψ_b in the hydrothermaltime variant) allowing for a more rapid accumulation of thermaltime and more rapid germination. This framework has been applied to include afterrippening (Meyer *et al.*, 2000), and cold stratification(Batlla & Benech-Arnold, 2003; Pritchard *et al.*, 1996), but has not been broadly applied outside of agriculture or horticulture, and its effectiveness for a diversity of taxa remains unknown (Steadman & Pritchard, 2004). By evaluating the change in germination rate at different dormancy break treatment, one can determine a coefficient which reflects the rate of change in T_b. The equation is formulated below:

$T_b = (\beta(t_s)) + T_b(o)$

where β is constant rate of decline, t_s is the stratification time, and $T_b(o)$ is the base temperature without dormancy treatment.

Chapter I: The significance of flower-leaf sequences in an era of global climate change

Introduction

Why do some tree species seasonally flower before leafing out? This sequence, known as hysteranthy, proteranthy, or precocious flowering is readily apparent in many ecologically and commercially important species and has been described as the characteristic flower-leaf sequence (FLS) of temperate deciduous forests (Rathcke & Lacey, 1985). Most of the current hypotheses regarding FLS's suggest that they are critical to the reproductive or physiological functioning of woody plants (Gougherty & Gougherty, 2018). Several authors suggest that the hysteranthous FLS is a trait critical for wind-pollination efficiency (Friedman & Barrett, 2009; Whitehead, 1969). Others suggest that flowering first is an adaptation to reduce water stress and maintain floral hydration (Franklin, 2016), though this hypothesis has emerged primarily from the dry-deciduous tropics where hysteranthy is also common (Franklin, 2016; Janzen, 1967). Still others suggest the hysteranthous FLS is an adaptation to allow for extremely early flowering and is correlated with other early flowering traits such as seed size, dispersal time and cold tolerance (Bolmgren *et al.*, 2003; Gougherty & Gougherty, 2018; Primack, 1987). It is also possible that FLS's are highly conserved, and the preponderance of hysteranthy in the temperate zone is a product of phylogenetic representation of the region rather than an adaptive quality to the trait. These functional hypotheses suggest that FLS's may have an important role in mediating both inter and intra-specific competition for pollen, and variation in FLS's could be considered a critical life history tradeoff.

Despite the rich theoretical attention FLS has received in the literature, data about FLS is limited. The most comprehensive source of data we have regarding FLS comes from categorical, qualitative descriptions in regional flora and guide books. A few long term empirical datasets in which FLS can be properly described as a continuous variable can be found, but these are rare, as flower and leaf phenology have generally been treated and observed separately (Wolkovich & Ettinger, 2014). In part I of this chapter I ask, given the available data:

1. What is the association between FLS and several other life history traits (pollination syndrome, shade tolerance, plant height, flowering time, duration of fruit maturation) pertinent to the established hypotheses? Are these results sensitive to data quality, observational ambiguity and modeling choices? Does treating FLS as a continuous rather than categorical variable result in a model more robust to the afore mentioned factors?

Treating FLS as a categorical trait masks important characteristics of FLS such as the range variability of FLS offset between species, individuals, populations and years. As far as I know, there have been no attempts to empirically quantify this variability. Improving our understanding of the variability of this pattern would aid significantly in evaluating the evolutionary hypotheses for FLS, and serve to generate hypotheses for the eco-physiological mechanisms behind these patterns. In part II of this chapter I ask:

1. What is the range in variability in FLS between species, years and individuals?
2. Is variability in FLS a product of differential sensitivities to environmental cue combinations between flower and leaf buds?

Methods:

