

1 Competition between native Honewort (*Cryptotaenia canadensis*)
2 and invasive Dame's Rocket (*Hesperis matronalis*) seedlings is
3 mediated by relative germination timing

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Abstract

Invasive plant species are often characterized by rapid germination and precocious phenology. Theory suggests that early germination may provide invaders with significant competitive advantage over slower germinating natives, but the relative contribution of rapid germination vs. other intrinsic competitive traits to the success of plant invaders is poorly characterized. Depending on the relationship between germination timing and competition, alterations to patterns of germination phenology due to anthropogenic climate change may either increase the dominance of invaders or buffer native communities against their impacts. Predicting invasion dynamics and the structure and function of plant communities of the future will require clarifying the relationship between climate variability, phenological differences among species and competitive outcomes.

To fill this gap, we performed a sequence of controlled environment experiments. First, we evaluated how variation in cold stratification duration and growing temperatures impacted the relative germination phenology of eleven temperate herbaceous species. We then leveraged these differences to indirectly manipulate the relative germination phenology of two species, the invasive *Hesperis matronalis* and native *Cryptotaenia canadensis*, planted in competition, in order to quantify the effects of their phenological differences on competitive outcomes.

We found that precocious germination phenology roughly doubled the competitive impact of *H. matronalis* relative to its intrinsic competitive traits, and that phenological advances of just two-three days relative to *C. canadensis* were enough to secure its competitive dominance at the seedling stage. We also found that the germination phenology of most native species in our study was strongly associated with cold stratification duration, suggesting that decreases in cold stratification conditions due to anthropogenic global warming will likely increase the phenological advantage of rapidly germinating invaders.

Add a sentence here. Our results suggest the phenological diversity may be an important factor for maintaining native plant communities into the future.

Introduction

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 long-term alternate stable states (Fukami & Nakajima, 2011). Yet 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, rather than the timing of the arrival of propagules, which in many cases occurs prior to the dormant season (Rudolf, 2019; Howe & Smallwood, 1982; Baskin & Baskin, 1988a).

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, native plants tend to exhibit more constrained germination cues (Marushia *et al.*, 2010). In many temperate systems, seeds of native plants are dispersed with deep physiological dormancy, requiring prolonged exposure to specific environmental conditions, such as cold stratification (cool temperatures 0-10

C) to break dormancy and stimulate germination (ten Brink *et al.*, 2013; Cavieres & Sierra-Almeida, 2017; Bradford & Nonogaki, 2007).

These differences in germination physiology can yield strong differences in the relative germination phenology of invasive and native plants, with invaders germinating well before their native competitors (Gioria & Pyšek, 2017). We refer to this difference in relative germination timing among species as **phenological advantage**. Phenological advantage can contribute 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). We refer to this effect of phenological advantage on interspecific competition as a **seasonal priority effect** (Wainwright *et al.*, 2011).

Yet, it is difficult to quantify the overall 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 (Dickson *et al.*, 2012; Milbau *et al.*, 2003; HAO *et al.*, 2009). One way that this problem has been addressed is through experiments that vary phenological advantage by sowing competing seeds at different time intervals (Young *et al.*, 2017). While these experiments have provided strong evidence that phenological advantage on the order of just days to weeks can yield substantial priority effects (Weidlich *et al.*, 2020), the highly artificial experimental set-up is difficult to translate into natural communities in which priority effects would be mediated by climate. As such, the relative importance of phenological advantage vs. other competitive traits to invasion success and competition dynamics in nature remains poorly characterized.

Understanding the role that phenological advantage plays in mediating the dynamics of interspecific competition is critical for predicting and managing the structure and function of plant communities in the face of anthropogenic climate change. Due to interspecific differences in germination responses to environmental variation, sustained alterations to environmental conditions are already shifting community-wide patterns of germination (Walck *et al.*, 2011). If patterns of germination are indeed tightly link the the competitive dynamics of communities than phenological re-organization is likely to shift the strength of species' interactions, change patterns of invasion, and strongly influence biological filtering of plant communities.

In this study, we leveraged among-species differences in germination phenological sensitivity to environmental variation in order generate contrasting levels of phenological advantage among competing species. This approach allowed us to assess the maximum variation in phenological advantage due to climate variation among co-occurring species and quantify the contribution of seasonal priority effects to the competitive outcomes of interacting species. First, we performed a series of germination assays in controlled environments under varying temperature regimes to estimate a realistic range of climate-driven variation in phenological advantage for a suite of widespread invasive and native forbs. We then performed competition trials for two focal species (the North American invasive *Hesperis matronalis* and native *Cryptotaenia canadensis*) under contrasting environmental conditions to indirectly manipulate the phenological advantage between them and quantify the contribution of seasonal priority effect to their competitive dynamics.

Through operationalizing unique species-level responses to environmental change, our experiments constrain the magnitude of variation in phenological advantage to biological realistic scenarios. This allow us to robustly estimate seasonal priority effects on plant competition in a way that is more readily translatable field conditions, and assess to what degree seasonal priority effects mechanistically contribute to the competitive success of invasive species. Through linking climate variation, phenological advantage and seasonal priority effects, our study has important implications for how anthropogenic climate change will alter phenological

assembly and, in turn, plant community interactions in the decades to come.

Methods

Focal species

Dames Rocket (*Hesperis matronalis*) is a herbaceous biennial/perennial species in the *Brassicaceae* family, originally from Eurasia, and introduced to North America in the 19th century (Francis *et al.*, 2009). It can rapidly invade meadows, forest edges and woodlands, forming thick stands and excluding native vegetation (Francis *et al.*, 2009). It is currently listed as a noxious or invasive weed in several states and provinces in the United States and Canada (Susko & Hussein, 2008). Honewort (*Cryptotania canadensis*) is a herbaceous perennial in the *Apiaceae* family, native to forests and woodlands of eastern North America (Hawkins *et al.*, 2007). The habitat overlap of these two species suggests that they may compete in nature. While their habitat requirements 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 deep physiological dormancy and require a substantial period of cold moist stratification to release dormancy and initiate germination (Baskin & Baskin, 1988b). While some reports suggest that cold stratification enhances germination in *H. matronalis* at low incubation temperatures, several studies have demonstrated that fresh and after-ripened (dry-stored) seeds of *H. matronalis* are capable of rapid and complete germination at a wide range of temperatures (Susko & Hussein, 2008). These contrasting germination dynamics among species suggest that phenological advantage between them is likely to be strong mediated by cold stratification and incubation variation.

