

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

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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 symbiont; 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 Grotel, 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

28 intensity or likelihood of host-symbiont interactions across host species geographic range is lim-  
29 ited.

30 Ecological studies of plant-microbe symbiosis usually investigate the interaction from the  
31 plant's perspective and rarely study how symbiont response to environmental variation might  
32 translate to its influence on host demographic performance across host range. These studies  
33 showed that symbionts increase their host's growth and reproduction rates, which outweigh the  
34 host's lower survival rates and result in an overall increase in host population size over time  
35 (Yule et al., 2013). Moreover, studies of plant-microbe symbiosis relied on methods such as inoc-  
36 uinating sterile soil, excluding endophyte fungal hyphae by using fine mesh or rotating cores, and  
37 adding fungicide (Bennett and Groten, 2022). Despite their value, all these approaches are often  
38 difficult to implement in field settings or on a large scale. As a result, the exact mechanisms by  
39 which symbionts drive host range limitation and expansion are not well understood, hindering  
40 our understanding of the potential cascading effects of symbionts on eco-evolutionary species  
41 demography and range limitation in the context of global change.

42 Working across a precipitation gradient in the south-central US, we asked how the demo-  
43 graphic effects of endophyte symbiosis varied from core to edge of the host range. We also asked  
44 how does fungal growth affect host demography from range core to range edge. To answer,  
45 these questions, we studied the symbiotic association between a cool-season grass species (*Ely-*  
46 *mus virginicus*) and its vertically transmitted fungal symbiont *Epichloë elymi*. [Describe ecology  
47 and natural history of grass-endophyte interactions]<sup>1</sup>. Our experiment was design to test the  
48 following hypotheses:

- 49 1. We hypothesized that stress associated with aridity and low precipitation would strengthen  
50 the plant-fungal mutualism, such that the fitness benefits of endophyte symbiosis are max-  
51 imized at the range edge.
- 52 2. We hypothesized that fungal growth in planta varied from range core to range edge. If

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<sup>1</sup>I am not sure If we need this

53 endophyte growth is limited by host photosynthesis, then environments that are stressful  
54 for hosts may correspond to poor endophyte growth. Alternatively, if active regulation by  
55 the host is required to keep symbionts “in check”, then environments that are stressful for  
56 hosts may correspond to high endophyte growth.

## 57 Materials and methods

### 58 Study species

59 *Elymus virginicus* (Poaceae) is a cool season perennial grass native to woodland and prairie  
60 habitats of eastern North America (Shaw, 2011). The westernmost range limits of this species  
61 correspond to the longitudinal aridity gradient in the central and southern Great Plains (fig.  
62 1). Throughout its range, the species is symbiotic with the seed-transmitted fungal endophyte  
63 (*Epichloë* spp.) (Rudgers and Swafford, 2009). Across natural populations in Texas, endophyte  
64 prevalence (fraction of plants that are endophyte-symbiotic) in *Elymus virginicus* ranged from  
65 10% to 100%, with a mean of 53% (Sneck et al., 2017). Fungal genotyping indicated that the  
66 endophytes are capable of synthesizing secondary compounds such as peramine, loline, and er-  
67 got alkaloids, which may confer resistance against drought and herbivory (Beaudry, 1951). In  
68 addition, the species is capable of both self-pollination and outcrossing (Church, 1958).

### 69 Study design

70 *Experimental Design.* To understand the demographic effects of endophyte symbiosis from core  
71 to edge of the host range, we established common gardens at 7 sites across the geographic range  
72 of *Elymus virginicus* (fig. 1). Experimental sites spanned an aridity gradient (temperature gradi-  
73 ent). Common gardens were established in 8 plots per site. Plots were 1.5m \* 1.5m and the area  
74 was tilled of existing vegetation to control for native plant competition. Plots were also selected  
75 in shaded areas under tree canopy or near shrubs to mimic the natural environmental of the  
76 species. In each plot, we planted 15 individuals of *E. virginicus* approximately 15 cm deep in an