Part I: I obtained species level descriptions of floral-foliate sequences and trait information from the regional guidebook Michigan Trees (Barnes & Wagner, 1981,2004) and its companion volume Michigan Shrubs and Vines (Burton V. Barnes, Christopher W. Dick, 2016) (hereafter: MTSV) and the Unites States Forest Services Silvic Manual Vol.2 (Burns *et al.*, 1990) (hereafter: USFS). All categorical traits were reclassified as binary (FLS (0 leaf first, 1 flower first), shade tolerance (0 intolerant, 1 tolerant), pollination syndrome (0 insect, 1 wind)). I developed three, alternative classifications for FLS, physiological (only taxa with "flowers before leaves" classified hysteranthous), intermediate (taxa with "flowers before leaves" and "flowers before and flowers before/with leaves" classified hysteranthous) and functional (taxa with "flowers before leaves", "flowers before/with leaves" and "flowers with leaves" classified hysteranthous). In total, 196 and 82 woody species, in MTSV and USFS respectively, were included in my analysis. To investigate the phylogenetic signal of hysteranthy and control for phylogenetic structure in the datasets, I used a published angiosperm phylogenetic tree (Zanne *et al.*, 2013) pruned to match the species list from the MTSV and USFS data. Species not in the tree were added at the generic root. I used a generalized linear modeling framework (Ives & Garland, 2010) to build a logistical regression model corrected for phylogenetic structure using the R package phylolm (Ho & Ane, 2014). The model was run with 599 bootstrapped re-sampling iterations for each dataset (Wilcox, 2010). Continuous predictors were rescaled by subtracting the mean and dividing by two standard deviations to allow for a reasonable comparison of effect sizes between the binary and continuous predictors in this model (Gellman & Hill, 2997). Models were run on each dataset for each classification of FLS for sensitivity analysis.

For the model with FLS as a continuous response, I used a long term phenological dataset from Harvard Forest in Petersham, MA (O'Keefe, 2015) (hereafter: HAFO) and calculated the average FLS lag time for 21 species which overlapped the MTSV species list. With the same modeling framework, I modeled the associated between the original MTSV predictors and the Harvard forest continuous FLS data. I also ran model where mean FLS lag time was coded as a binary varaible (FLS< 0 = leaf first, FLS> 0 = flower first) to directly assess the effect of binary vs. continuous FLS data.

Part II: I first determined descriptive statistics (mean, standard deviation and range) and plotted the seasonal dynamics of FLS offset in the HAFO dataset using the R base statistical package to establish a basic understanding of the typical variability in FLS. In October of 2017, I obtained cutting of dormant twigs from 12 woody plant species from Harvard Forest in Petersham MA. Twigs were transported to the Weld Hill Research Building in Boston, MA, re-cut in water, and placed into 250 ML Erlenmeyer flasks. 6 replicates of each species were randomly assigned to different growth chamber temperature and photoperiod combinations. Twigs received 1 of 2 levels of chilling (4 or 8 weeks at 4 °C), combined with 1 of 2 forcing treatments (24 °C/18 °Cor 18 °C/12 °C day/night temperatures on 12 hour cycles) and 1 of 2 photoperiod treatments (8/16 or 12/12 day/night) for a 12 way full factorial design. Flasks were moved between chambers every 2 weeks to reduce the influence growth chamber effect artifacts on the results. Twigs were monitored for flower and leaf phenology using the BBCH scale (Finn *et al.*, 2007) every 2-3 days for four months.

I currently use a multilevel, Bayesian, survival analysis framework to analyze these data. Survival analysis is the appropriate framework for this experiment as there was a considerable number of twigs that did not burst buds by then end of the experiment, but were determined to be living. Dead twigs, or those which were determined to have had no flower buds at the outset of the experiment are excluded from the analysis.

I predict that the FLS offset will vary significantly between treatment combinations and that this variation will be traceable to flowering and leaf phenology being differentially sensitive to the environmental factors. My initial model is a varying slope/intercept model by species with the following predictor and response formulation:

$D_e \sim \beta_1(photoperiod : phase) + \beta_2(chill : phase) + \beta_3(force : phase) + E$

Where D_e is the days from initation in forcing conditions to phenological event, and the interaction between phase and each predictor will reveal differences in response to the treatments between floral and leaf phenophases.

Status and Preliminary Results:

What is the association between FLS and several other life history traits? Are these results sensitive to data quality, observational ambiguity and modeling choices?: In the categorical models, there isa consistant negative relationship between flowering time and hysteranthy, indicating that early flowering is more likely to be associated with a hysteranthous FLS. Seed developement time also showed this negative relationship, suggesting that more rapid seed development is associated with hysteranthy. Pollination syndrome showed a general positive trend with hysteranthy, indicated the likelihood of hysteranthy is great in wind pollinated taxa. Increasing height and shade tolerance display a weak positive association with hysteranthy, but there is little confidence in these estimates. All traits except flowering time varied considerably in significance and strength depending on data source and modeling choices. See figure 1 for effect size plots.