Germination Assays

To investigate the relationship between environmental variation and relative germination timing between competing species, we obtained seeds of 14 spring-germinating herbaceous species common to temperate forest-edges, including our focal species *C. canadensis* and *H. matronalis*, from domestic plant nurseries (see Tab. ?? for details). 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 (0,2,4,5,6,7,8,9,11,13 weeks at 4C) and two levels of incubation temperature (warm— 25C:15C (day/night), cool— 20C:10C (day/night)).

Prior to applying experimental treatments we performed a “float test” in which all seeds were placed in distilled water, and unfilled seeds (floating) were removed from the experiment (Baskin & Baskin, 2014). We imbibed the remaining seeds in distilled water for 24 hours and then placed 20 seeds per species/ treatment combination in petri dishes 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 period by performing

a “crush test” in which we applied pressure to the intact seed to evaluate its condition (Baskin & Baskin, 2014). We excluded any 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 inter-specific differences in the relationship between germination rate and temperature variability, we fit a Bayesian mixed-effect accelerated failure time model (AFT) with weeks of stratification and incubation temperature as fixed effects and species as a random effect. Three species, (*Carex grisea*, *Impatiens capensis* and *Phlox cuspidata*), did not germinate at high enough fractions in our assays to analyze, so our final analysis consisted of 11 species.

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 or time to 50% germination) even among treatments with different final germination percentages in the time frame of the experiment (Soltani *et al.*, 2015). One drawback of this approach is that this class of models assume that all viable seeds will eventually germinate, which we would not expect to be true in nature. For this reason, we considered any estimated t50 values greater than 40 days to indicate that seeds would not reach 50% germination under those conditions.

In addition to our full species model described above, we fit an additional AFT model on a subset of data including only *C. canadensis* and *H. matronalis* in order to obtain higher resolution estimates for the germination dynamics for our two focal species. In this model we included species as a fixed effect in addition to incubation temperature and stratification duration.

We fit the models using the R package “brms” (Bürkner, 2018) 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.

Competition Trials

To quantify the contribution of seasonal priority effects to inter-specific competition dynamics of our focal species we performed competition trials under controlled condition in a research greenhouse at the Arnold Arboretum from October 2020-February 2021. We planted seeds of *C. canadensis* and *H. matronalis* into 3.5 inch 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 total 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.

We randomly assigned half of the pots low (45 days) and high (72 days) cold stratification treatments at 4

C. 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 15-25C with 14 hours of supplemental light. Germination was observed daily from 24 December 2020 - 13 January 2021 and every two days from 15 January 2021 to 01 February 2021. The locations of each pot in the greenhouse were randomly reassigned every three 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-ground biomass from all pots, dried it 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 phenological advantage 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 evaluate the effect phenological advantage with a regression design (Cottingham *et al.*, 2005), with advantage values 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*).

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. For this calculation we obtained estimates of seed mass for our focal species from the Kew Gardens Seed Information Database ().

We then modeled the effect of seedling density of *C. canadensis*, *H. matronalis* and phenological advantage using Bayesian linear regression using the R packages “brms” (Bürkner, 2018). Using weakly informative priors, we ran this model on 4 chains, with 4000 iterations per chain and a warm up of 3000 iterations, for a total of 4,000 posterior samples per parameter. The model is written below:

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

where β_1 and β_2 are the known as the species influence parameters, or the estimated effect of increasing the seedling density of each species by one individual on the RGRD (Connolly & Wayne, 2005), and β_3 is the

priority effect, or the effect of increasing the difference in MGT between *H. matronalis* and *C. canadensis*. In this formulation, α is an un-interpretable intercept (Connolly & Wayne, 2005).

To assess whether the rapid germination phenology of *H. matronalis* modified the germination niche of *C. canadensis* we performed two additional Bayesian regression analyses. We assessed the influence of planting type (single species vs. mixed competition) on the the likelihood of *C. canadensis* germination using a Bernoulli distribution and the mean germination time of *C. canadensis* using a Gaussian distribution. In both models we included stratification treatment as a fixed-effect co-variate. As above, we ran these models using the R packages “brms” (Bürkner, 2018), with weakly informative priors.

Results

Germination advantage

In our germination assays, cold stratification duration has a significant effect on germination phenology, advancing the time to 50% germination across species (mean: -0.14, $CI_{95}(-0.23, -0.067)$, all reported estimates are on the log scale), though the strength of this effect varied significantly among species (Fig. 1a., Fig. ??). The overall estimated effect of incubation temperature on germination phenology was weak (mean: 0.03, $CI_{95}(-0.06, 0.12)$) but this was due to strong inter-specific differences in the directional response to increasing incubation temperatures. The germination phenology of 5 species advanced with increasing incubation temperature, and was delayed for 6 species (Fig.1 b.).

Considering our focal species, *H. matronalis* reached 50% germination in under five days for all environmental treatments, always exceeded 75% germination regardless of environmental conditions (Fig. 2, Tab. ??). Increasing cold stratification duration and incubation temperature only marginally enhanced the germination rate of this species (Fig. 2). By contrast, increasing incubation temperature had a delaying 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. 2). 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. 2, Tab. ??). However, under high levels of cold stratification, (>7 weeks with cool incubation) germination rates of *C. canadensis* began to converge on those of *H. matronialis*, 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. matronialis* (Fig. 2, Tab. ??).

Given the strong inter-specific differences in phenological sensitivity to stratification and incubation, our results indicate that climate strongly shapes patterns of phenological assembly, and patterns of phenological advantage can highly variable due to climate variation.

Germination priority effects

In our competition trials, both competitor influence parameters and phenological priority effect had a substantial impact on the competitive interactions between *C. canadensis* and *H. matronalis*. The species influence parameters for *H. matronalis* and *C. canadensis* were 0.126 ($CI_{95}: 0.0767, 0.174$) and -0.40 ($CI_{95}: -0.474, -0.341$) respectively, indicating that with no phenological advantage, the influence of adding one individual of *H. matronalis* is almost 4x less than adding one individual of *C. canadensis* to the plot level RGRD. The estimated effect of phenological advantage (ie the priority effect) was 0.146 ($CI_{95}: 0.082, 0.209$) indicating that an increase of just one day of *H. matronalis* advantage has approximately the same influence on shifting the community biomass composition towards *H. matronalis* as adding an individual of that species to the community and weakens the species influence parameter of *C. canadensis* by 35% (Fig. 3, Tab. ??). Together, these result suggest that *H. matronalis* will come to dominate the community biomass composition unless *C. canadensis* is at high relative abundance or the phenological advantage of *H. matronalis* is small (Fig. 4).

Priority effects and germination niche modification

We found no significant differences in the final germination fraction or mean germination time of *C. canadensis* between monoculture and competition plots at both low or high levels of stratification (Fig. 5). These results do not provide evidence that the rapid germination of *H. matronalis* modified the germination environment of *C. canadensis*.

