

Variation in the demographic effects of grass-endophyte symbiosis along an aridity gradient

Jacob K. Moutouama^{1,*}

Julia Martin¹

Ulisses Rizo¹

Malcolm Sherwood¹

Emily Chong¹

Dajanae Pearson¹

Alexandra Jimenez Martín¹

Josh Fowler¹

Ali Campbell¹

Chris Oxley¹

Karl Schrader¹

Tom E.X. Miller¹

1. Program in Ecology and Evolutionary Biology, Department of BioSciences, Rice University, Houston, TX USA;

2. University of Miami, Department of Biology, Miami, Florida;

* Corresponding author; e-mail: jmoutouama@gmail.com.

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Abstract

Introduction

Plant-microbe symbioses are widespread and ecologically important. These interactions are famously context-dependent, where the direction and strength of the interaction outcome depends on the environment in which it occurs (Bronstein, 1994; Fowler et al., 2023; Hoeksema and Bruna, 2015). Under biotic stress (e.g., herbivory), endophyte symbiosis can benefit host plants by facilitating the production of secondary compounds that deter feeding or cause direct toxicity, thereby reducing insect growth, survival, and oviposition (Atala et al., 2022; Bastias et al., 2017; Vega, 2008). Similarly under abiotic stress (e.g., drought), symbionts can increase their host tolerance to drought (Clay and Schardl, 2002). However, in many plant-microbe interactions, host protection is not guaranteed solely by the presence of a symbionts; rather, the density of the symbiont can determine the effectiveness of this protection (Laughton et al., 2014). Having a greater endophyte density could lead to high resource exploitation by the symbiont, which may be costly (reduction in growth or in reproductive) for the host (Faeth, 2009). Ultimately, these context-dependent costs and benefits may underlie the observed distribution of host species.

Context-dependence raises the hypothesis that plant-microbe interactions are likely to vary across environmental gradients, from range-core to range-edge, which could have significant consequences for host range expansion. If the benefits of microbial symbiosis strengthen under environmental stress then symbionts could make range-edge environments more suitable, possibly extending the host's range limits (Allsup et al., 2023; Rudgers et al., 2020). For instance, fungal endophytes improve the survival of *Bromus laevipes* populations in dry conditions, enhancing their resistance to drought stress at the range edge and thereby extending the species' geographic range (Afkhami et al., 2014; David et al., 2019). In contrast, if microbial symbiosis is costly for the host at range edge, then symbionts could limit host range (Bennett and Groten, 2022; Benning and Moeller, 2021a,b). Although context dependence, along with spatio-temporal variations in abiotic environmental conditions may reduce the effectiveness of the benefits provided by the symbiont to the host species, our understanding of the mechanisms that alter the

intensity or likelihood of host-symbiont interactions across host species geographic range is limited.

Ecological studies of plant-microbe symbiosis usually investigate the interaction from the plant's perspective and rarely study how symbiont response to environmental variation might translate to its influence on host demographic performance across host range. Moreover, studies of plant-microbe symbiosis relied on methods such as such as inoculating sterile soil, excluding endophyte fungal hyphae by using fine mesh or rotating cores, and adding fungicide (Bennett and Groten, 2022). Despite their value, all these approaches are often difficult to implement in field settings or on a large scale. As a result, the exact mechanisms by which symbionts drive host range limitation and expansion are not well understood, hindering our understanding of the potential cascading effects of symbionts on eco-evolutionary species demography and range limitation in the context of global change.

Working across a precipitation gradient in the south-central US, we asked how the demographic effects of endophyte symbiosis varied from core to edge of the host range. We also asked how does fungal growth affect host demography from range core to range edge. To answer, these questions, we studied the symbiotic association between a cool-season grass species (*Elymus virginicus*) and its vertically transmitted fungal symbiont *Epichloë elymi*. [\[Describe ecology and natural history of grass-endophyte interactions\]](#). Our experiment was design to test the following hypotheses:

1. We hypothesized that stress associated with aridity and low precipitation would strengthen the plant-fungal mutualism, such that the fitness benefits of endophyte symbiosis are maximized at the range edge.
2. We hypothesized that fungal growth in planta varied from range core to range edge. If endophyte growth is limited by host photosynthesis, then environments that are stressful for hosts may correspond to poor endophyte growth. Alternatively, if active regulation by the host is required to keep symbionts "in check", then environments that are stressful for

hosts may correspond to high endophyte growth.