The phylogenetic signal for FLS also varied depending on the data and modeling choices with estimated D stastics of 0.05,0.16 0.29, 0.64,0.11, 0.12 for MTSV-functional,MTSV-intermediate, MTSV-physiological, USFS-functional, USFS-intermediate and USFS-physiological respectively. Since values close to 0 indicate a high likelihood of Brownian phylogenetic structure and values closer to 1 indicate random structure, no clear characterization of the phylogenetic structure can be made to to the variablity accross datasets and FLS classifications.

In the continuous model, pollination syndrome was the strongest predictor of FLS, with a strong postive relationship between wind pollination and hysteranthy. Shade tolerance also displayed a strong postive association with hysteranthy and the negative relationship between flowering time and hysteranthy obtained in the binomial models persisted in the continuous one (see figure 2). The continous model was robust to FLS classification with all three classification scheme yeilding comparable effect sizes. This was not the case when the mean FLS offset values in HAFO were classified as binary These findings demonstrate the advantage of treating FLS as a continous response varaibles, and also lend support to the pollination syndrome, early flowering hypotheses. It is not surprising that multiple hypotheses find support in this analysis, as FLS is a complex trait that may have developed independently in different selection environments.

Is variability in FLS a product of a differential sensitivity to environmental cue combinations between flower and leaf buds?: As seen in the example plots of *Quercus rubra* phenology at Harvard Forest (Fig. 3), there is considerable interannual and inter-individual variation in FLS. Additionally, for this species, there appears to be a temporal trend in which FLS has shifted towards a hysteranthous pattern over time. This phenomenon could be correlated with climate change, and should be investigated further.

Preliminary analysis of my experimental result confirms there is considerable variability in FLS offset in different environments (see figure 4). There seems to be support for the hypothesis that this variability is a product of differential sensitivity between flowering and leafing (note in particular, *Comptonia peregrina* and *Corylus cornuta*, in figure **??**), but because of many non-response values in the data spread unevenly across species and treatments, this finding has low confidence and other modeling approaches need to be attempted. Below I present other modeling frameworks I will attempt:

1. Rather than modeling flowering and leafing sensitivities as interactions, use offset (as determined in part I) as a response variable.

2. Use a logistic regression framework with phenology as a binary response.

3. Run individual species models for only the species with the most data.

Chapter II: The germination response to varying stratification regimes of a suite of temperate herbaceous species

Introduction

The cold stratification requirement for dormancy release has been identified in a large number of North American temperate plant taxa, and stratification treatments are employed widely in both plant science and industry (Hartmann *et al.*, 2011). The required stratification period has been shown to differ between species, ranging from just a few days to many months (Luna *et al.*, 2009), vary significantly depending on temperature and between seed populations (Steadman & Pritchard, 2004), and affect the germination speed and final germination percentage of a cohort. These effects strongly influence other life history traits,resource use, and the seedling competitive environement, and are thus an important element of community interactions (Koerner *et al.*, 2008). While cold stratification is commonly found as an experimental treatment in the literature, and has been shown to advance germination, studies which evaluate the germination response across a range of stratification periods or temperatures are rarer (Batlla *et al.*, 2009). As a result, the dynamics of the germination response to variable stratification regimes are poorly characterized for the vast majority of plant species.

Cold stratification in the lab, serves as a proxy for the natural exposure to chilling conditions that a seed would experience while overwintering in the field. With global climate change, changes to the severity and duration of winter will alter the natural stratification period experienced by seeds (Walck *et al.*, 2011). While winters are generally predicted to be warmer and shorter (Pachauri & Meyer, 2014), the number of days which the stratification conditions are met may increase, decrease, or shift temporally, differentially affecting the germination phenology plant species depending on their geographic position, and the dynamics of their response to temperature (Walck *et al.*, 2011). These shifts in germination may in turn alter plant competition through priority effects (Gioria *et al.*, 2018) and plant demography through seed bank dynamics, and multi-trophic interactions.

To better predict the effect of warming winters on seed germination, it is imperative to better characterize the germination response to variable stratification regimes for a more broad range of plant species. In this chapter I ask:

1. How do varying stratification periods effect the germination rate of plant species?
2. How do stratification periods and incubation temperatures interact in germination time courses?
3. Is the stratification requirement best characterized by an optimum (can seeds be over stratified) or a threshold?
4. To what degree is germination rank between species affected by varying stratification regimes?