Discussion

Germination advantage as a seasonal priority effect

In this study, we found that climate driven differences in germination advantage was a key mediator of the competitive dynamics between our two focal species *H. matronalis* and *C. canadensis*. In the absence of phenological advantage, the species influence parameter estimates from our model (representing the intrinsic competitive abilities of seedlings of each species) suggest that *C. canadensis* is the stronger competitor (Fig. 3, the influence parameter of *C. canadensis* is significantly more negative than that of *H. matronalis* is positive). However, the influence of one day of phenological advantage for *H. matronalis* virtually doubled the influence of this species on the final community composition, suggesting that seasonal priority effects play a major in the competitive success of *H. matronalis* (Fig. 3). Using the species influence parameters and priority effect we estimated in our model we determined that *C. canadensis* can compete with the invasive *H. matronalis*, but only at high relative abundance levels and when phenological advantage is low (Fig. 4). While it is certainly possible that *H. matronalis* might interact differently with other species, the results of our pair-wise competition trials suggest that both seasonal priority effects manifested through rapid germination phenology and propagule pressure are mechanistically related to the competitive dominance, and ultimately invasion success of *H. matronalis*.

While we found that seasonal priority effects strongly impacted competition among seedlings, our experiment

was not able to quantify the role of seasonal priority effects in influencing the long-term, among year dynamics of these perennial species, which may be quite different from within-season seedling interactions (). 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 (Weidlich *et al.*, 2020). In perennial communities, these long term dynamics are even more difficult to assess as many perennial herbs, *C. canadensis* included, rely heavily on vegetative reproduction (Hawkins *et al.*, 2005). Because of this, the kind of seedling to seedling competition we observed 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 (). Understanding how phenological differences across life stages of long-lived perennial plants affects within season competition is an important next step for predicting how communities may be impacted by climate change.

Our competition trials did not suggest any evidence that the rapid germination phenology of *H. matronalis* impacted the germination niche of *C. canadensis* in any regard (Fig. 5). This indicates that the mechanism underlying the seasonal priority effect if *H. matronalis* is likely niche preemption, though we can not rule out the possibility that niche modification occurs at the seeding stage as well.

Environmental drivers of seasonal priority effect

Our results join a growing body of experiments demonstrating that relative germination phenology can function as a seasonal priority effect, enhancing the performance of the earliest germinating species at the expense of later germinants (Koerner *et al.*, 2008; Dickson *et al.*, 2012; Ross & Harper, 1972). While this effect has been primarily commonly demonstrated in experiments in which the planting of competing seeds is staggered at increasing intervals (Young *et al.*, 2017; Weidlich *et al.*, 2020), our experimental results advance the understanding of the role of seasonal priority effects on competition by identifying a natural mechanism, species' differential sensitivity to climate, that can generate seasonal priority effects.

We found that climate variation, especially the duration of cold stratification was tightly linked to mediating the phenological advantage between *H. matronalis* and *C. canadensis*, and that small changes in advantage, of as few two days (Fig. 3). Interestingly, the difference in phenological advantage among our focal species was much higher in our germination assays than in our competition trials even at comparable levels of stratification. There are likely several explanations for these differences.

Perhaps the most important is that we used different metrics of germination speed, time to 50% germination (t50) and mean germination time (MGT) in each experiment. While the metrics are related and often confused, there are important differences between them that make one or the other more appropriate for the two types of experiments we ran. The t50 is an estimate of the time to 50% germination of all seeds planted, while MGT is a measure of the time to 50% germination of only individuals that actually germinated (Soltani *et al.*, 2015). In comparative germination assays, t50 is a better metric because is standardized phenological estimates across variable germination fractions. In our competition trials it would not have made sense to account for the the germination speed of individuals that did not germinate, because only germinated individuals were influencing the competitive dynamics of our species. Because MGT is sensitive to the final germination fraction, it is not surprising the MGT measurements in the competition trials were lower than

the t50 estimates in the germination assays.

Additionally, the incubation temperatures in our greenhouse competition trials were more variable than in our growth chamber germination assays. The lower germination fractions we observed in *C. canadensis* under greenhouse conditions suggests that the temperature range was likely supra-optimal for this species, and the lower germination fraction increased the difference between t50 estimates and MGT measurements. Finally, germination assays and competition were conducted in different growth media (filter sand vs. potting soil), which have different moisture retention and light transmissible capacities. Germination media can strongly affect germination rates (), which may further explain differences among our two experiments.

However, despite these differences, the relationships between cold stratification germination phenology and phenological advantage were robust across our experiments, with increased cold stratification strongly advancing the germination phenology of *C. canadensis* and weakly that of *H. matronalis*, resulting in weaker phenological advantage at higher stratification levels. (Fig. 2, Fig. ??).

Seasonal priority effects and anthropogenic climate change

The implications for the role of climate variability in mediating seasonal priority effects is two fold. First, our results suggest that inter-annual climate variability should generate both among and within season variation in competition strength among species, potentially driving species coexistence via the storage effect (). Second, the key role we observed of climate in generating germination advantage and therefore seasonal priority effects suggests that sustained alteration to historic patterns of climate variability, like those driven by anthropogenic climate change, are likely to strongly alter the dynamics of competing seedlings which will have downstream effects on the structure and function of plant communities.

In our study, the priority advantage of *H. matronalis* was maximized under lower stratification treatments and warmer incubation temperatures. This suggests that the warming temperatures associated with anthropogenic climate change may increase the magnitude of seasonal priority effects, largely due to the delay of germination in more climate sensitive native species like *C. canadensis*.

Importantly, invaders invade plant communities, not single species populations, and while we were only able to quantify the impact of seasonal priority effects for two species, our multi-species germination assays allow us to make some inference about how patterns of germination advantage may shift at the community level. At low stratification duration, less climate sensitive invaders can exploit the early season germination niche with less competition from natives than at when stratification is higher. Projections based on our germination time models suggest, that under low stratification (6 weeks) that ratio of native to invasive species we studied that germinate well within the first 20 days of the climatic growing season is small (Fig. 6, 3:2 and 4:2 under cool (15C) and warm (20C) growing temperatures respectively), while under longer stratification duration (12 weeks), the ration is much higher (6:2 and 5:2 cool vs. warm) and the phenological advantage of the invaders significantly reduced (Fig. 6). While the conversion of phenological advantage into seasonal priority effects is likely species specific, the strong positive relationship between advantage and competitive impact we observed in our study suggests that the phenological advantage that comes with warmer conditions is likely to increase the impact of rapid germinating invaders through seasonal priority effects.