Materials and methods

Study species

Elymus virginicus (Poaceae) is a cool season perennial grass native to woodland and prairie habitats of eastern North America (Shaw, 2011). The westernmost range limits of this species correspond to the longitudinal aridity gradient in the central and southern Great Plains (fig. 1). Throughout its range, the species is symbiotic with the seed-transmitted fungal endophyte (*Epichloë* spp.) (Rudgers and Swafford, 2009). Across natural populations in Texas, endophyte prevalence (fraction of plants that are endophyte-symbiotic) in *Elymus virginicus* ranged from 10% to 100%, with a mean of 53% (Sneck et al., 2017). Fungal genotyping indicated that the endophytes are capable of synthesizing secondary compounds such as peramine, loline, and ergot alkaloids, which may confer resistance against drought and herbivory (Beaudry, 1951). In addition, the species is capable of both self-pollination and outcrossing (Church, 1958).

Study design

Experimental Design. To understand the demographic effects of endophyte symbiosis from core to edge of the host range, we established common gardens at 7 sites across the geographic range of *Elymus virginicus* (fig. 1). Experimental sites spanned an aridity gradient (temperature gradient). Common gardens were established in 8 plots per site. Plots were 1.5m * 1.5m and the area was tilled of existing vegetation to control for native plant competition. Plots were also selected in shaded areas under tree canopy or near shrubs to mimic the natural environmental of the species. In each plot, we planted 15 individuals of *E. virginicus* approximately 15 cm deep in an evenly spaced 4*4 grid pattern, with positions randomly assigned. For each plot, we randomly assigned a starting endophyte frequency (80%, 60%, 40%, 20%)¹ and herbivory treatment (herbi-

¹Do we need a schematic of one replicate of the experimental design?