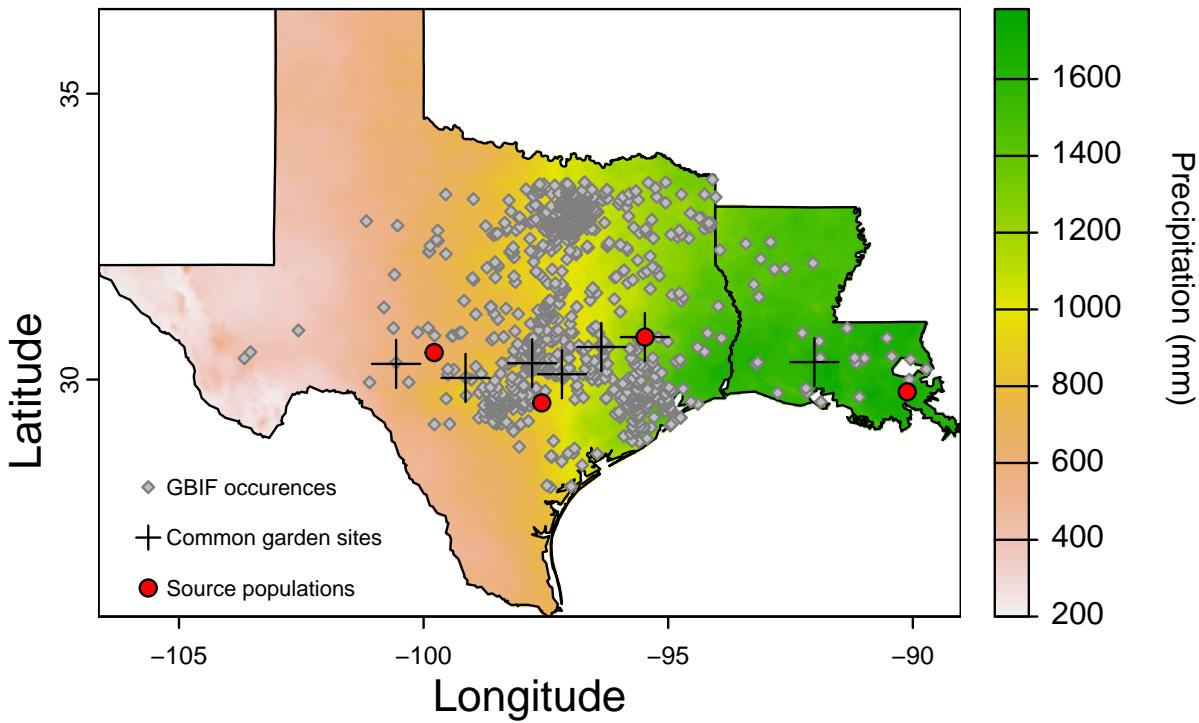


Figure 1: Distribution of common garden sites across the longitudinal aridity gradient in the central and southern Great Plains. Red dots represent the locations of source populations, while grey dots represent the GBIF locations of the species across the study area.

evenly spaced 4\*4 grid pattern, with positions randomly assigned. For each plot, we randomly assigned a starting endophyte frequency (80%, 60%, 40%, 20%)<sup>2</sup> and herbivory treatment (herbivores exclusion and herbivores accessibility). We ensured that all plots had comparable quantities of source populations. After establishing the plots, we watered the plants and recorded initial tiller counts, flowering status and plot position, endophyte status, source population of each individual plant. For herbivory exclusion plots, we enclosed them with 1.2m tall mesh fencing to prevent browsing by vertebrate herbivores and sprayed the plots with insecticide. For herbivores

<sup>2</sup>*Do we need a schematic of one replicate of the experimental design?*

84 accessibility plots (control treatment), we half enclosed the plots with the mesh netting. We sta-  
85 tioned one HOBO MX2307 data logger at each site to collect temperature and volumetric water  
86 content in the soil every hour.

87 *Source populations and Identification of individual endophyte status.* Plants used in the common gar-  
88 den experiment were derived from natural populations throughout the native range in the south-  
89 central US (fig.1, [Table X](#)<sup>3</sup>). At each of these natural populations we collected seeds. Some of the  
90 seeds of *E. virginicus* were heat treated to produce endophyte negative plants ( $E^-$ ) . To do so, we  
91 placed these seeds in a drying oven set at 60°C for approximately five days (120 hours). While  
92 this method eliminates endophytes from all individuals, it does not affect seed viability. All  
93 seeds (both heat-treated and non-heat-treated) were planted in the Rice University greenhouse.  
94 Seedlings were regularly fertilized every two weeks. The seedlings were then vegetatively prop-  
95 agated to produce enough individuals for your experiment (N = 840). Before planting in the  
96 field, we confirmed the endophyte status ( $E^+$  or  $E^-$ ) of all seedlings using either microscopy or  
97 an immunoblot assay. This was necessary due to the varying success of the heat treatment and  
98 differences in the prevalence of endophytes between the natural populations. Leaf tissues were  
99 stained with aniline blue lactic acid and viewed under a compound microscope at 200x-400x  
100 to identify fungal hyphae. The immunoblot assay (Phytoscreen field tiller endophyte detection  
101 kit, Agrinostics Ltd. Co.) uses monoclonal antibodies that target proteins of *Epichloë* spp. and  
102 chromagen to visually indicate presence or absence. Both methods yield similar detection rates.