One important caveat is that in our experiment, there was no cost to germinating too early. It is generally

accepted that optimum germination phenology is driven by a trade-off 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 (Wainwright *et al.*, 2011). 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 community interactions by experimentally manipulating this trade-off as well.

Because we found that seasonal priority effects strongly mediated competition for at least early ontological stages (germination, seedling), our findings may be most relevant in the context of native plant establishment, whether colonizing new areas due to range shifts, recovering from disturbance or ecological restoration. In fact, there has been an increasing call to increase phenological diversity in restoration planning (Hess *et al.*, 2019). Studies have found that including early active species in plantings can suppress the abundance of invaders in both grassland (Cleland *et al.*, 2013) and forest ecosystems (Schuster *et al.*, 2020). At the same time, restoration mixes tend to lack species which fill the early season phenological niche (Havens & Vitt, 2016). The results of our study suggest the minimizing the priority effect advantage conferred to invasive species to due rapid germination and early phenology by including species with similar, early phenological traits could be a powerful tool for managing plant invasions and restoring native ecosystems.

Conclusion

By opporationalizing the differential germination sensitivities to environmental cues of two competing species to manipulate phenological advantage between them, we were able to quantify the contribution of seasonal priority effects gained through rapid phenology on the competitive ability of the invasive species *H. matronalis*. We found that priority effects were approximately as strong as the intrinsic competitive traits of *H. matronalis* in influencing its competitive dominance over the native forest herb *Cryptotaenia canadensis*, suggesting seasonal priority effects mechanistically increase the invasion success of *H. matronalis*. For a larger suite of temperature herbs, variation in germination phenology was strongly mediated by differences in how species respond to temperature cues, suggesting that climate variation and sustained climate change will alter patterns of phenological advantage, potentially strengthening the seasonal priority effects on invaders as climate warms. Our finding highlight the important role of phenological diversity in the invasion resistance of native plant communities, implying that measures of phenological diversity should be incorporated into plant community assessments and ecological restoration.

References

- Baskin, C. & Baskin, J. (2014) *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Elsevier Inc.
- Baskin, C.C. & Baskin, J.M. (1988a) Germination ecophysiology of herbaceous plant species in a temperate region. *American Journal of Botany* **75**, 286–305.
- Baskin, J.M. & Baskin, C.C. (1988b) The ecological life cycle of *cryptotaenia canadensis* (l.) dc. (umbelliferae), a woodland herb with monocarpic ramets. *The American Midland Naturalist* **119**, 165–173.

- Bradford, K.J.K.J.. & Nonogaki, H. (2007) *Seed development, dormancy, and germination*. Blackwell Pub., Oxford, OX, UK; Ames, Iowa, USA.
- Bürkner, P.C. (2018) Advanced bayesian multilevel modeling with the r package brms. *R Journal* **10**, 395–411.
- Cavieres, L. & Sierra-Almeida, A. (2017) Assessing the importance of cold-stratification for seed germination in alpine plant species of the high-andes of central chile. *Perspectives in Plant Ecology, Evolution and Systematics* **30**.
- Cleland, E.E., Larios, L. & Suding, K.N. (2013) Strengthening invasion filters to reassemble native plant communities: Soil resources and phenological overlap. *Restoration Ecology* **21**, 390–398.
- Connolly, J. & Wayne, P. (2005) Assessing determinants of community biomass composition in two-species plant competition studies. *Oecologia* **142**, 450–457.
- Cottingham, K.L., Lennon, J.T. & Brown, B.L. (2005) Knowing when to draw the line: designing more informative ecological experiments. *Frontiers in Ecology and the Environment* **3**, 145–152.
- Dickson, T.L., Hopwood, J.L. & Wilsey, B.J. (2012) Do priority effects benefit invasive plants more than native plants? an experiment with six grassland species. *Biological Invasions* **14**, 2617–2624.
- Francis, A., Cavers, P.B. & Warwick, S.I. (2009) The biology of canadian weeds. 140. *hesperis matronalis* l. *Canadian Journal of Plant Science* **89**, 191–206.
- Fukami, T. (2015) Historical contingency in community assembly: Integrating niches, species pools, and priority effects. *Annual Review of Ecology, Evolution, and Systematics* **46**, 1–23.
- Fukami, T. & Nakajima, M. (2011) Community assembly: alternative stable states or alternative transient states? *Ecology Letters* **14**, 973–984.
- Gioria, M. & Pyšek, P. (2017) Early bird catches the worm: germination as a critical step in plant invasion. *Biological Invasions* **19**, 1055–1080.
- Gioria, M., Pyšek, P. & Osborne, B.A. (2018) Timing is everything: does early and late germination favor invasions by herbaceous alien plants? *Journal of Plant Ecology* **11**, 4–16.
- HAO, J.H., QIANG, S., LIU, Q.Q. & CAO, F. (2009) Reproductive traits associated with invasiveness in *conyza sumatrensis*. *Journal of Systematics and Evolution* **47**, 245–254.
- Havens, K. & Vitt, P. (2016) The importance of phenological diversity in seed mixes for pollinator restoration. *Natural Areas Journal* **36**, 531–537.
- Hawkins, T.S., Baskin, J.M. & Baskin, C.C. (2005) Life cycles and biomass allocation in seed- and ramet-derived plants of *cryptotaenia canadensis* (apiaceae), a monocarpic species of eastern north america. *Canadian Journal of Botany* **83**, 518–528.
- Hawkins, T.S., Baskin, J.M. & Baskin, C.C. (2007) Seed morphology, germination phenology, and capacity to form a seed bank in six herbaceous layer apiaceae species of the eastern deciduous forest. *Castanea* **72**, 8–14.