Proposed methods

Experimental Protocols: In the summer of 2018, I procured seeds of 15 temperate Eastern North America herbaceous plant species of both native and non-native origins from plant nursery stock or collections (see figure 5) and dry-stored until the start of the experiment. In mid-August 2018, I checked all seeds for the presence of an embryo using a float test (Baskin & Baskin, 2014), and imbibed them in distilled water for 20 hours.I then, randomly divided seeds of each species into cohorts of 15-20, depending on seed availability, and placed them onto wetted sterile sand in 8 cm plastic petri dishes. I assigned three replicates of each cohort to a combination of stratification duration (10 levels: 0, 14, 28, 35, 42, 49, 56, 63, 77, 100 days) and incubation (2 levels, low temperature: 20 °C day/10 °C night or high: 25 °C day/15 °C night) treatments, making for a 20-level full factorial treatment design. For stratification, I wrapped petri dishes in aluminum foil and placed them in a germination chamber in the dark at 4 °C. At the end of each stratification duration, I transferred

cohorts to incubation conditions in growth chambers. I observed germination fractions every other day for 25 days, and checked petri dishes regularly and moistened as needed. By measuring the germination fraction over time, I will generate germination time courses, plots describing changes in germination percentage over time, for each species and each treatment.

Statistical analysis: Using the germination time courses for each species, I will calculate the rate of change for T_b as a function of stratification duration. From these data, I will assess each species sensitivity to stratification, and use this information to predict how germination rank may change under different stratification scenarios. I will also examine whether species with different characteristics such as life history and habitat requirements differ in the strength of their response to the environmental treatments.

Status/preliminary results

Project Status: The experimental procedures are underway, and expected to conclude in December 2018.

OEGREs: A meta-analysis of Observed Environmental Germination Responses in Experiments

Introduction and Questions:

Seed germination is a critical life history stage for plant life, and as such, there is a long history, over 2000 years, of germination research (Baskin & Baskin, 2014; Fenner, 2000). More comptemorary work has produced a large body of literature detailing the germination requirements and dormancy classes (physiological, morphological, physical, morpho-physiological dormancy and their subclasses) for a vast number of species across an array of taxonomic and geographic space (see Baskin & Baskin (2014) for examples). Many comprehensive books and review papers have been written on the subject, and germination research continues at a rapid pace around the globe, but there are still large gaps in our knowledge (Baskin & Baskin, 2014). As mentioned above, our understanding of kinetics of the germination response to environmental conditions remain in its infancy. Without a better understanding of a more complete range of germination responses to different environmental states, it is difficult to predict the extent of climate change impact on plant regeneration. While few individual studies systematically investigate responses to a wide range of environmental conditions, the large body of germination literature could be leveraged to this end. In this chapter, I propose a meta-analysis to more broadly address the questions I laid out in chapter II:

1. How do varying environmental treatments effect the germination time courses of plant species?
2. How do various environmental treatments interact to effect seed germination?
3. Are there any broad patterns in the germination response to variable environmental conditions at the phylogenetic, geographic, or life-history level?

In addition to these fundamental biological question I will also use this project to address several important questions about germination research methodologies.

Proposed methods:

Due to the acknowledged importance of temperature in mediating seed dormancy and germination, my primary interest is to evaluate the effects of stratification and incubation treatments on germination, but I intend to also include other environmental factors, such as water status, soil properties, afterripening, and scarification treatments in my analysis.

Data collection: I performed a search of the Web of Science database using the keywords "germination" and "stratification", and excluded meeting abstracts, abstracts of published items (abstract only) and proceedings papers. The search returned 1,208 papers. For each paper, I read the abstract, methods and

figures to identify studies that fit for inclusion in my analysis.

Inclusion criteria: To be included in the study, papers were required to:

- Report a temporal germination response in addition to a final germination percentage.
- Manipulate a temperature variable (cold or warm stratification).

Currently I exlcued papers that were not fully accessible were also from my analysis, but intend to access them through the inter-library loan system.

Because of the large volume of papers, I will randomly sample 200 papers that meet the selection criteria to build a database, using ImageJ to scrape data from the relevant figures. The response variables I will capture include any measurement or index of germination rate as well as final germination percentages. The predictors I capture in addition to the specific environmental treatments in the paper will include: seed provenance (continent, latitude, longitude, altitude, biome), seed age, year of collection and maternal environment, dormancy class (if applicable), non-environmental treatments (chemical application), life history and population native status.

Analysis: Upon completion of the database, I will use a multilevel, bayesian modeling framework to assess the impact of varying temperature regimes on germination behavior. I intend to run several models with different grouping factors, including taxonomic and regional. I will also query the database to address the methodological questions addressed above.

