

Competition between native *Cryptotaenia canadensis* and invasive *Hesperis matronalis* seedlings is mediated by relative germination timing

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A central tenet of community assembly theory is that the order of arrival of species to a community mediates inter-specific interactions and can dictate the trajectory of community structure in the long term (Fukami, 2015). These historical contingencies, known as priority effects, have been shown to alter the structure and function of communities, driving communities to alternate stable states (Fukami & Nakajima, 2011).

In many ecosystems, plant communities must re-assemble each year after a period of dormancy. In these communities, priority effects are the products of phenology, the timing of seasonal life cycle events, and the rate at which dormant plants and seeds respond to their environment and resume growth or germinate when favorable conditions return (Rudolf, 2019). These seasonal, or short-term priority effects (Wainwright *et al.*, 2011; Young *et al.*, 2017), may be important mediators of plant interactions, and have been invoked to explain the competitive dominance of some species (when strong competitors also have rapid/early germination) (Gioria *et al.*, 2018), and inter-specific coexistence (when weaker competitors have rapid/early germination and/or priority varies over time) (Towers *et al.*, 2020).

Invasive plants are often characterized by rapid germination and precocious phenology under a wide variety of environmental condition (Gioria *et al.*, 2018; Gioria & Pyšek, 2017; Wolkovich & Cleland, 2011; Smith, 2013). By contrast, in many temperate regions of North America with seasonal climates, native plants, especially perennials, produce seed with physiological dormancy that is released by exposure to environmental cues including temperature (both cool stratification temperatures to break dormancy and warm incubation temperatures to stimulate germination), moisture and light availability (Bewley, 1997; Fenner, 2000).

These differences in germination physiology suggest that the germination phenology of invasive relative to their native competitors manifests a seasonal priority effects, contributing significantly to the competitive abilities, and ultimately invasion success of invasive plants by allowing them to begin drawing down seasonal resources and modifying their environment before their native competitors emerge (Kardol *et al.*, 2013).

Yet, it is difficult to quantify the contribution of seasonal priority effects to the competitive success of invaders. Germination is notorious difficult to monitor in the field, and rapid phenology often co-varies with other competitive traits such as high fecundity, growth rates, and stress tolerance () and the relative importance of of germination phenology to invasion success, and competition dynamics in general is unknown.

Understanding the role that differences in germination phenology plays in mediating the dynamics inter-specific competition is critical for predicting and managing the structure and function of plant communities in the face of anthropogenic climate change. The link between climate and germination dynamics is certain, and because of inter-specific differences in germination response to environmental variation, the sustained alterations to environmental conditions are already shifting-community wide patterns of germination. If the patterns are indeed tightly link the the competitive dynamics of communities than phenological reorganization is likely to shift balances of species' interactions, change patterns of invasion, and strongly influence biological filtering of communities.

To address this gap, we leveraged the difference in species germination response to quantifying how the phenological lag between species changes under realistic climate variation. First we performed a series of germination assays in controlled environments temperature regimes to estimate a realistic range of climate-driven variation in germination phenology between an widespread invasive and native forb species. With this understanding in hand, we then performed competition trial for these species under contrasting environmental regimes to manipulate the relative germination phenology and quantify the contribution of seasonal priority effect to competitive dynamics among them.

Methods

0.1 Focal species

Dames Rocket (*Hesperis matronalis*) is a herbaceous biennial/perennial species originally from Eurasia, and introduced to North America in the 19th century (?) It can rapidly invade meadows, forest edges and woodland, forming dense, monotypic stands and excluding native vegetation (). It is currently listed as a noxious or invasive weed is several states and provinces in the United States and Canada. Honewort (*Cryptotania canadensis*) is a herbaceous perennial in the *Apiaceae* family, native to forests and woodland of North America. The habitat overlap of these two species suggests that they may compete in nature. While their apparent niche may be similar, the two species display a substantial different germination niche, making them a suitable model for our study. *C. canadensis* seeds are classified with non-deep physiological dormancy and require a substantial period of cold moist stratification to release dormancy and initiate germination. While some reports suggests that cold stratification enhances germination in *H. matronalis* at low incubation temperatures () several studies have demonstrated that this fresh and after-ripened (dry-stored) seeds of *H. matronalis* are capable of rapid and complete germination at average spring temperatures in the temperate zone (). The dynamics suggest that the phenolgical differences in germination among the species have potential to be strong mediated by cold stratification and incubation.