- Hess, M.C.M., Mesléard, F. & Buisson, E. (2019) Priority effects: Emerging principles for invasive plant species management. *Ecological Engineering* **127**, 48–57.
- Howe, H.F. & Smallwood, J. (1982) Ecology of seed dispersal **13**, 201–228.
- Inouye, B. (2001) Response surface experimental designs for investigating interspecific competition. *Ecology* **82**, 2696–2706.
- Kardol, P., Souza, L. & Classen, A.T. (2013) Resource availability mediates the importance of priority effects in plant community assembly and ecosystem function. *Oikos* **122**, 84–94.
- Koerner, C., Stoecklin, J., Reuther-Thiebaud, L. & Pelaez-Riedl, S. (2008) Small differences in arrival time influence composition and productivity of plant communities. *New Phytologist* **177**, 698–705.
- Marushia, R.G., Cadotte, M.W. & Holt, J.S. (2010) Phenology as a basis for management of exotic annual plants in desert invasions. *Journal of Applied Ecology* **47**, 1290–1299.
- Milbau, A., Nijs, I., Van Peer, L., Reheul, D. & De Cauwer, B. (2003) Disentangling invasiveness and invasibility during invasion in synthesized grassland communities. *New Phytologist* **159**, 657–667.
- Ross, M.A. & Harper, J.L. (1972) Occupation of biological space during seedling establishment **60**, 77–88.
- Rudolf, V.H.W. (2019) The role of seasonal timing and phenological shifts for species coexistence. *Ecology Letters* **22**, 1324–1338.
- Schuster, M.J., Wragg, P.D., Williams, L.J., Butler, E.E., Stefanski, A. & Reich, P.B. (2020) Phenology matters: Extended spring and autumn canopy cover increases biotic resistance of forests to invasion by common buckthorn (*rhamnus cathartica*). *Forest Ecology and Management* **464**, 118067.
- Smith, L.M. (2013) Extended leaf phenology in deciduous forest invaders: mechanisms of impact on native communities. *Journal of Vegetation Science* **24**, 979–987.
- Soltani, E., Ghaderi-Far, F., Baskin, C.C. & Baskin, J.M. (2015) Problems with using mean germination time to calculate rate of seed germination. *Australian Journal of Botany* **63**, 631–635.
- Susko, D.J. & Hussein, Y. (2008) Factors affecting germination and emergence of dame's rocket (*hesperis matronalis*). *Weed Science* **56**, 389–393.
- ten Brink, D.J., Hendriksma, H.P. & Bruun, H.H. (2013) Habitat specialization through germination cueing: a comparative study of herbs from forests and open habitats. *Annals of Botany* **111**, 283–292.
- Wainwright, C.E., Wolkovich, E.M. & Cleland, E.E. (2011) Seasonal priority effects: implications for invasion and restoration in a semi-arid system. *Journal of Applied Ecology* **49**, 234–241.
- Walck, J.L., Hidayati, S.N., Dixon, K.W., Thompson, K. & Poschlod, P. (2011) Climate change and plant regeneration from seed. *Global Change Biology* **17**, 2145–2161.
- Weidlich, E.W.A., Nelson, C.R., Maron, J.L., Callaway, R.M., Delory, B.M. & Temperton, V.M. (2020) Priority effects and ecological restoration. *Restoration Ecology* **n/a**, e13317.
- Wolkovich, E.M. & Cleland, E.E. (2011) The phenology of plant invasions: a community ecology perspective. *Frontiers in Ecology and the Environment* **9**, 287–294.

439 Young, T.P., Stuble, K.L., Balachowski, J.A. & Werner, C.M. (2017) Using priority effects to manipulate
 440 competitive relationships in restoration. *Restoration Ecology* **25**, S114–S123.

441 Figures

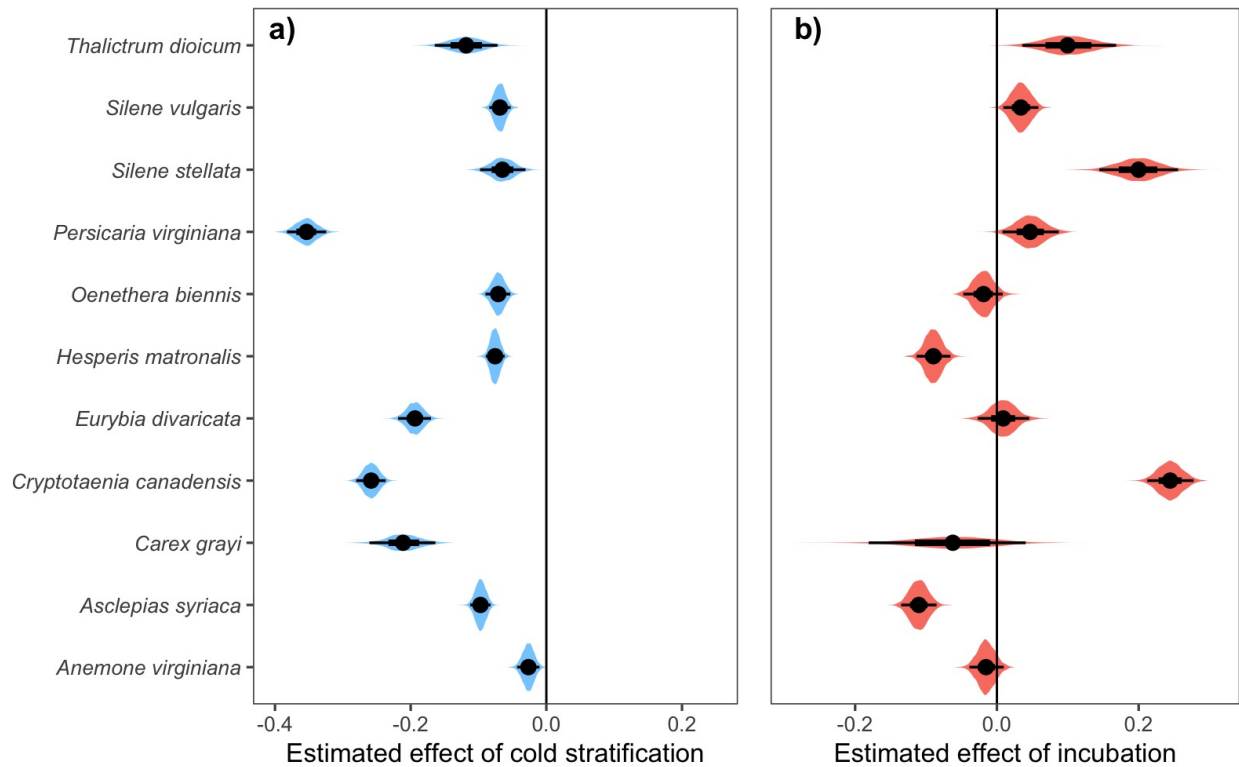


Figure 1: Estimated effects of weeks of cold stratification (a)) and incubation temperature (b) on the time to 50% germination (T50) for a suite of temperate herbaceous species. Negative estimates describe an advance in T50 and positive values a delay, estimates are on the log scale. The points indicate the mean estimated effect of each parameter, thicker and thinner bars the 50% and 95% credible intervals respectively. The full posterior distribution for each parameter is also depicted