103 *Climatic data*

104 To characterize the stress gradient with respect to climatic conditions, we collected the hourly  
105 temperature and soil moisture at each site using the HOBO MX2307 data loggers. We used this  
106 hourly variable to calculate the daily mean temperature (°C) and soil moisture (%)(fig. 3). [We](#)  
107 [calculated the mean soil moisture and the coefficient of variation from the time the plants were](#)

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<sup>3</sup>[We need this table in the Appendix](#)

108 placed on the ground to the time we collected demographic data<sup>4</sup>. The coefficient of variation of  
109 soil moisture was estimated to capture season variability in climatic data (Medvigi and Beaulieu,  
110 2012; Meshram et al., 2017).

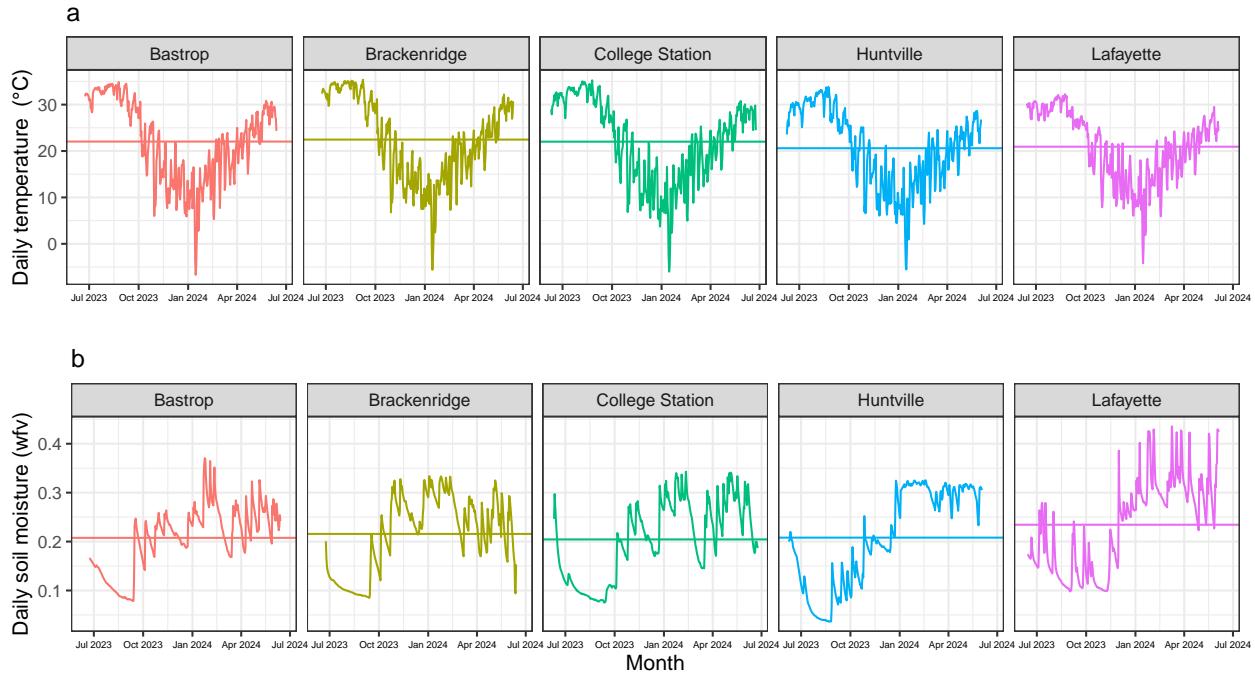


Figure 2: Climate variation across common garden sites. (a) Daily temperature, estimated from average hourly data collected by HOBO data loggers. (b) Daily soil moisture, estimated from average hourly data collected by HOBO data loggers.

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### Demographic data

112 We collected demographic data including survival, growth, and reproduction during June 2023,  
113 which coincided with the flowering season of *E. virginicus*. On each individual, survival of  
114 plants was recorded as a binary (death or alive) and the size of the plant was recorded as the  
115 number of living tillers, indicated by the presence of green coloration. We recorded the number  
116 of inflorescences per plant and the number of spikelets on up to three inflorescences from three

<sup>4</sup>I will change this later

117 reproducing plants. We limited the spikelets count to three reproducing tillers per plot due to  
118 the time consuming nature of this measurement process. We used the number of spikelets for  
119 these three tillers to estimate the number the average number of spikelets per plants.