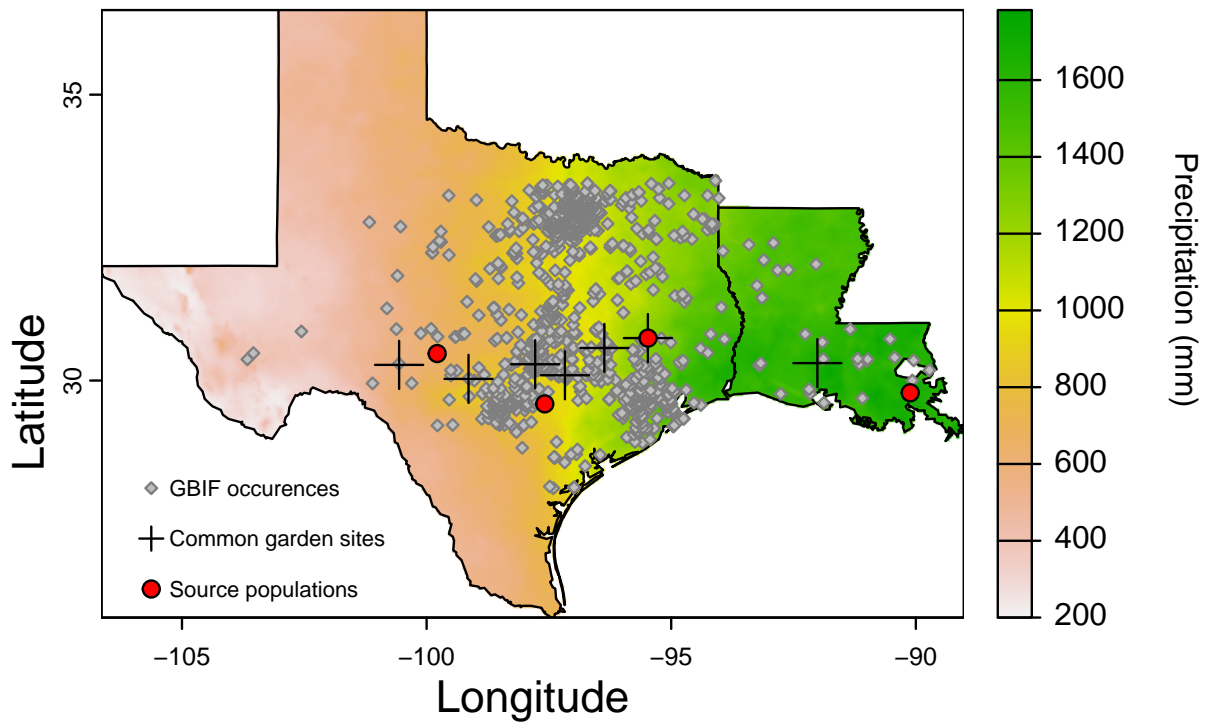


Figure 1: Distribution of common garden sites across the longitudinal aridity gradient in the central and southern Great Plains. Red dots represent the locations of source populations, while grey dots represent the GBIF locations of the species across the study area.

77 vore exclusion and herbivores accessibility). We ensured that all plots had comparable quantities
 78 of source populations. After establishing the plots, we watered the plants and recorded initial
 79 tiller counts, flowering status and plot position, endophyte status, source population of each in-
 80 dividual plant. For herbivory exclusion plots, we enclosed them with 1.2m tall mesh fencing to
 81 prevent browsing by vertebrate herbivores and sprayed the plots with insecticide. For herbivores
 82 accessibility plots (control treatment), we half enclosed the plots with the mesh netting. We sta-
 83 tioned one HOBO MX2307 data logger at each site to collect temperature and volumetric water
 84 content in the soil every hour.

Source populations and Identification of individual endophyte status. Plants used in the common garden experiment were derived from natural populations throughout the native range in the south-central US (fig.1, Table X²). At each of these natural populations we collected seeds. These seeds were planted at Rice University greenhouse. Seedlings were regularly fertilized every two weeks. The seedlings were then vegetatively propagated to produce enough individuals for your experiment (N = 840). Before planting in the field, we confirmed the endophyte status (E^+ or E^-) of all seedlings using either microscopy or an immunoblot assay. This was necessary due to the varying success of the heat treatment and differences in the prevalence of endophytes between the natural populations. Leaf tissues were stained with aniline blue lactic acid and viewed under a compound microscope at 200x-400x to identify fungal hyphae. The immunoblot assay (Phytoscreen field tiller endophyte detection kit, Agrinostics Ltd. Co.) uses monoclonal antibodies that target proteins of *Epichloë* spp. and chromagen to visually indicate presence or absence. Both methods yield similar detection rates.

Demographic data

We collected demographic data including survival, growth, and reproduction during June 2023, which coincided with the flowering season of *E. virginicus*. On each individual, survival of plants was recorded as a binary (death or alive) and the size of the plant was recorded as the number of living tillers, indicated by the presence of green coloration. We recorded the number of inflorescences per plant and the number of spikelets on up to three inflorescences from three reproducing plants. We limited the spikelets count to three reproducing tillers per plot due to the time consuming nature of this measurement process. We used the number of spikelets for these three tillers to estimate the number the average number of spikelets per plants.