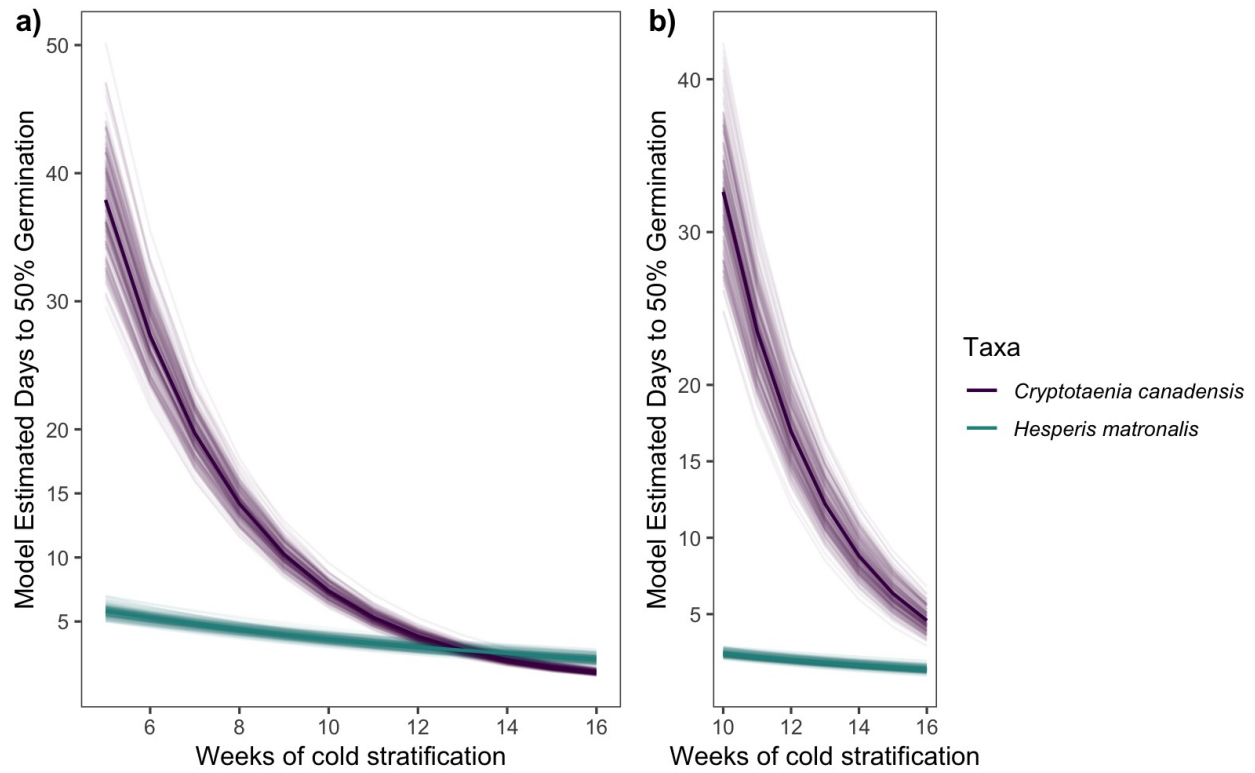


Figure 2: The effects of weeks of cold stratification at 4C on the time to 50% germination of *Cryptotaenia canadensis* and *Hesperis matronalis* under a) cool and b) warm (20/10C vs. 25/15C day/night) incubation conditions, estimated with accelerated failure time model. Only stratification treatment levels which allowed both species to reach 50% germination in less than 50 days are depicted here. The solid lines indicated the mean estimate, while lighter lines depict uncertainty with 100 random draws from the posterior distribution.

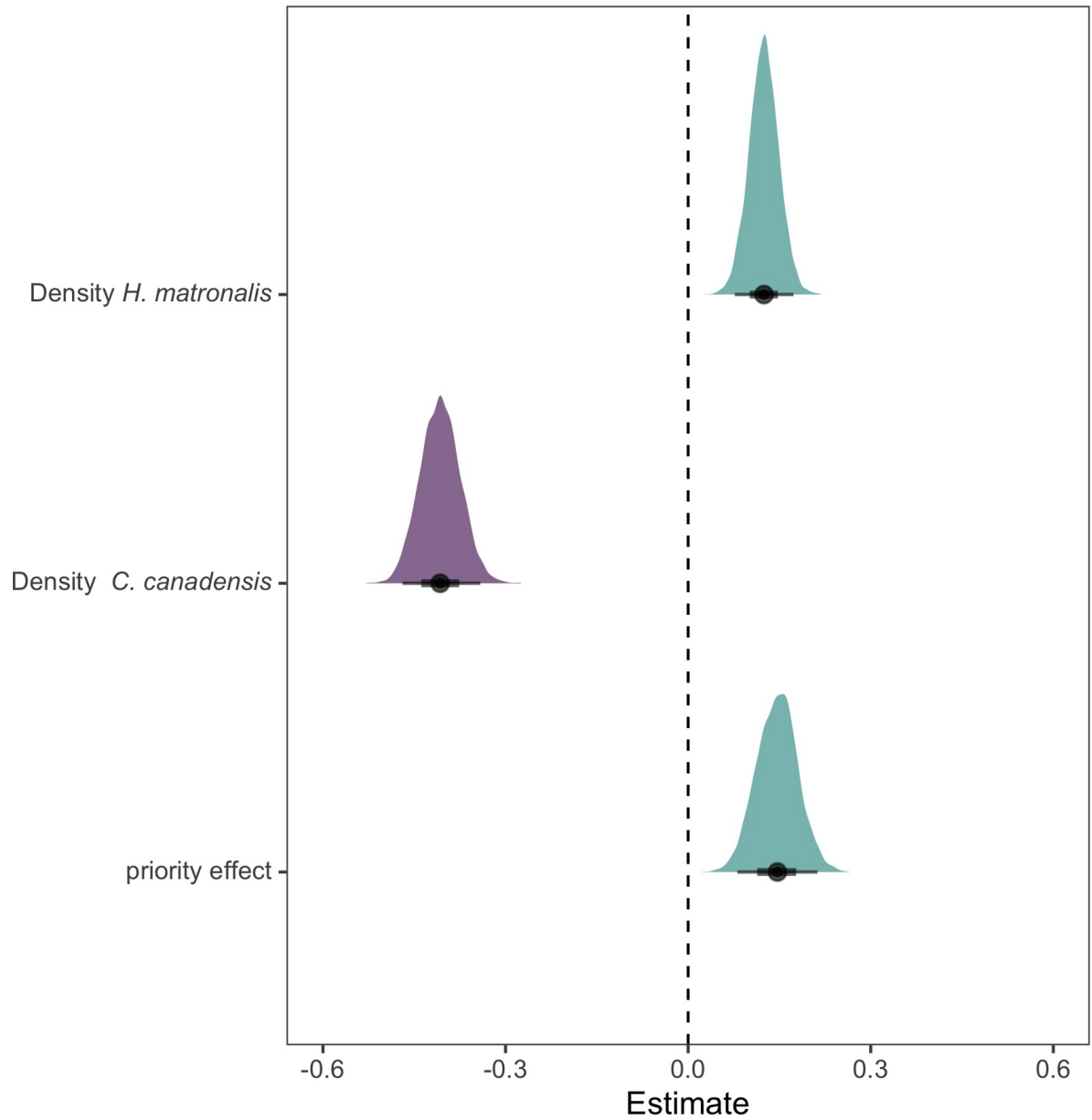


Figure 3: Estimated effects of species' abundance and phenological advantage on the relative growth rate difference between *H. matronalis* and *C. canadensis*. Negative parameter estimates indicate the community biomass composition shifts to favor *C. canadensis* while positive estimate towards dominance by *H. matronalis*. The points indicate the mean estimated effect of each parameter, thicker and thinner bars the 50% and 95% credible intervals respectively. The full posterior distribution for each parameter is also depicted.

