

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

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## Abstract

## Introduction

3 Plant-microbe symbioses are widespread and ecologically important. These interactions are fa-  
4 mously context-dependent, where the direction and strength of the interaction outcome depends  
5 on the environment in which it occurs (Bronstein, 1994; Fowler et al., 2023; Hoeksema and Bruna,  
6 2015). Under biotic stress (e.g., herbivory), endophyte symbiosis can benefit host plants by fa-  
7 cilitating the production of secondary compounds that deter feeding or cause direct toxicity,  
8 thereby reducing insect growth, survival, and oviposition (Atala et al., 2022; Bastias et al., 2017;  
9 Vega, 2008). Similarly under abiotic stress (e.g., drought), symbionts can increase their host tol-  
10 erance to drought (Clay and Schardl, 2002). However, in many plant-microbe interactions, host  
11 protection is not guaranteed solely by the presence of a symbiont; rather, the density of the  
12 symbiont can determine the effectiveness of this protection (Laughton et al., 2014). Having a  
13 greater endophyte density could lead to high resource exploitation by the symbiont, which may  
14 be costly (reduction in growth or in reproductive) for the host (Faeth, 2009). Ultimately, these  
15 context-dependent costs and benefits may underlie the observed distribution of host species.

16 Context-dependence raises the hypothesis that plant-microbe interactions are likely to vary  
17 across environmental gradients, from range-core to range-edge, which could have significant  
18 consequences for host range expansion. If the benefits of microbial symbiosis strengthen under  
19 environmental stress then symbionts could make range-edge environments more suitable, pos-  
20 sibly extending the host's range limits (Allsup et al., 2023; Rudgers et al., 2020). For instance,  
21 fungal endophytes improve the survival of *Bromus laevipes* populations in dry conditions, en-  
22 hancing their resistance to drought stress at the range edge and thereby extending the species'  
23 geographic range (Afkhami et al., 2014; David et al., 2019). Even if the symbiont does not improve  
24 host survival, it could still increase the host's population growth rate over time by enhancing the  
25 host's relative growth and reproduction, potentially outweighing the negative impact of lower  
26 survival rates (Yule et al., 2013). However, if microbial symbiosis is costly for the host at range  
27 edge, then symbionts could limit host range (Bennett and Groten, 2022; Benning and Moeller,

28 2021a,b). Although context dependence, along with spatio-temporal variations in abiotic envi-  
29 ronmental conditions may reduce the effectiveness of the benefits provided by the symbiont to  
30 the host species, our understanding of the mechanisms that alter the intensity or likelihood of  
31 host-symbiont interactions across host species geographic range is limited.

32 Ecological studies of plant-microbe symbiosis usually investigate the interaction from the  
33 plant's perspective and rarely study how symbiont response to environmental variation might  
34 translate to its influence on host demographic performance across host range. Moreover, studies  
35 on plant-microbe symbiosis often overlook the synergistic effects of abiotic and biotic factors  
36 on host dynamics across species ranges. Finally, studies of plant-microbe symbiosis relied on  
37 methods such as inoculating sterile soil (Peacher and Meiners, 2020), excluding endophyte fungal  
38 hyphae by using fine mesh or rotating cores (Chung et al., 2019), and adding fungicide (Bennett  
39 and Groten, 2022). Despite their value, all these approaches are often difficult to implement in  
40 field settings or on a large scale. As a result, the exact mechanisms by which symbionts drive  
41 host range limitation and expansion are not well understood, hindering our understanding of  
42 the potential cascading effects of symbionts on eco-evolutionary species demography and range  
43 limitation in the context of global change.

44 Working across a precipitation gradient in the south-central US, we asked how the demo-  
45 graphic effects of endophyte symbiosis varied from core to edge of the host range. We also asked  
46 how does fungal growth affect host demography from range core to range edge. To answer,  
47 these questions, we studied the symbiotic association between a cool-season grass species (*Ely-*  
48 *mus virginicus*) and its vertically transmitted fungal symbiont *Epichloë elymi*. [Describe ecology  
49 and natural history of grass-endophyte interactions]<sup>1</sup>. Our experiment was design to test the  
50 following hypotheses<sup>2</sup> :

- 51 1. We hypothesized that stress associated with aridity and low precipitation would strengthen  
52 the plant-fungal mutualism, such that the fitness benefits of endophyte symbiosis are max-

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

<sup>2</sup> I need to update these hypotheses by adding the herbivory effect

53 imized at the range edge.

54 2. We hypothesized that fungal growth in planta varied from range core to range edge. If  
55 endophyte growth is limited by host photosynthesis, then environments that are stressful  
56 for hosts may correspond to poor endophyte growth. Alternatively, if active regulation by  
57 the host is required to keep symbionts “in check”, then environments that are stressful for  
58 hosts may correspond to high endophyte growth.

