The ARNOLD ARBORETUM of HARVARD UNIVERSITY

Dear Dr. Hector,

Please consider our revised manuscript, now titled "Contrasting responses to climate variability generate seasonal priority effects between native and invasive forest herbs" as a "research article" in *Journal of Ecology*.

Rapid germination is a trait common to many invasive plant species, which may give invaders a competitive advantage over slower-germinating natives. Because the germination timing of many forest plant species is closely linked to environmental cues, the impact of these germination differences may be exacerbated by climate change. It has been difficult, however, to quantify the overall contribution of rapid germination to the competitive success of invaders, and few studies to date have mechanistically linked these kinds of competitive outcomes to climate. We addressed these questions with a series of controlled-environment experiments in which we indirectly varied the germination timing of native and invasive herbs by manipulating climate variables. We found that precocious germination doubled the competitive impact of the invader relative to its other intrinsic competitive traits; a germination advantage of just two to three days, was enough to secure competitive dominance.

Comments from the Handling Editor, Dr. Amy Iler, and two reviewers suggested that the topic of our manuscript was timely and important but pointed out a need to better present our rationale for selecting our focal species and to qualify how our laboratory results translate into complex forest ecosystems.

Based on their comments we have made important changes to the manuscript. These changes include the addition of a new figure in the main text and an additional data table in the Supporting Information, which show that our focal species co-occur frequently in eastern forests, as well as a new sub-section in our Methods laying out the specific selection criteria we considered. We have also changed our Introduction to more clearly state the specific goals of this study, and added a new subsection to our Discussion "A research agenda for assessing the role of seasonal priority effects in plant communities of temperate forests" to place our finding in a broader context of temperate forest community ecology. We have also changed our title as requested.

We feel that the editor's and reviewers' input has yielded a new submission that is much improved, and we detail our specific changes in the following pages with reviewer comments in *italics* and our responses in regular text.

The main text of this manuscript is 6,147 words in length and now contains four figures. It is co-authored by E.M. Wolkovich, and is not under consideration elsewhere. We hope that you will find it suitable for publication in *Journal of Ecology*, and look forward to hearing from you.

Best,

Daniel Buonaiuto

Reviewer comments are in italics. Author responses are in plain text. In-text citations mentioned here can be found in the References section on the main manuscript.

Handling Editor Comments for Authors:

This paper has received two consistent external reviews. Both reviewers applaud the question and topic of the paper, which is centered on how priority effects might contribute to the success of invasive plants in terms of germination phenology. This is a relevant question in the context of climate change and modern coexistence theory. The main result is that the native species is a better competitor in the absence of any phenological advantage; however, with only one day of germination advantage, the invasive species becomes superior. The main concern with the paper is that it is vague about whether the two study species ever actually co-occur in nature, so it is unclear how much relevance this result has for natural systems (I also shared this concern with reviewers). Without more information on co-occurrence of the study species, it is not clear whether the paper has a broad enough reach for Journal of Ecology. Another concern that brings into question the relevance of the result for natural systems is the fact that the two study species have different types of dormancy. Please thoroughly address these concerns and others raised by the reviewers.

We are grateful to the Handling Editor and two Reviewers for their comments and feedback on our initial submission. With your input, we feel that we have much improved this manuscript. In particular, have added considerable text about our process for species selection, a new main text figure, and two new analyses (and associated text) to demonstrate that our focal species co-occur widely and interact in the natural systems. We appreciate the reviewers and editors highlighting this as it is clearly critical for our study.

We have also clarified how studying species with contrasting dormancy types in the context of our study was an intentional decision that contributes to relevance of our experiments to applications in real communities, including a number of new citations to support this. We also have substantially adjusted our Discussion to more directly address the limitations of lab experiments like ours for translation to real ecological communities, and outlined future research needs to bridge these gaps. The changes are detailed below.

Line 100: Please elaborate more about this. Have you ever observed them co-occurring together, or can you find some GIS layers, iNaturalist occurrence data, or something similar to further suggest that they co-occur?

We thank the editor for raising this point. One of reasons we chose these focal species for our study was our *a priori* knowledge that they co-occur in nature, and we can now see that we did not clearly present this critical information in our initial version of this manuscript. We are grateful to the editor and both reviewers for calling attention to this omission.

We also thank the editor for their suggestions for potential data sources to verify our assertion these species co-occur. In this version of the manuscript we have added two new data presentations to demonstrate that our focal species frequently co-occur in natural systems.