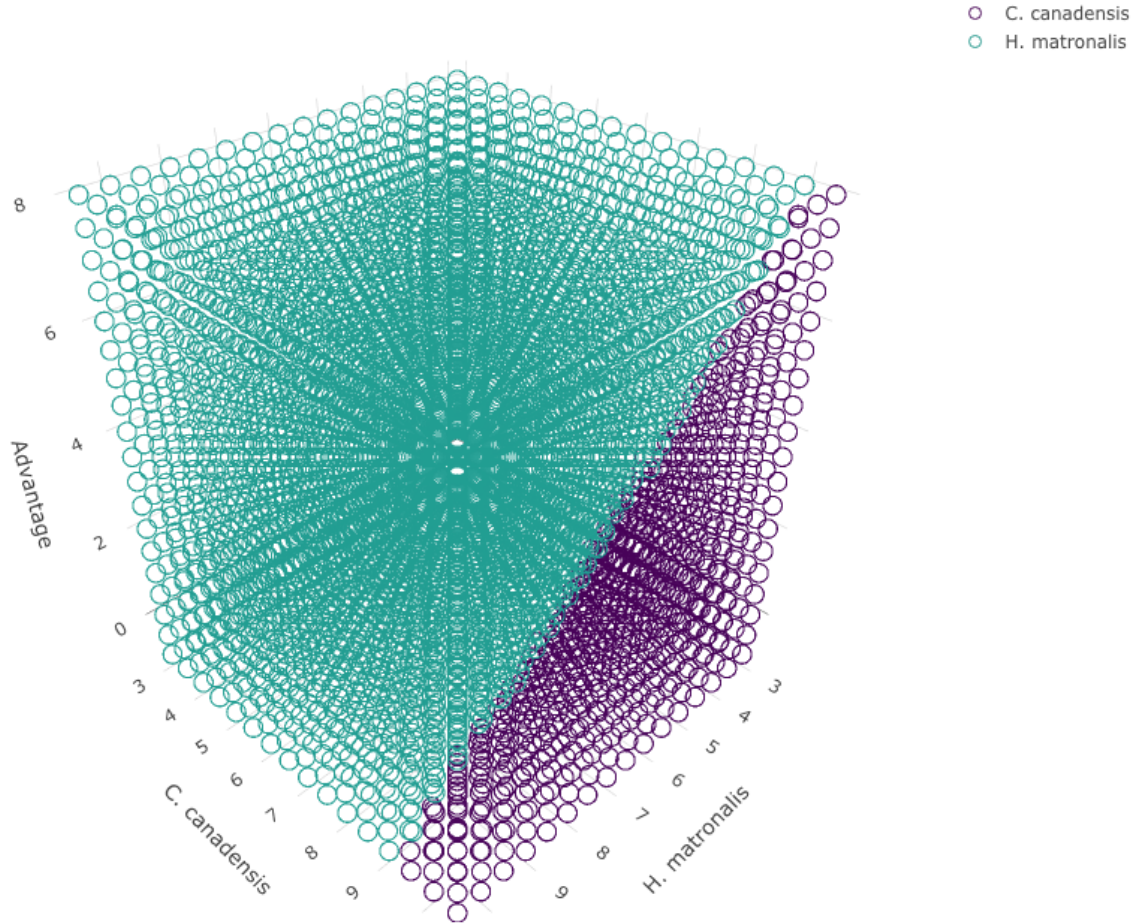


Figure 4: Predicted outcome of competition under differing combinations of *C. canadensis* and *H. matronalis* abundance and phenological advantage of *H. matronalis*. Purple dots indicate conditions that favor *C. canadensis* in community biomass composition while green dot conditions favor *H. matronalis*. Estimates are based on multiple regression models estimating the effect of each variable on the relative growth rate difference among species.