Project status:

As of August 16, I have evaluated 610 papers of which 241 were determined fit for inclusion. Assuming this 39.5 percent inclusion rate remains consistent, I expect that 450-500 studies will be fit for inclusion.

Chapter IV: Seasonal priority effects: Germination phenology as a mediator of plant competition

Introduction

Priority effects, a class of interspecific interaction in which the effect of species on one another depends on the order in which they arrive at a site, are a cornerstone of community assembly theory (Fukami, 2015). These historical contingencies have been shown to alter the structure and function of communities, driving communities to alternate stable states (Fukami & Nakajima, 2011). Recent theory has suggested that it is not just species' arrival times that determine the course of interactions, but significant priority effects can be determined by the phenological differences between species already resident to a site, a subcategory of historical contingency known as seasonal priority effects (SPE) (Wainwright *et al.*, 2012). Like traditional priority effects, SPE's can operate though niche preemption, in which the species with earlier phenology reduce the amount of resources available for species with more delayed phenological activity, or niche modification, in which earlier phenological initiators modify the environment, determining the growth conditions that the later species will experience (Fukami, 2015).

Most of the evidence for seasonal priority effects comes from observation studies correlating earlier phenology with competitive dominance or invasion success (Gioria *et al.*, 2018). However, early phenology may be associated with other traits associated with superior competitive ability (Dickson *et al.*, 2012), and the strength of priority effects have been shown to vary based on species identities (Cleland *et al.*, 2015) and competition environments (Kardol *et al.*, 2013). While some studies have succeeded in experimentally linked seasonal priority effects with competitive dominance (Wainwright *et al.*, 2012), or inferred seasonal priority effects through sequential planting studies (Koerner *et al.*, 2008), to my knowledge, the relative strength of SPE's influence on competition between species has yet to have been quantified in any systems.

With the evidence for significant interspecific differences in phenological sensitivity to variable climates, it is likely that climate change will alter the germination lag times or even rank between species. If SPE's do

indeed significantly mediate species interactions, these phenological shifts would be expected to have strong implications for community dynamics. In this final chapter I ask:

- What are the effects of varying SPE's on the competitive dynamics between two species in a controlled environment?

Proposed Methods

Based on results from chapter II, I will select two species for a pairwise competition experiment. The two species will be selected based on the following criteria:

1. They have similar growth requirements and would be likely to be found in the same habitat in nature.
2. Their germination rank changes or the lag between their respective germination phenology shifts by more than 10-15 days given different stratification/incubation combinations.

I will sow seeds of each species in a soil medium at varying relative and overall densities following a response surface design detailed in Inouye (2001), (see figure 6). This design has been shown to be the most effective for differentiating between the effects of intra vs. interspecific competition and integrating data with theoretical models of competition. I will randomly assign replicates of each density to two different stratification/incubation regimes that have previously been show to alter the germination rank of the species, thus manipulating the strength of the seasonal priority effects in the competitive system.

After the given stratification period and 25 days of incubation, I will transfer all pots to a greenhouse for the duration of the experiment (12 weeks). Every 4 weeks, I will measure the height of each plant amd take standardized photos of each pot to allow for an estimation of percent cover of each species. The measurement will be applied for a biomass estimation using models found in Axmanová *et al.* (2012). At each measurement interval, I will measure, harvest, dry and weigh five plants of each species, not included in the response surface to better calibrate the biomass models. At the conclusion of the experiment, I will harvest, dry and weigh all plants for a final biomass calculation.

Analysis: I will use the repeat measures of biomass to calculate and compare the relative growth rate (RGR) (Connolly & Wayne, 2005) of each species under the different priority effect manipulations. I predict that first species to germinate in each treatment will have a higher relative growth rate and suppress the growth rate of the second species. If no switch in germination rank is possible, I expect more pronounced priority effect (great lag between germination), to produce a greater differential in relative growth rate than the weaker priority effect treatment.