0.2 Germination Assays

To investigate the relationship between environmental variation and relative germination timing between our two focal species, we obtained seeds of *C. canadensis* from Prairie Moon Nursery (Winona, MN) and seed of *H. matronalis* from American Meadows (Shelburne, VT). We performed germination assays in the growth facilities of Arnold Arboretum in Boston MA (42.3074N, 71.1208W). We assigned seeds to a fully crossed set of

twenty experimental treatments; 10 levels of cold stratification duration (0,14,28,35,42,49,56,63,77,91 days at 4C) , two levels of incubation temperature (warm— 25C:15C (day/night), cool— 20C:10C (day/night)).

Prior to applying experimental treatments we applied a “float test” in which all seeds were placed in distilled water and unfilled seeds (floating) were removed from the experiment (Baskin & Baskin, 2014). The remaining seeds were imbibed in distilled water for 24 hours after which we placed 20 seeds per species/ treatment combination in petri dish on moist pool filter sand. We replicated each treatment combination three times. For the cold stratification treatments, we wrapped petri dishes in aluminum foil to prevent light exposure and placed them in a growth chamber at 4C. After each stratification interval, we transferred the petri dishes to their assigned incubation chamber for 25 days, moistening the germination substrate as necessary to maintain maximum saturation of the medium without flooding the seeds. We check for new germinates every 2 days, defining a seed as germinated when its radical or cotyledon tissue was visible (Baskin & Baskin, 2014). We assessed the viability of any seeds that did not germinate in the 25 day incubation trial by performing a “crush test” in which we applied pressure to the intact seed to evaluate its condition (Baskin & Baskin, 2014). We excluded any subsequent seeds deemed unviable from all subsequent analyses. Due to the staggering of our stratification treatments the experiment took place between 27 August- 12 December 2018.

Statistical analysis

To assess interspecific differences in the relationship between germination rate and temperature variability, In addition to reporting descriptive statistics for each treatment (maximum germination, mean germination time). we fit a Bayesian mixed-effect accelerated failure time model (AFT) with weeks of stratification and incubation temperature and species as fixed effects. We chose an AFT model as it allowed for us to account for viable seeds that did not germinate during our incubation window letting us robustly compare germination timing (t50 time to 50% germination) even among treatments with different final germination percentages in the time of the experiment (Soltani *et al.*, 2015). One drawback of this approach is that these classes models assume that all viable seeds will eventually germination, an assumption which we would not expect to be true in nature. For this reason, we considered any estimate t50 values greater than 60 days to suggest that particular seed lot would not reach 50% germination under those conditions.

We fit the model using the R package “brms” (?) using a weibull distribution for the model’s likelihood function. We ran the model on four chains with 4000 iterations and a 3000 iteration warm up for a total of 4000 posterior draws for each parameter using weakly informative priors. We assessed model performance through ensuring \hat{R} s were between 1 and 1.01 and bulk and tail effective sample sizes were high.

0.3 Competition Trials

To quantify the contribution of SPEs to inter-specific competition dynamics, we chose two species from our germination trial *Cryptotaenia canadensis* and *Hesperis matronalis* for competition trials. We chose these species because the germination of *C. canadensis* advanced strongly with increasing cold stratification, while seeds of *H. matronalis* germinated rapidly under all conditions suggesting that under low stratification treat-

ments there would be a strong priority effect between the species that would diminish as stratification time increased. Additionally *H. matronalis*, originally from Eurasia, is considered an invasive species or noxious weed in many parts of North America and so evaluating the role of SPE's in this species's competitive ability has potential applied benefits for the management of this species.

Competition trials took place under controlled condition in a research greenhouse at the Arnold Arboretum in October 2020-February 2021. We planted seeds into 3.5 in square pots, employing a response surface design where we varied both the overall density of seeds and proportion of each species in each pot (Inouye, 2001). High and low density treatments consisted of 14 and 8 seeds respectively. Our proportion treatments (100:0%, 25:75%, 50:50%, 75:25%, 0:100% (species A :species B)) Each density by proportion treatment was replicated six times. This design allows us to evaluate effects of inter- and intra- specific competition and density dependence independently and in association with our experimental treatment.