120 *Models building and models selection*

121 To assess how stress associated with aridity affect plant-fungal mutualism, we developed four  
122 candidates models for each vital rate (survival, growth, flowering, fertility). Each vital rate was  
123 modeled with the grand mean intercept ( $\beta_0$ ), slopes for variation in each covariate ( $\beta_1 \dots \beta_3$ ) as well  
124 as the interaction between covariates ( $\beta_4 \dots \beta_6$ ): Each model includes normally distributed random  
125 effects for site-to-site variation ( $\phi \sim N(0, \sigma_{site})$ ), plot to plot variation ( $\rho \sim N(0, \sigma_{plot})$ ), and  
126 source-to-source variation that is related to the provenance of the transplants used to establish  
127 the common garden ( $\omega \sim N(0, \sigma_{source})$ ) (Eq.1).

$$\begin{aligned} model1 &= \beta_0 + \beta_1 T_{mean} + \beta_2 E + \beta_3 H + \beta_4 E * T_{mean} + \beta_5 H * E + \beta_6 H * T_{mean} + \phi + \omega + \rho \\ model2 &= \beta_0 + \beta_1 T_{CV} + \beta_2 E + \beta_3 H + \beta_4 E * T_{CV} + \beta_5 H * E + \beta_6 H * T_{mean} + \phi + \omega + \rho \\ model3 &= \beta_0 + \beta_1 M_{mean} + \beta_2 E + \beta_3 H + \beta_4 E * M_{mean} + \beta_5 H * E + \beta_6 H * M_{mean} + \phi + \omega + \rho \\ model4 &= \beta_0 + \beta_1 M_{CV} + \beta_2 E + \beta_3 H + \beta_4 E * M_{CV} + \beta_5 H * E + \beta_6 H * M_{CV} + \phi + \omega + \rho \end{aligned} \quad (1)$$

128 We modeled survival using a Bernoulli distribution, growth with a Gaussian distribution,  
129 flower with a negative binomial, fertility (number of spikelet) with a negative binomial distri-  
130 bution. To check whether the fitted models are compatible with the observed data, we used the  
131 posterior predictive checks (Berkhof et al., 2000; Gelman et al., 2000). All models make sense to  
132 explain the observed data (fig.A1, fig.A2, fig.A3, fig.A4).

133 To select the best model for each vital rate, we compared the four models using the leave-  
134 one-out cross-validation (LOOCV) (Vehtari et al., 2017). LOOCV combines both validation and  
135 training methods. In this approach, one observation is used for validation while the training  
136 set consists of n-1 observations. This process is repeated for each observation, resulting in n  
137 estimated models (Silva and Zanella, 2024). The estimate of test error from LOOCV is calculated

<sup>138</sup> by averaging the errors across these n models (Eq.2).

$$CV_n = \frac{1}{n} \sum_{i=1}^n (y_I - \hat{y}_I)^2 \quad (2)$$

<sup>139</sup> All models were performed in R (R Core Team, 2023) and Stan (Stan Development Team,  
<sup>140</sup> 2024).

## Results

Table 1: Candidate models of *E. virginicus* vital rates (growth, flowering, spikelet and survival).

Vital rate	Model	$\Delta elpd$	$\Delta se$
<b>Survival</b>	<b>model3</b>	<b>0.0</b>	<b>0.0</b>
Survival	model2	-0.5	0.5
Survival	model4	-0.9	0.9
Survival	model1	-2.2	1.9
<b>Growth</b>	<b>model2</b>	<b>0.0</b>	<b>0.0</b>
Growth	model4	0.0	0.8
Growth	model3	-0.3	0.4
Growth	model1	-0.4	0.4
<b>Flowering</b>	<b>model2</b>	<b>0.0</b>	<b>0.0</b>
Flowering	model4	-4.3	1.0
Flowering	model3	-6.5	1.4
<b>Spiket</b>	<b>model2</b>	<b>0.0</b>	<b>0.0</b>
Spiket	model3	-0.5	0.6
Spiket	model1	-0.7	1.2
Spiket	model4	-1.2	1.2