Timeline

Time	Task
Fall 2018	
ch.1	Write article for part 2
ch.2	Complete experiment data collection
ch.3	Complete OEGRES initial inclusion survey
Spring 2019	
ch.1	Analysis of part 2
ch.2	Data analysis
ch.3	Begin data scraping
ch.4	Select species and treatment for response surface
Summer 2019	
ch.1	Write article for part 2
ch.2	Continue data analysis
ch.3	Continue data scraping
ch.4	Continue preparations for response surface trials
Fall 2019	
ch.2	Continue data analysis
ch.3	Continue data scraping
ch.4	Initiate treatments, begin competition experiment
Spring 2020	
ch.2	Data analysis
ch.3	Data cleaning
ch.4	Continue experiment
Summer 2020	
ch.2	Conclude Data analysis
ch.3	Data cleaning
ch.4	Begin data analysis
Fall 2020	
ch.2	Write article
ch.3	Modeling
ch.4	Continue data analysis
Spring 2021	
ch.3	Continue Modeling
ch.4	Continue data analysis
Summer 2021	
ch.3	Write article
ch.4	Continue data analysis
Fall 2021	
ch.4	Write article
Spring 2022	Defense

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Figures

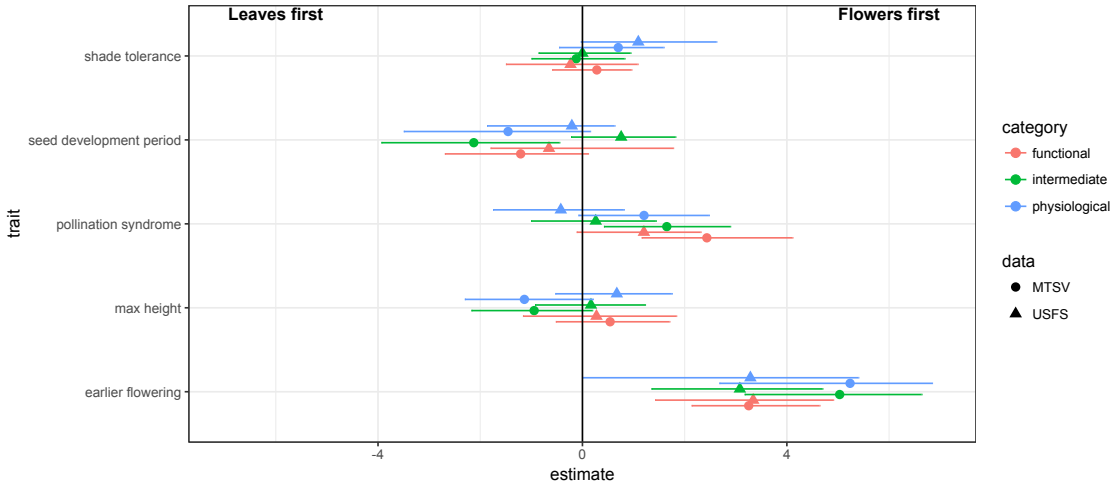

Figure 1: Predictor effect size comparisons (means and 95% bootstrap intervals, scaled predictors) with two different data sources (USFS and MTSV) and three different binary classifications of flower-leaf sequences (flower first= 1, leaf first= 0). Results are sensitive to both data source and modeling choices.

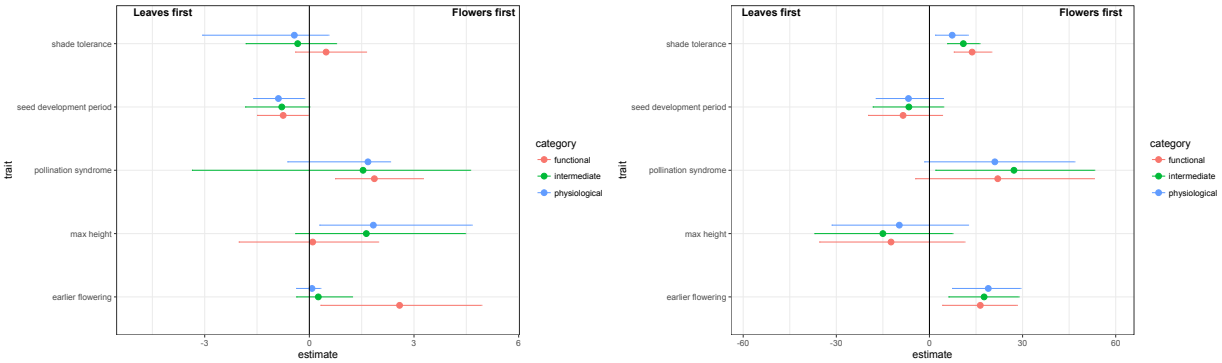

Figure 2: Model effect sizes (means and 95% bootstrap intervals) for a model using scaled MTSV predictors with mean FLS offset (in days) for overlapping species from Harvard Forest with three different classifications of FLS. In panel (A) mean offset was re-coded as a binary response (offset < 0 = seranthous, > 0 hysteranthous) and and in panel (B) mean offset in days is continous data. Treating FLS data as continuous reduces model sensitivity to FLS classification choices.