To test the effects of temporal priority on plant growth, we randomly assigned half of the pots low (45 days) and high (72 days) cold stratification treatments at 4C. We staggered the start of the treatments, so that at the conclusion of the pre-treatment, all pots were transferred to a heated greenhouse maintained at 15-25 C with 14 hours of supplemental light. Germination was observed daily from 24 December- Jan 13 and every two days from 15 Jan to 1 Feb. The locations of each pot in the greenhouse were randomly reassigned every 3 days to minimize any blocking effects on germination or growth.

After 35 days, we added 1 tsp per 1 gallon of water of Peter's 20-10-20 liquid feed fertilizer to all pots. After 62 days, we harvested the above group biomass from all pots, dried them in a oven for 48 hours at 60C, and recorded the dry weight of each species/pot using a Mettler balance.

Statistical analysis

We quantified the temporal priority between the species by subtracting the mean germination time (MGT) of *H. matronalis* from that of *C. canadensis* in each pot. This allowed us to apply priority treatment as a regression design () with priority levels ranging from -1.3 to 9.5 (*C. canadensis* mean germination time 1.3 days earlier to 9.5 days later than that of *matronalis*).

We assessed the impact of competitor density and relative germination timing on species biomass with two different frameworks.

First we calculated the average per-capita biomass of each species per pot, by divided each species' plot level biomass by its number of germinants. We then set up a system of pair-wise equations in which the per-capita biomass of each species was regressed against the density of conspecific seeds, density of competitor seeds, and the difference in mean germination time among them using Bayesian linear models. The equation is written below.

$$\text{biomass}_{C.canadensis} = \alpha + \beta_1 * \text{density}_{C.canadensis} + \beta_2 * \text{density}_{H.matronalis} + \beta_3 * \delta \text{MGT} + \epsilon$$

$$\text{biomass}_{H.matronalis} = \alpha + \beta_1 * \text{density}_{C.canadensis} + \beta_2 * \text{density}_{H.matronalis} + \beta_3 * \delta \text{MGT} + \epsilon$$

In the formulation, the β_1 is the estimated effect of intra-specific competition, β_2 is the estimated effect of inter-specific competition, and β_3 is the estimated priority effect. From these estimates we calculated the competition coefficients (c) (ratio of intra- to inter-specific competition strength) for each species, with and without the additive effects of germination phenology differences.

$$c_{sp1} = \frac{b_1}{b_2}$$

$$c_{prioritysp1} = \frac{(b_1 + b_3)}{b_2}$$

We also assessed difference in biomass among density and phenology treatments by calculating the relative growth rate differences among species at the plot level . Like the per capita methods described above, this metric can be used to quantify the effects of initial species' abundance, species identity and the environment on competition outcomes, but is designed to evaluate biomass composition at the community level (Connolly & Wayne, 2005).

For each plot, we calculated the relative growth rate difference among species using the equation below modified from Connolly & Wayne (2005).

$$\text{RGRD} = \ln\left(\frac{Y_{Cc}}{y_{Cc}}\right) - \ln\left(\frac{Y_{Hm}}{y_{Hm}}\right)$$

where Y_{Hm} and Y_{Cc} are the final biomass of the species at the end of the experiment and y_{Hm} and y_{Cc} are the initial biomass of the seeds planted at the outset of the experiment. In this formulation α is an un-interpretable intercept (Connolly & Wayne, 2005)

We then modeled the effect of seed density of *C. canadensis*, *H. matronalis* and δ mean germination time using Bayesian linear regression.

$$\text{RGRD} = \alpha + \beta_1 y_{Hm} + \beta_2 y_{Cc} + \beta_3 \text{MGT} + \epsilon$$

where β_1 and β_2 are the estimated effect of changing the initial biomass of seeds of each species on the RGRD, and β_3 is the effect of increasing the difference in MGT between *H. matronalis* and *C. canadensis*.