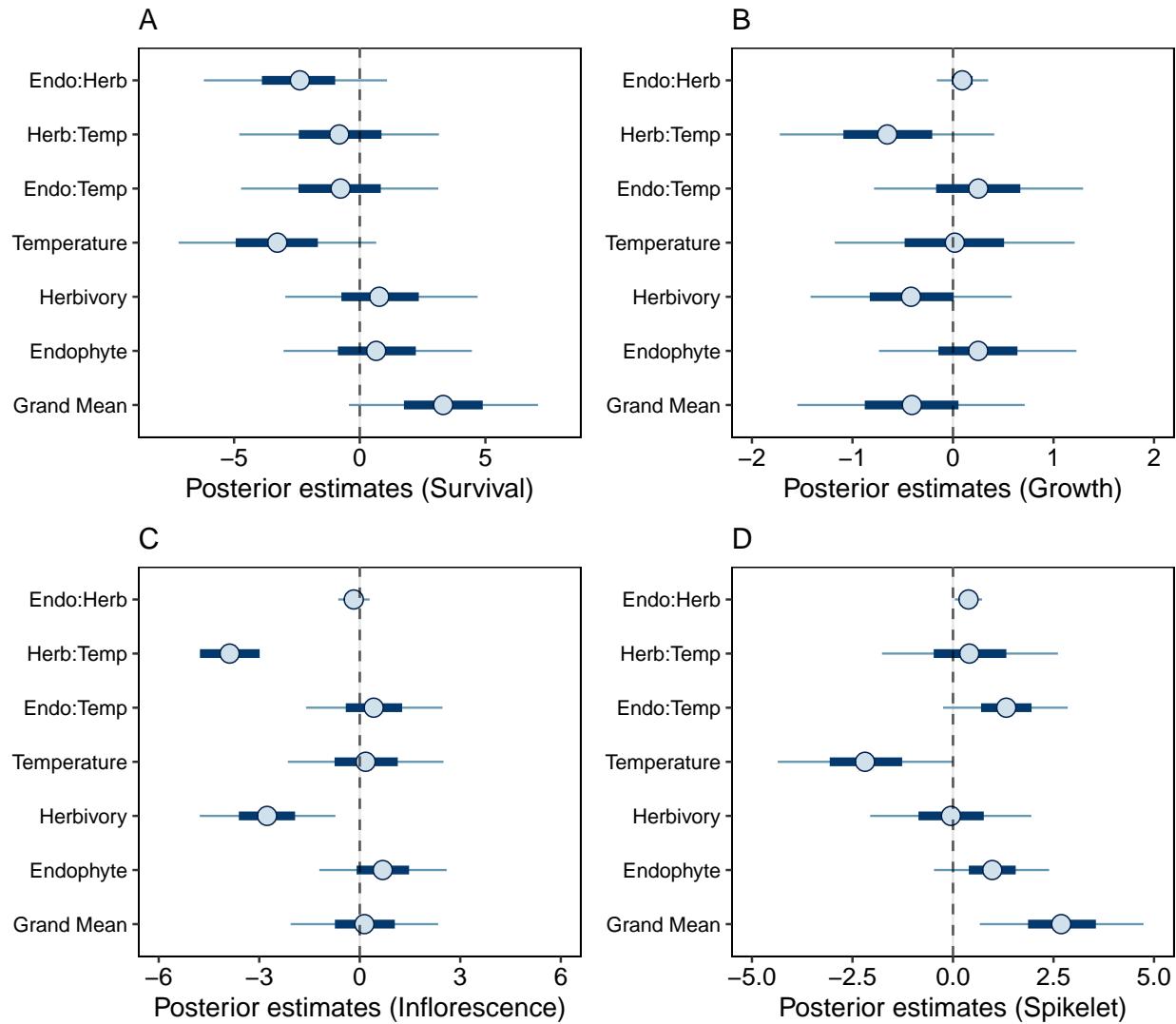


Figure 3: Posterior mean estimates for each vital rate. (a) Daily temperature, estimated from average hourly data collected by HOBO data loggers. (b) Daily soil moisture, estimated from average hourly data collected by HOBO data loggers.

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## Acknowledgments

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## Statement of Authorship

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## Data and Code Availability