First, we have added a new figure to the main manuscript (Fig. 1) that highlights that our focal species frequently occur in proximity to each other based on all geo-located North America observations of the two species from the Global Biodiversity Information Facility (GBIF; 18,571

observations total).

Second, we queried a recently published database that compiles over 80,000 observational plots of plant communities from federal and state agencies across the United States for plots in which these species co-occur. Of the 136 plots in the temperate forest region in which *H. matronalis* was detected, *Cryptotaenia canadensis* was present at 24% of them, and present this statistic in our Supporting Information. This was the 9th highest occurrence-overlap of the 463 native forbs species found in any plots where *H. matronalis* was present.

We now present these two examples within in a broader "Species selection" sections of our Methods (lines 116-117), and in a new data table and description in our Supporting Information (Table S1).

On a related note, why were the seeds collected from different habitats, and what implications does this have for interpretation of the results?

We appreciate this concern. Restrictions to research activity during the COVID-19 pandemic necessitated that we purchase our seeds rather than collect them ourselves (this also caused the large gap between the series of reported experiments as our labs were shut down for several months). This meant that matching our source environments was restricted by commercial availability of plant materials.

As the Editor points out, it is clear from the literature that site specific factors (e.g. transgenerational plasticity, local adaptation, etc) could influence the patterns we observed in our study, and we should have explained this better in our original submission. In the updated version of the manuscript, we address this within a new section of our Discussion called: "A research agenda for assessing the role of seasonal priority effects in plant communities of temperate forests". In this section we write in lines 318-323:

> Factors like local adaptation and trans-generational plasticity can influence the germination behavior of seeds (Donohue *et al.*, 2010; Baughman *et al.*, 2019). In our study, we obtained seeds of each species from different source populations. While many germination traits are conserved as the species level, intra-specific variation that we did not capture in our study design could moderate the patterns we observed in our experiments. Follow-up studies that collect seeds from co-occurring populations from a gradient of habitats, could at once account for and estimate variation at the population level.

Line 153: change 'and' to 'an'

Thanks. We have made this change.

Line 215: I would save this interpretation for the Discussion section.

Agreed. We have removed our interpretation of this result form this section. This statement is now paraphrased in the Discussion at line 244

Line 233: I'm not convinced you can say this unless you know that these species actually co-occur. Otherwise, it remains unknown how important this actually is in nature. Same for lines 237–239. Additionally, another caveat is that we don't know about all of the other species interactions that would occur in a natural setting, which could modify the pairwise effects of this study.

We hope that the additional analyses we include in our revised submission provide stronger support for these assertions. The editor's point about how presence additional species could modify the interactions between our focal species is well taken. We made several changes to our text to better articulate the limitations of our approach and research directions needed to robustly apply our results to natural systems.

We fully agree that the complexity of multi-species interactions could substantial modify the dynamics we observed in our simple pairwise study. Given that studies of seasonal priority effects in temperate forest communities are very limited, we felt there was not a sound basis to speculate on how the presence of additional species might affect these dynamics. We have added a paragraph to the Discussion section of our new submission to be more upfront about this uncertainty, and have added recommendations for a multi-species study framework to evaluate the role of seasonal priority effects in more realistic experimental arrays. This addition can be found in lines 324-329 and reads:

> Additionally, ground-layer plant communities of temperate forests are species rich (Whigham, 2004), and understanding the the role of priority effects in a multi-species context is a major need. While our study focused on pair-wise differences, future studies could address this limitation by performing trials with more species, both in controlled environments and under field conditions, to capture the complexity of phenological assembly in multi-species plant communities that comprise of diverse functional types, germination niches and life-history traits like temperate forests.

One potentially relevant paper that was not cited is Kimball et al. (2010). Contemporary climate change in the Sonoran Desert favors cold-adapted species. Global Change Biology 16: 1555-1565

We thank the editor for providing this citation. We found it very useful for thinking about how net warming could also benefit species with high stratification requirement and cooler germination niches in our temperate forest system. We have added this point and the citation to our discussion in lines 277-281.

Reviewers' comments:

Reviewer: 1

COMMENTS FOR THE AUTHOR The manuscript contains good information. But the problem statement is not done well. Why should this study be done? Is this invasive species a problem for the native species? Is there a report on this? In the materials and methods section, I saw that you wrote that the seeds were collected from two different habitats. If they have different habitats, why should the competition between them be compared?! Another question I have is that these two species differ in their type of dormancy. One has physiological dormancy and the other morphological/morphophysiological dormancy. How would you expect these two species not to differ in terms of germination, especially germination speed and response to stratification. You can see some of my other comments in the attached file. With these problems I have to reject the manuscript.