Both models were run using the R packages “brms” using weakly informative prior. We ran all models of 4 chains, with a 400 iterations per chain and a warm up of 3000 iterations, for a total of 4,000 posterior samples per parameter.

Results

Relative germination phenology

In our germination assays, *H. matronalis* reach 50% germination in under five days for all environmental treatments, always exceeded 85% germination regardless of environmental conditions (Fig. ??). Increasing cold stratification duration and incubation temperature only marginally enhanced the germination rate of this species (Fig. ??). By contrast, increasing incubation temperature had a negative effect of the germination rate of *C. canadensis*, suggesting that the mean 20C temperatures of our warm incubation treatment are supra-optimal for the species (Fig. ??). Without sufficient cold stratification (<5 weeks for low incubation and < 7 weeks for high incubation temperatures), seeds of *C. canadensis* did not reach 50% germination during the duration of our experiment (Fig. ??). However, under high levels of cold stratification, (>7 weeks with cool incubation) germination rates of *C. canadensis* began to converge on those of *H. matronalis*, and at levels of stratification greater than 10-12 weeks and low incubation temperatures, the germination rate and fraction of *C. canadensis* was well matched to that of *H. matronalis* (Fig. ??).

Effects of relative germination phenology on competition

Both competitor density and phenological priority effects had a substantial influence on the competitive interactions between *C. canadensis* and *H. matronalis*. When considering the effects of competition of per capita growth rates of each species, in the absence of phenological priority (ie both species germinate at the same time) competition coefficients for both species were less than 1 ($C. canadensis=X$, $H. matronalis=Y$, Fig. 2), suggesting that intra-specific competition was strong than inter-specific competition, suggesting co-existence between the species (). However, with just an increase in one day of phenological priority, the competition coefficient for describing the effect of *H. matronalis* on the per capita growth of *C. canadensis* became positive (Z, Fig. 2), suggesting the eventual competitive exclusion of *C. canadensis*.

When considering our plot level metric relative growth rate difference among the species, we found increasing the density of *H. matronalis* seeds shifted the community composition towards *H. matronalis*, while increasing the density of *C. canadensis* seed shifted the community composition towards *C. canadensis*, the density effect of *C. canadensis* seeds was almost three times higher than that of *H. matronalis* (Fig. 3). Priority effects

shifted community composition strongly towards *H. matronalis*, and this effect was X times higher than the density effect of *H. matronalis* (Fig. 3).

Discussion

Germination as a seasonal priority effect

In this study, we found that climate driven differences in the germination timing among species has strong impacts on their competitive dynamics. In our study, it was differences in germination timing that tipped the interactions among species from potential coexistence to the path towards competitive exclusion by the invasive *H. matronalis*. We found that seasonal priority effects of germination phenology were considerably stronger than density effect of competitors, suggesting that precocious germination is indeed an important mechanism for competitive advantage.

Our results join a growing body of experiments demonstrating that relative germination phenology can function as a seasonal or short term priority effect, enhancing the performance of the earliest germinating species at the expense of later germinants (). While this effect has been primarily commonly demonstrated in experiments in which the planting of competing seeds is staggered at increasing intervals (), we were able to generate substantial variation in relative germination timing among our competing species though by operationalizing their differential sensitivity to environmental cues.

This link between environmental conditions, relative germination phenology and competitive dynamics suggest that shifts in environment due to anthropogenic climate change has potentially to strongly alter early stage competition among species with deeper dormancy like *C. candensis* and consistent rapid germinating species like *H. matronalis*. Because stratification conditions occur at intermediately low temperature (usually 0-10 ()), global warming is likely to increase the extent of stratification conditions in some locations, while decreasing it in others (). In regions with decreasing stratification, the rapid phenology of many invasives may allow them to exploit an unoccupied phenological niche, exerting stronger seasonal priority effects on the native community and increase the relative abundance. By contrast, in regions where stratification conditions are being maintained or increasing, native communities may be more likely to fill the early season phenological niche, more effectively resisting invasion from species with rapid germination phenology.