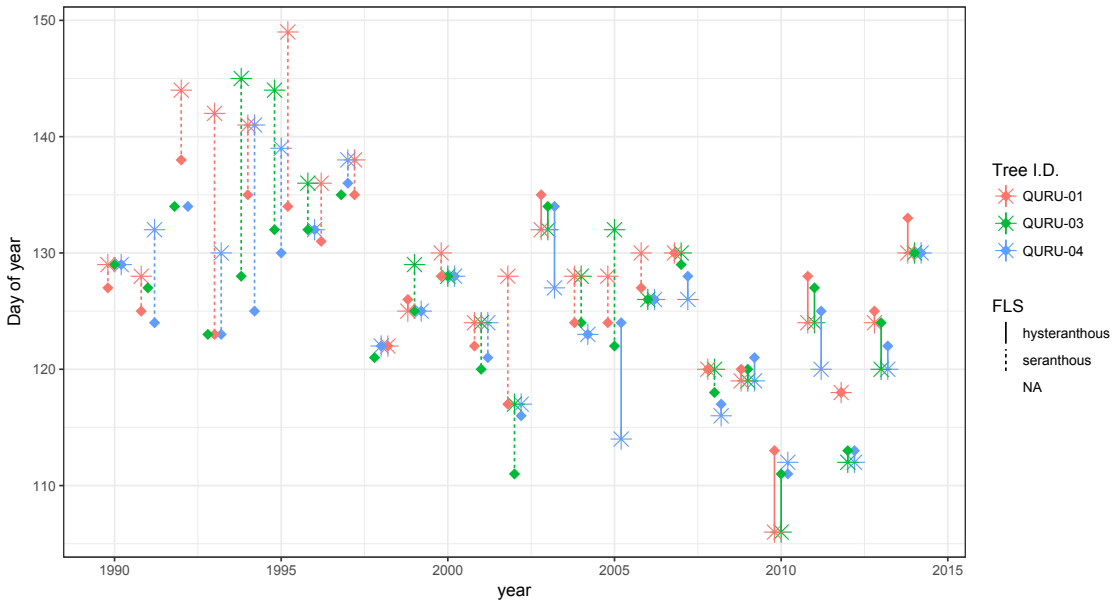

Figure 3: Plots showing interannual variability in FLS for three *Quercus rubra* individuals at Harvard Forest from 1990-2015. Red points indicated flower budburst, and green circles leaf budburst. Solid offset lines indicate years of flowering buds bursting first (hysteranthy) and dashed lines indicate years in which leaf buds burst first (seranthy)