## Appendix A

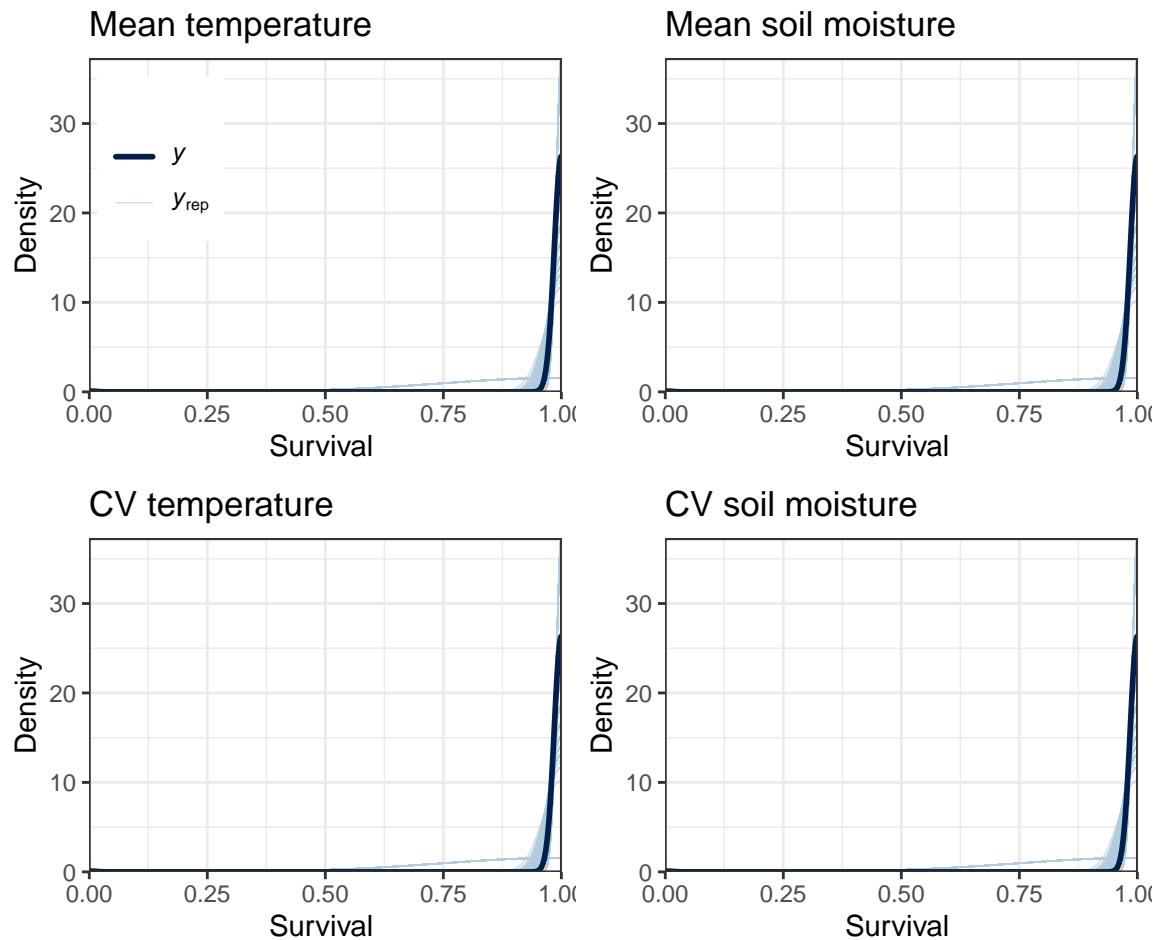


Figure A1: Comparison of the observed survival data with the posterior predictions from the survival models for each climate variable