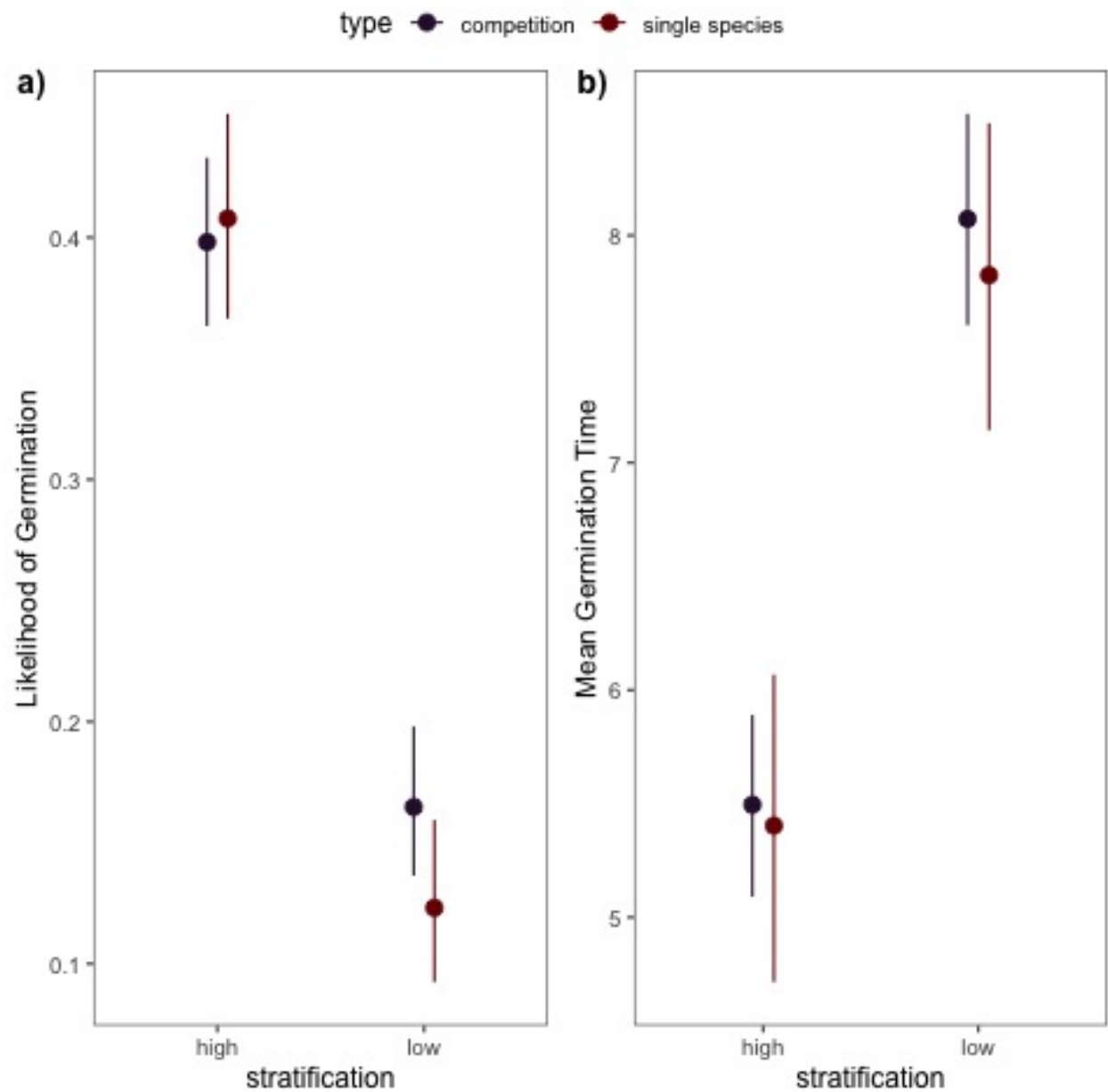


Figure 5: Estimated effects of intra-vs. inter-specific competition on the germination dynamics of *Cryptotania canadensis* under 6 (low) and 10 weeks (high) of cold stratification at 4C. Panel **a)** depicts differences germination likelihood and **b)** shows the estimated mean germination time in single species mono-cultures vs. competition plot. Colored dots represent the mean estimate under each planting type and bars represent 80% credible intervals.

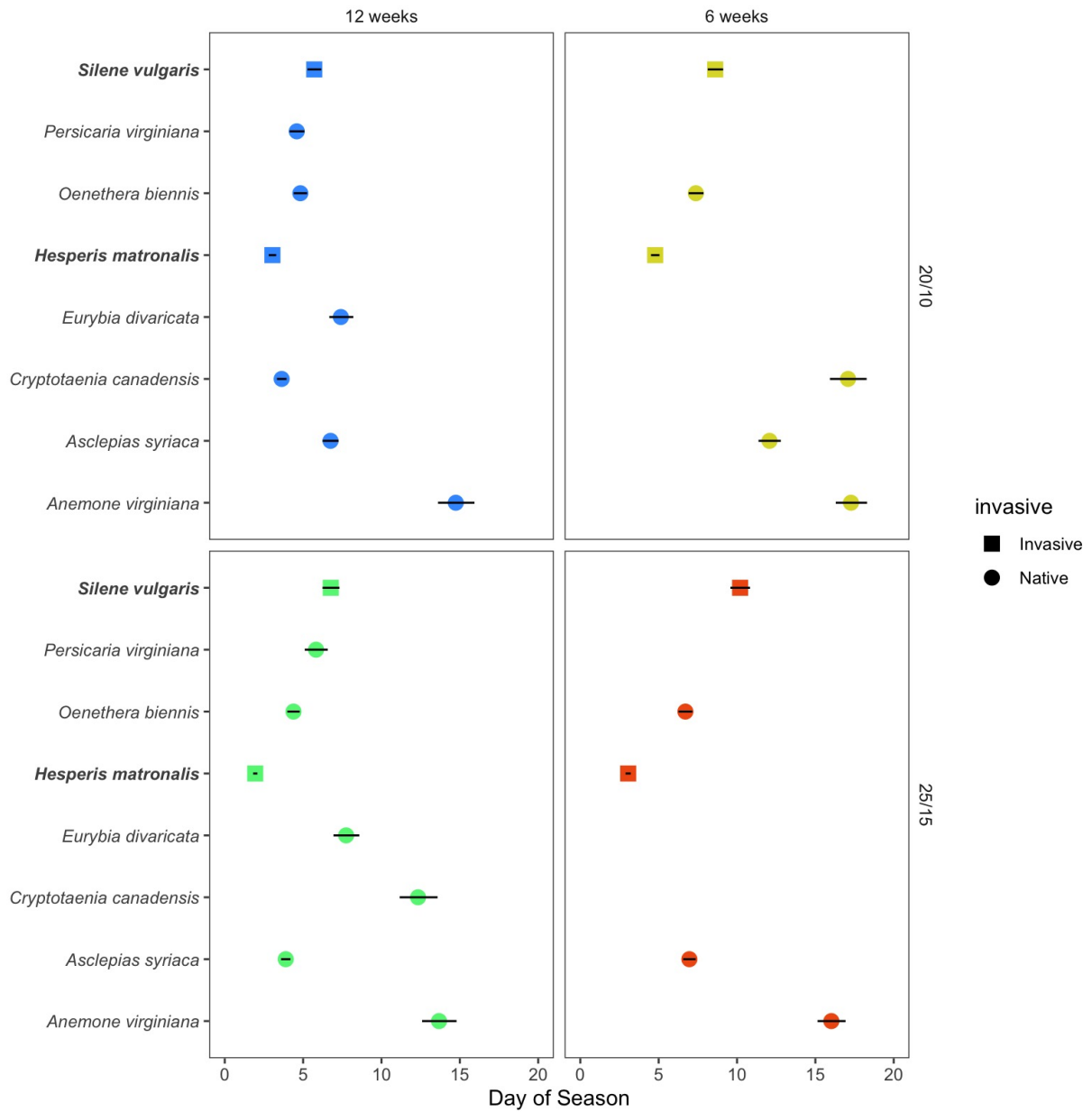


Figure 6: Predicted germination phenology of a suite of temperate herbs under varying cold stratification (6 vs. 10 weeks at 4C) and incubation (20/10C vs. 25/15C day/night) levels within the first 20 days of the growing period. Longer stratification periods increased the ratio of native to invasive species (circles vs. squares) that germinate within 20 days of the growing period, and reduced the phenological advantage of the invasive species in this study