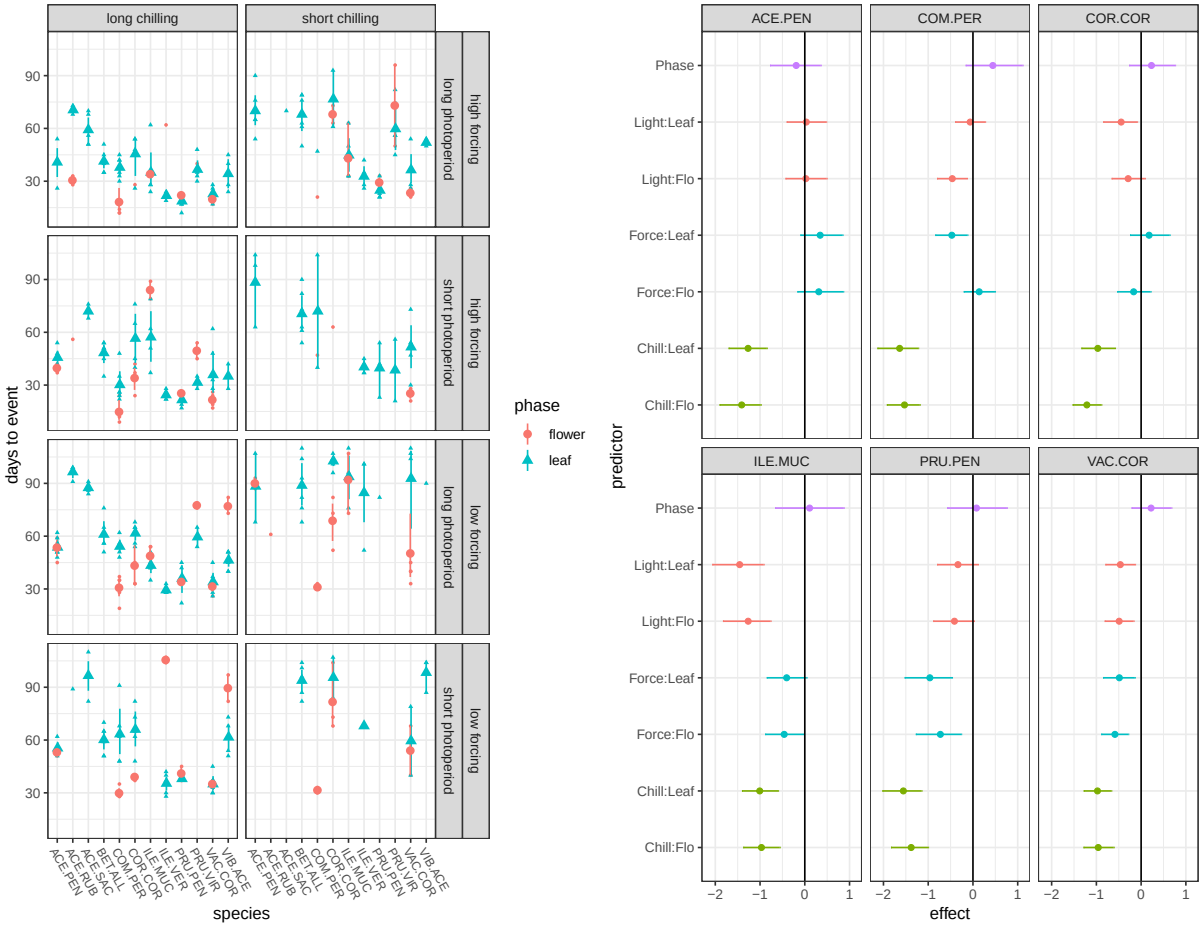

Figure 4: FLS variability (mean and standard deviation flowering and leafout time) for 12 species under different temperature and phtotoperiod regimes.

Species	Provenance	Life history	Habitat
Achillea millefolium	New Jersey	perennial	O/E
Anemone virginiana	Minnesota	perennial	E
Asclepias syriaca	New York	perennial	O
Bromus latiglumus	Minnesota	perennial	E
Carex grayi	Minnesota	perennial	E/F
Carex grisea	Minnesota	perennial	E/F
Centurea cyanus	U.S.	annual	O
Cryptotaenia canadensis	Minnesota	perennial	E/F
Eurybia divaircata	New York	perennial	E/F
Hesperis matronalis	U.S.	biennial	O/E
Hieracium kalmii	New York	perennial	O/E
Impatiens capensis	Minnesota	annual	E/F
Oenothera biennis	New York	biennial	O/E
Phlox divircata	Texas	Perennial	E/F
Polygonum virginianum	Minnesota	Perennial	E/F
Silene stellata	Minnesota	Perennia	E/F
Silene vulgaris	Massachusetts	Perennial	O/E
Solidago altissima	New York	Perennial	O/E
Solidago juncea	New York	Perennial	O/E
Thalictrim dioicum	Minnesota	Perennial	E/F

Figure 5: Species information: species, seed provenance, life history, and habitat preference (O=Open, E=Edge, F=Forested)

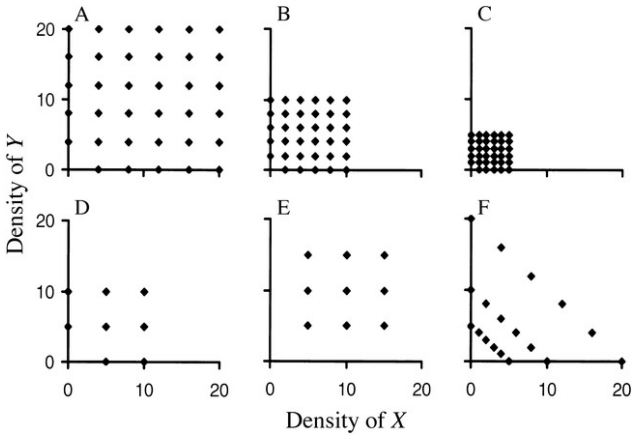

Figure 6: Example of different response surface experimental designs from Inouye, 2001.