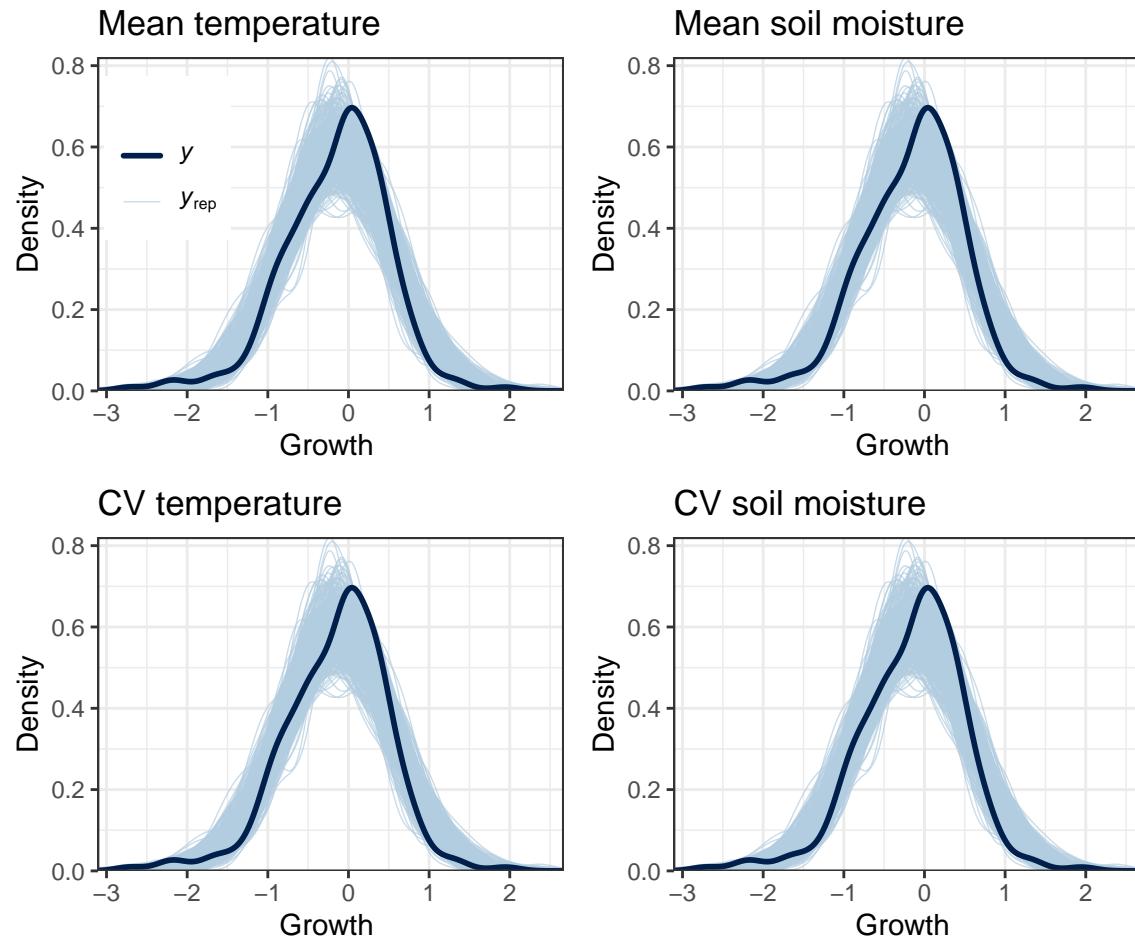


Figure A2: Comparison of the observed growth data with the posterior predictions from the growth models for each climate variable