Considering the impacts of seasonal priority effects in forest communities

While most studies on seasonal priority effects focus on grassland environments with annual taxa () we explicitly conducted our experiment with forest perennials to better understand the generality of these effects to other ecological systems. While we found priority effects impacted within-year dynamics of seedlings, our experiment was not designed to the longevity of these priority effects on the longer term, among year dynamics of our focal species. Many studies suggest that these short term priority effects may be transient, though several studies that used staggered planting methods at similar scale to the phenological lags we observed in our trials saw the influence of these initial priority effect on community composition several seasons later (). In perennial communities, these long term dynamics are even more difficult to assess.

Many perennial forest herbs, *C. canadensis* included, rely heavily on vegetative reproduction (), and as such, the kind of seedling to seedling competition we produced in our experiment, may be less common, and therefore less important to overall community demography than competition among vegetative ramets, or between ramets and seeds ().

Additionally, in our experiment, there was no cost to germinating too early. It is generally accepted that optimum germination phenology is driven by a tradeoff between maximizing the growing season and the risk of exposure to damaging environmental episodes when germinating too early (). In dry grassland ecosystem, it has been demonstrated that the precocious germination of invasive has a substantial cost in water availability is too low (). In temperate forest ecosystems, the primary risk of early phenology is damage from late season frost (). Future work could further clarify the contribution of seasonal priority effects to forest community interactions by experimentally manipulating this trade-off as well.

Given these caveats, we cannot assert our study could accurately predict the long term outcomes of competition in average forest communities, even between our focal species. However, our findings are likely to be most critical use conditions where seedling competition may be most important, including colonizing new habitats through range shifts, recovering from large scale disturbances, and perhaps most importantly ecological restorations.

Phenological diversity in ecological restoration

Our pairwise competition study indicates the phenological differences in germination time can strongly influence competitive outcomes. While empirical work with more species will help, if phenology is a mechanism of competitive dominance and invasion, it can also be a mechanism of invasion resistance. There is an extensive literature

. Read a bit about the invasibility literature and suggest considering phenological diversity as a metric for assessing communities may be critical. Comparing across phases and life forms. I.e germinating seeds also competed with adults. This could be particularly important for design ecological restorations.

From germination requirements to germination responses

Both predicting germination dynamics as a response to climate change and design phenologically diverse ecological restoration communities will require a conceptual shift in how germination is studied and reported. Centuries of research of seed germination has greatly advanced our understanding of the ecology and evolution of different classes of seed dormancy and the environmental requirements that allow for dormancy break and ultimately germination (). This fundamental research is generally well integrated into applied field like plant propagation and nursery production (). Today it is almost universal for native plant growers to collect and provide information on the conditions needed to facilitate dormancy break and successful germination (). The guidance is often