We thank the Reviewer for the comments and are pleased they found the information in this study to be useful. The Reviewer provided us with several important questions that we have addressed in our new version of the manuscript that we feel has resulted in a much improved submission. We've

separated out each question and responses below for clarity.

The manuscript contains good information. But the problem statement is not done well. Why should this study be done?

Based on their feedback and guidance in the attached file, we have re-written the concluding paragraph of our Introduction to more clearly articulate the questions this study addresses as well the scope of inference, assumptions and limitations of our design and inference in lines 82-99. Our problem statement now reads:

> In this study, we perform a sequences of experiments in controlled environments to link climate variation, phenological advantage, and seasonal priority effects to the competitive interactions of two woodland herbaceous species (the invasive *Hesperis matronalis* and native *Cryptotaenia canadensis*) that frequently co-occur in the understory of temperate forest regions of North America. First, we performed a series of germination assays under varying temperature regimes to address the question:
>
> > How does phenological advantage between two species with contrasting responses to environmental cues shift due to variable climate conditions?
>
> We then used competition trials under contrasting environmental conditions to indirectly manipulate the phenological advantage between these two taxa to address a second question:
>
> > To what extent do seasonal priority effects generated by varying patterns of phenological advantage influence the competitive dynamics of seedlings?

We also include information about the assumptions of our approach and the potential scope of inference from our finding in lines 93-99.

Is this invasive species a problem for the native species? Is there a report on this?

This question was shared by both reviewers and the editor, so was clearly a major omission in our first submission. We now address this by providing a new figure (Fig. 1) demonstrating high degrees of overlap in patterns of occurrence among these species, and analyses of plant community data indicating the frequently interaction (Tab. S1, lines 116-117). These changes are detailed more fully above in the responses to the editor's comments.

In the materials and methods section, I saw that you wrote that the seeds were collected from two different habitats. If they have different habitats, why should the competition between them be compared?!

We thank the reviewer for highlighting this point, which was a concern also raised by the editor. The COVID-19 pandemic necessitated that we purchase our seeds, and the seeds of these species were not available from the same providers. We have elaborated on how using different seed sources may impact the inference from our study in lines 318-323.

Another question I have is that these two species differ in their type of dormancy. One has physiological dormancy and the other morphological/morphophysiological dormancy. How would you expect

these two species not to differ in terms of germination, especially germination speed and response to stratification.

We agree with the reviewer—one of the reasons we specifically choose these species because we were seeking species that would have different responses to stratification. Our study was designed to specifically leverage these differences to generate the sufficient amount of variation in phenological advantage, which was one of the main cruxes of our experiment. In our re-submission we have added new text and relevant references in several places to emphasize that this was a choice that enhances the study rather than a limitation (lines 124-126).

An additional, interesting result of our study was that despite having different dormancy classes, these two species can display nearly identical germination behavior under certain ranges of environmental conditions—including environmental conditions that are likely to occur where these species are found. We have address this in text with the following addition to our Discussion (lines 260-263):

You can see some of my other comments in the attached file.

We thank the reviewer for these additional details. We have addressed all of the points above, and rechecked the citation for the "crush test" detailed in line 144, which is described in Baskin & Baskin (2014) on p. 32.

Reviewer: 2

The present article studies de effects of temperature variation, due to climate change, which may affect germination phenology and competitive interactions between native and introduced plant species under controlled conditions. This temperature variation may favor the germination and establishment success of introduced species, which may affect the composition of native plant communities.

I really appreciate the focus on priority effects, climate change, and introduced vs. native plant species. I admit that when first reading the title, I did not think about all this topics together. However, they are well presented in the article.

This article has the potential to significantly contribute to the scientific literature. However, it will first require some changes to clarify:

1) It is not clear how the authors selected the focal species. The authors need to justify their decision.

2) The title does not match the content

We thank the reviewer for their comments are are pleased that they found our the link between priority effect, climate change and invasion biology well presented, and see the potentially for this study to contribute to the literature. We agree that we could, and should, have described the criteria for our species selection better in our initial submission, and that the title should better reflect the content of the paper. In this version we have incorporated an additional section about species selection in our Methods section, and changed the title. We detail these changes, as well as the other points raised by the reviewer below.

Other comments:

Title: I suggest you to improve it, because when reading it first I did not get the idea that it will talk about warming climate, priority effects, and native vs. invasive species.