²We need this table in the Appendix

Candidate Models

To assess how stress associated with aridity and low precipitation affect plant-fungal mutualism, we developed five mixed-effects models for each vital rate. Each vital rate was modeled with 10 candidates models. These included XXX

In each of the mixed-effects models, we specified two random effects to account for heterogeneity among plot within site and heterogeneity among host source populations. The first random effect was a nested random effect (plot within site) and the second one was a random intercept effect (population). We modeled growth with a Gaussian distribution using the package `lmerTest` (Source). We modeled fertility (number of spikelet) with a zero-inflated negative binomial distribution using the package `glmmTMB` (Source). We preferred the negative binomial in which the variance was modeled as a non-linear function of the mean ($\text{variance} = m(1 + m/k)$). For each vital rate, the first model was the intercept only model which is the null model. The second and the third models regressed vital rate against the additive effect of endophyte status and mean of soil moisture or coefficient of variation of soil moisture. The fourth and fifth models regressed vital rates against the interaction effect of endophyte status and mean of soil moisture or coefficient of variation of soil moisture (Appendix S1). We compared the five models using the Akaike Information Criterion (AIC) to select the best model (Source).

Model Fitting, Model Selection, and Parameter Estimation

Acknowledgments

OEC would like to thank Madlen Wilmes, Gyuri Barabás, Flo Débarre, Vlastimil Křivan, and Greg Dwyer for their comments and suggestions on this template.

Statement of Authorship

OEC conceived the experiments, collected the data, and wrote the original draft. GHC provided specimens and analyzed the model. AQE oversaw data analysis and developed the code. All authors reviewed and edited the writing at all stages of revision.

Data and Code Availability

On initial submission, you may use this section to provide a URL for editors and reviewers that is ‘private for peer review’. After acceptance, this section must be updated with correct, working DOIs for data and code deposits (such as in Zenodo, Dryad, or DataVerse). An example statement could resemble the following: All data and code for this work are available from the Dryad Digital Repository, ?).

Appendix A: Additional Methods and Parameters

Fox–dog encounters through the ages

The quick red fox jumps over the lazy brown dog. The quick red fox has always jumped over the lazy brown dog. The quick red fox began jumping over the lazy brown dog in the 19th century and has never ceased from so jumping, as we shall see in figure A1. But there can be surprises (figure A2).

If the order and location of figures is not otherwise clear, feel free to include explanatory dummy text like this:

[Figure A1 goes here.]

[Figure A2 goes here.]

148

Further insights

149 Tables in the appendices can appear in the appendix text (see table A1 for an example), unlike
150 appendix figure legends which should be grouped at the end of the document together with the
151 other figure legends.

Table A1: Various rivers, cities, and animals

River	City	Animal
Chicago	Chicago	Raccoon
Des Plaines	Joliet	Coyote
Illinois	Peoria	Cardinal
Kankakee	Bourbonnais	White-tailed deer
Mississippi	Galena	Bald eagle

Note: See table 1 below for further table formatting hints.

152 Lorem ipsum dolor sit amet, as we have seen in figures A1 and A2.

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Tables

Table 1: Founders of *The American Naturalist*

Early editor	Years with the journal
Alpheus S. Packard Jr.	1867–1886
Frederick W. Putnam	1867–1874
Edward S. Morse	1867–1871
Alpheus Hyatt	1867–1871
Edward Drinker Cope ^a	1878–1897
J. S. Kingsley	1887–1896

Note: Table titles should be short. Further details should go in a ‘notes’ area after the tabular environment, like this.

^a Published the first description of *Dimetrodon*.

Figure legends

Figure 1: Figure legends can be longer than the titles of tables. However, they should not be excessively long—in most cases, they should be no more than 100 words each.

Figure 2: In this way, figure legends can be listed at the end of the document, with references that work, even though the graphic itself should be included for final files after acceptance. Instead, upload the relevant figure files separately to Editorial Manager; Editorial Manager should insert them at the end of the PDF automatically.

Figure A1: *A*, the quick red fox proceeding to jump 20 m straight into the air over not one, but several lazy dogs. *B*, the quick red fox landing gracefully despite the skepticism of naysayers.

Figure A2: The quicker the red fox jumps, the likelier it is to land near an okapi. For further details, see ?.

Video S1: Video legends can follow the same principles as figure legends. Counters should be set and reset so that videos and figures are enumerated separately.