References

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Figures

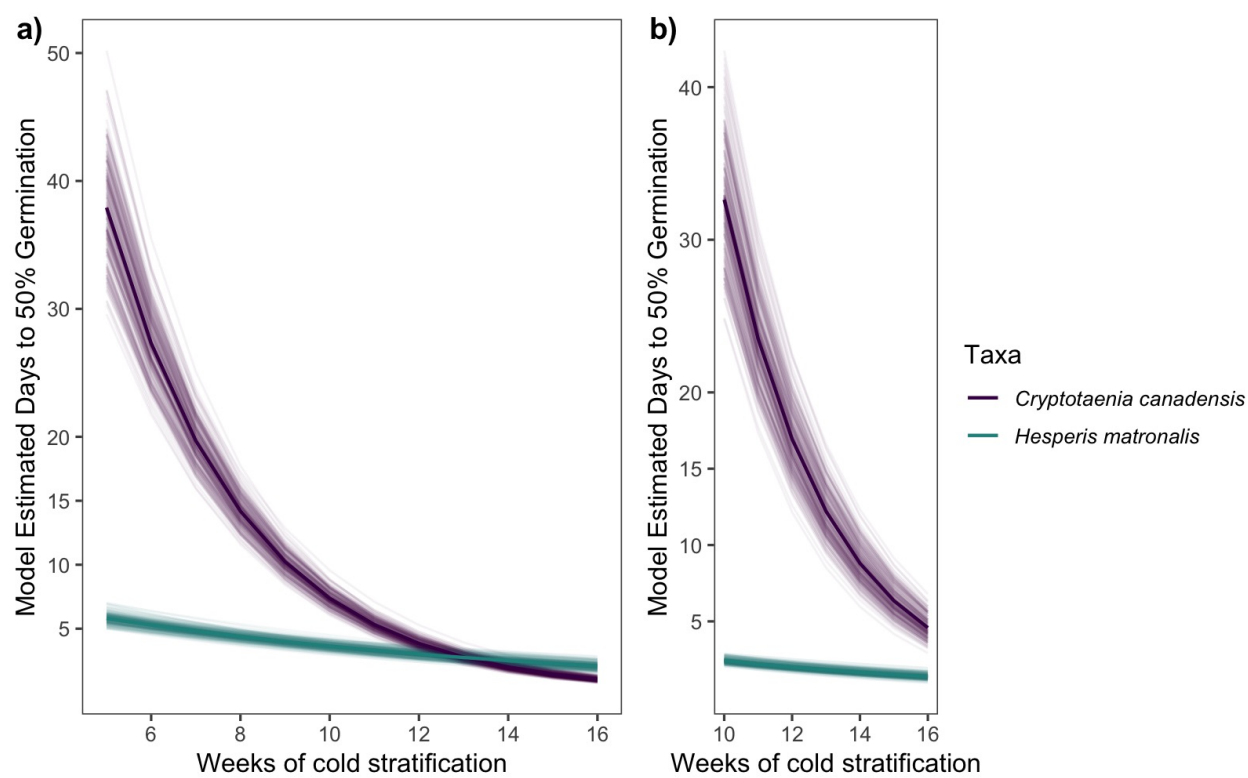


Figure 1: AFT

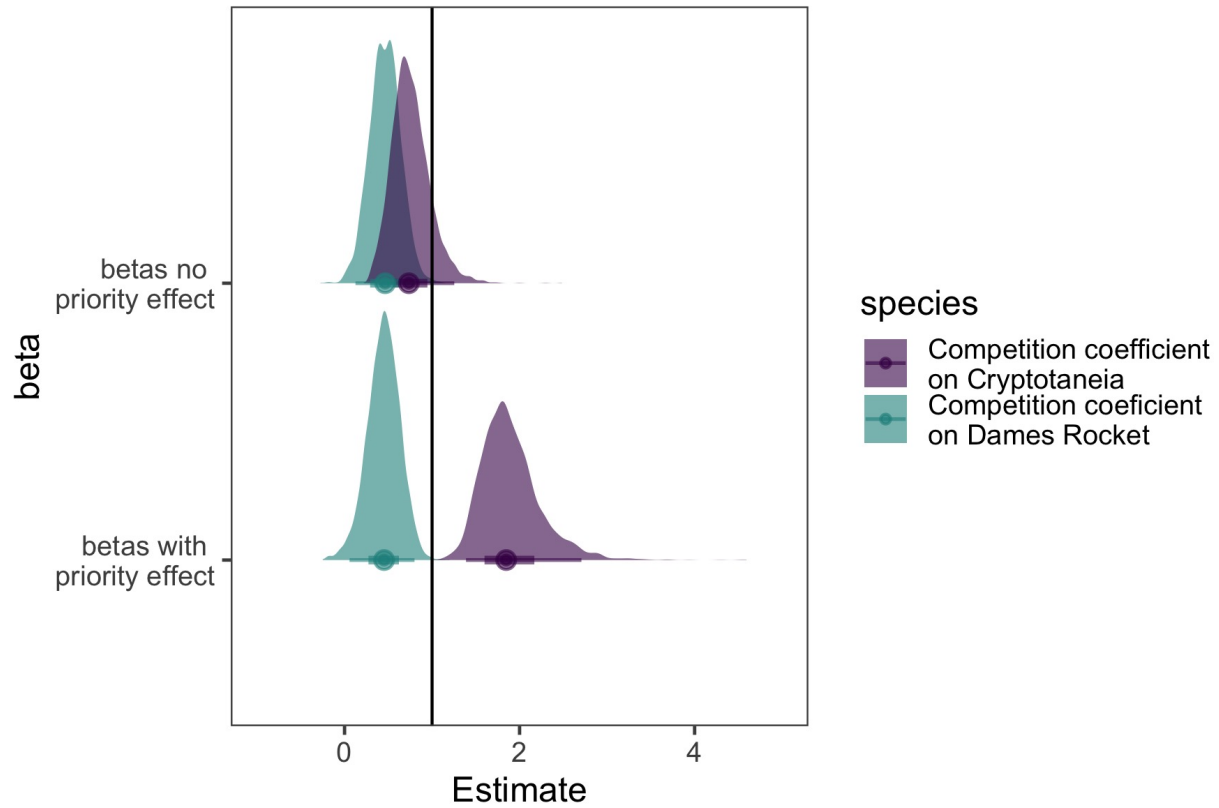


Figure 2: Estimated of competition coefficients with and without priority effect. Without priority effect, species would be expected to coexist (for both species, intra-specific competition is higher than interspecific i.e. coefficients are less than one). When just one day of priority effects are included in the calculation Dames rocket's interspecific competition increases relative to its intraspecific competition strength, suggesting it will ultimately competitively exclude Honewort.