We have changed the title to better reflect the links between climate, priority effects, and invasion biology.

Abstract:

L22: "germination phenology for two North American herbaceous species" makes me think that you are going to study 2 native species. Afterwards, it says the invasive and the native, then it makes me guess if the invasive is introduced/exotic or not. Please make it clear.

Thank you for pointing this out. We have changed these descriptors. This line now reads:

> ...two herbaceous species found in North American woodlands, the non-native *Hesperis matronalis* and native *Cryptotaenia canadensis* (Line 23)

L16: what are other intrinsic competition traits can you mention?

We have added examples of other competitive traits here (line 16).

Body:
L55-57: I suggest characterized instead of dispersed.

Done. We have made this change in line 57.

L82: "the North American invasive" for me this sounds like Hesperis matronalis is native to North America and that does not belong to the habitat of Cryptotaenia canadensis, due to that it is invasive.

Thanks again for helping us to sharpen our descriptors of these species. We have changed this sentence to be more clear about the native/introduced status of these species in line 83.

L100-103: You mention that it is known that C. canadensis need a substantial period of cold moist stratification to release dormancy and germinate. Also you said that they have different germination niche. Then, if all this is known why do we need to run an experiment to test it? Please justify better the selection of these focal species.

We thank the reviewer for highlighting this point. Our study intentionally leveraged species with different responses to the environment to generate the sufficient amount of variation in phenological advantage to run a meaningful experiment. We can see that we did not make this clear in our initial submission, and have made several changes to our method second to highlight this.

First, we have changed the "Focal species" sub-section of out methods to be "Species selection" and more comprehensively laid out our criteria for choosing species in lines 103-108. Second, we have added information explaining why including species with contrasting responses to cold stratification was important to our study (lines 124-126).

We feel these changes, combined with the inclusion of the new analyses demonstrating our focal species often co-occur in nature that we detailed above, substantially improved our presentation of context of this study, and we are grateful to the reviewer for pushing us to do this.

L104: "suggest" instead of suggestS

Thank you. We've made this change.

L112: remove one OF

Thank you for catching this typo. It is corrected in the updated submission

L113-114: The authors use low or cool incubation temperature and high or warm incubation temperature. I would suggest deciding for one and using it consistently all over the manuscript.

We appreciate this point and have changed all mentions of incubation temperature to "cool" and "warm" for consistency.

L128: Please mention what you have used as substrate in your pots.

We have added this information to line 152.

L133: I have seen that seed sizes of both species are really different, and also I have seen that they have different sizes when adults. Please justify why you are using those seed densities, in terms of natural history of your focal species, in your competition trial.

We appreciate this point. We have added more information about our basis for choosing these densities to the manuscript in lines 154-157 and added several relevant citations. In designing our destiny treatment levels, we balanced our understanding of seed bank densities in temperate forests—which can be highly variable (Leckie *et al.*, 2000; Bossuyt *et al.*, 2002; Decocq *et al.*, 2004) and the recommendation of Inouye (2001) to use relatively high planting densities in competition experiments. Based on reported densities in Leckie *et al.* (2000); Bossuyt *et al.* (2002); Decocq *et al.* (2004) we assessed that a reasonable average seedbank density for temperate forests is around 12 seeds/cm2. Our high and low density treatments straddle this mean.

L191-192: C. Canadensis in italics.

We have made this change.

L249: "lower stratification treatments" I would suggest you to specify, something like "less time/days/weeks of stratification"

We have changed this description to "shorter stratification periods".

References

Baskin, C. & Baskin, J. (2014) *Seeds: Ecology,Biogeography, and Evolution of Dormancy and Germination*. Elsevier Inc.

Bossuyt, B., Heyn, M. & Hermy, M. (2002) Seed bank and vegetation composition of forest stands of varying age in central belgium: consequences for regeneration of ancient forest vegetation. *Plant Ecology* **162**, 33–48.

Decocq, G., Valentin, B., Toussaint, B., Hendoux, F., Saguez, R. & Bardat, J. (2004) Soil seed bank composition and diversity in a managed temperate deciduous forest. *Biodiversity & Conservation* **13**, 2485–2509.

Inouye, B. (2001) Response surface experimental designs for investigating interspecific competition. *Ecology* **82**, 2696–2706.

Leckie, S., Vellend, M., Bell, G., Waterway, M.J. & Lechowicz, M.J. (2000) The seed bank in an old-growth, temperate deciduous forest. *Canadian Journal of Botany* **78**, 181–192.