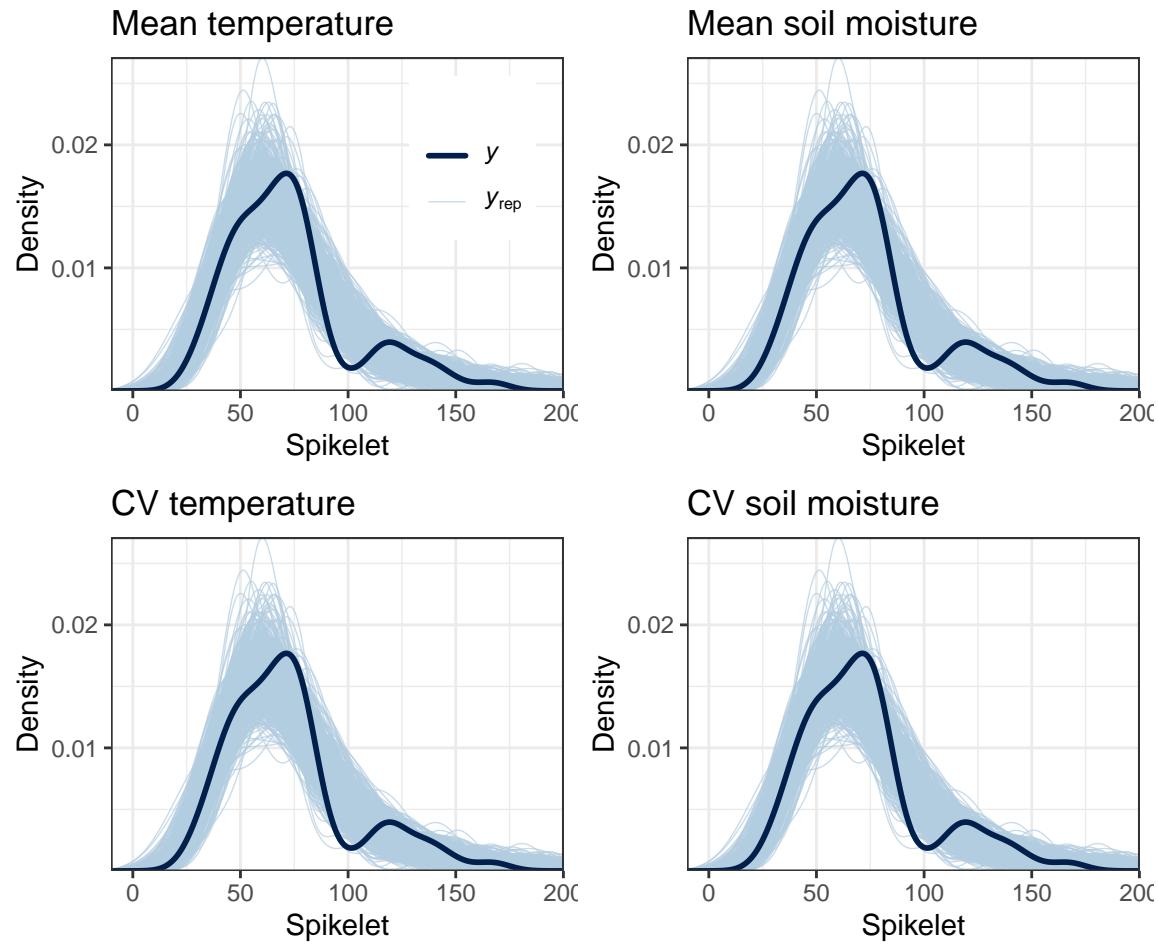


Figure A3: Comparison of the observed spikelet data with the posterior predictions from the spikelet models for each climate variable

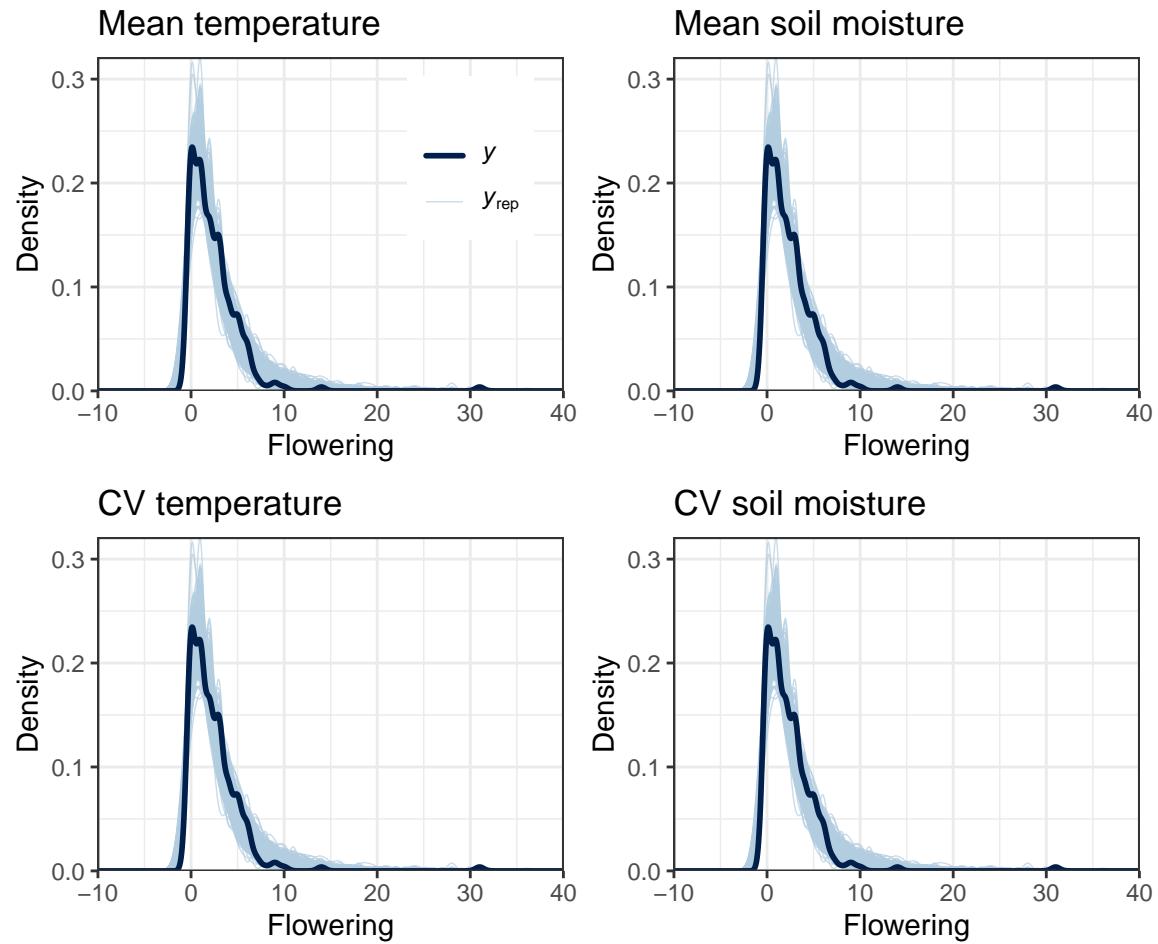


Figure A4: Comparison of the observed flowering data with the posterior predictions from the flowering models for each climate variable

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