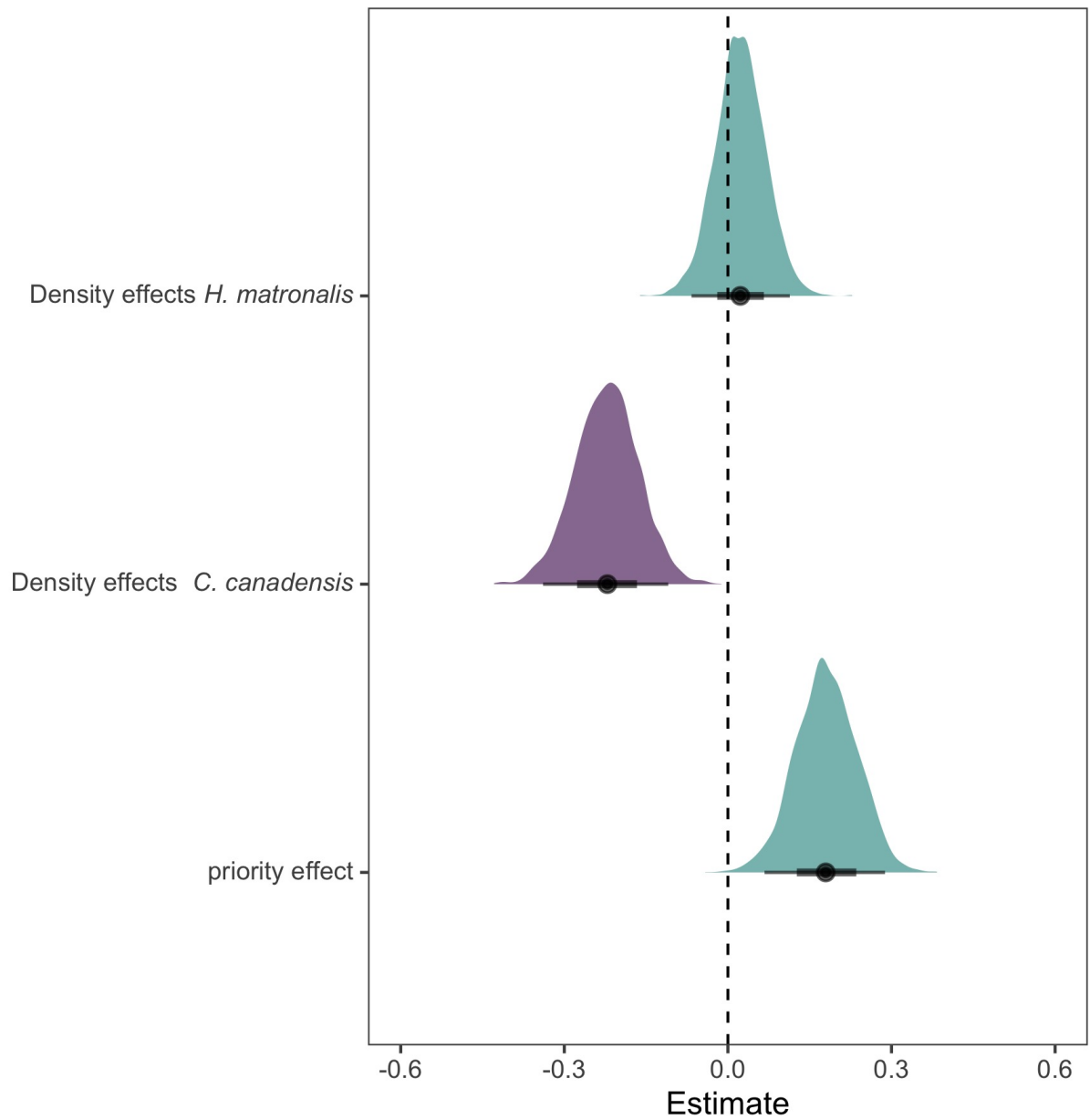


Figure 3: Estimated effects of density influence parameters and temporal priority effect on the relative growth rate difference between *H. matronalis* and *C. canadensis*. As per Connolly and Wayne 2005, The positive estimate of priority and density of *Hesperis matronalis* tip competitive balance towards it, while the negative estimate of density of *C. canadensis* favor that species. Like Fig. 2, this suggests that priority effects are a key mediator of competitive success in *H. matronalis*.

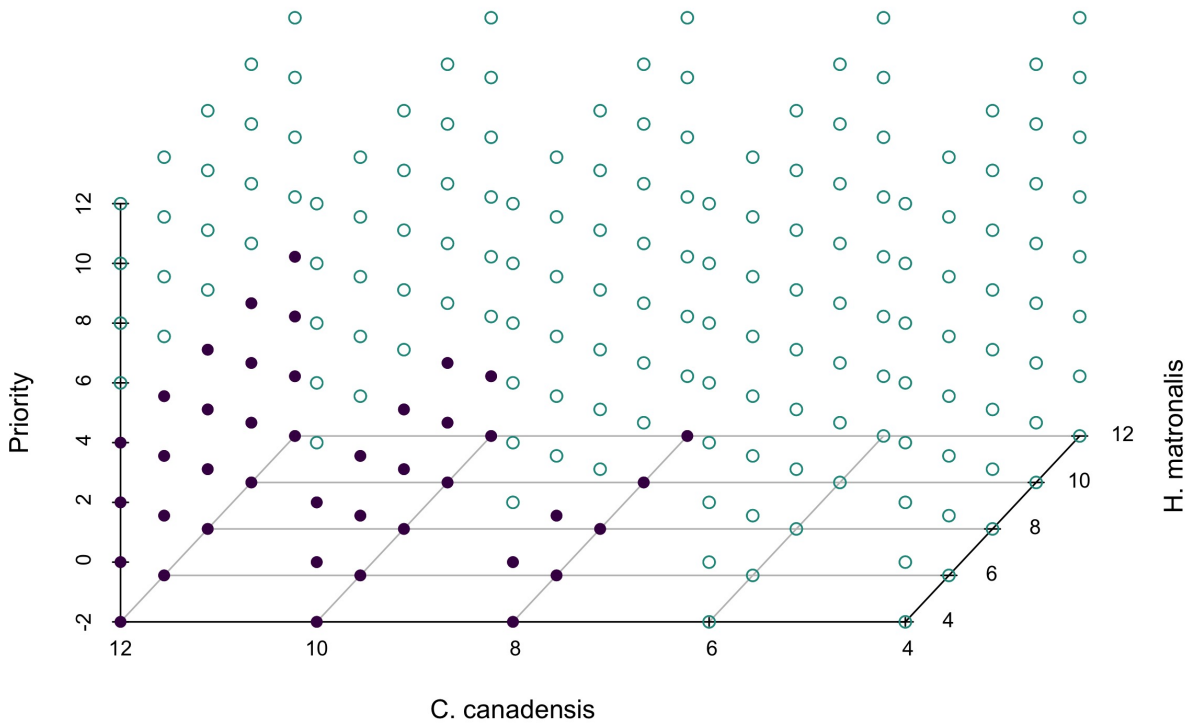


Figure 4: Predicted outcome of competition under vary inter-specific densities and temporal priority. Purple is *C. canadensis* and green *H. matronalis*. Need to add legend here. As can be seen *C. canadensis* is predicted to compete with *H. matronalis* only at low priority effects or high densities.