## 59 Materials and methods

### 60 Study species

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

### 71 Study design

72 *Experimental Design.* To understand the demographic effects of endophyte symbiosis from core  
73 to edge of the host range, we established common gardens at 7 sites across the geographic range  
74 of *Elymus virginicus* (fig. 1). Experimental sites spanned an aridity gradient (temperature gradi-  
75 ent). Common gardens were established in 8 plots per site. Plots were 1.5m \* 1.5m and the area

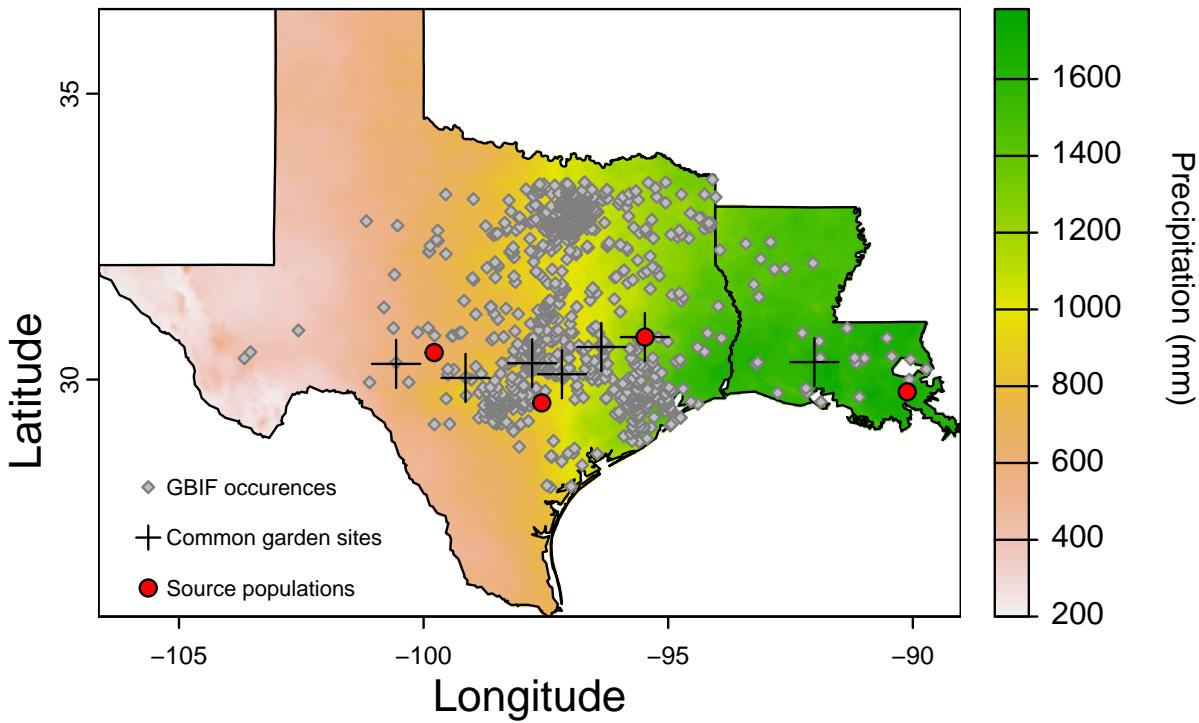


Figure 1: Distribution of common garden sites across the longitudinal aridity gradient in the central and southern Great Plains. Red dots represent the locations of source populations, while grey dots represent the GBIF locations of the species across the study area.

76 was tilled of existing vegetation to control for native plant competition. Plots were also selected  
 77 in shaded areas under tree canopy or near shrubs to mimic the natural environmental of the  
 78 species. In each plot, we planted 15 individuals of *E. virginicus* approximately 15 cm deep in an  
 79 evenly spaced 4\*4 grid pattern, with positions randomly assigned. For each plot, we randomly  
 80 assigned a starting endophyte frequency (80%, 60%, 40%, 20%)<sup>3</sup> and herbivory treatment (herbi-  
 81 vores exclusion and herbivores accessibility). We ensured that all plots had comparable quantities  
 82 of source populations. After establishing the plots, we watered the plants and recorded initial

<sup>3</sup>*Do we need a schematic of one replicate of the experimental design?*

83 tiller counts, flowering status and plot position, endophyte status, source population of each in-  
84 dividual plant. For herbivory exclusion plots, we enclosed them with 1.2m tall mesh fencing to  
85 prevent browsing by vertebrate herbivores and sprayed the plots with insecticide. For herbivores  
86 accessibility plots (control treatment), we half enclosed the plots with the mesh netting. We sta-  
87 tioned one HOBO MX2307 data logger at each site to collect temperature and volumetric water  
88 content in the soil every hour.

89 *Source populations and Identification of individual endophyte status.* Plants used in the common gar-  
90 den experiment were derived from natural populations throughout the native range in the south-  
91 central US (fig.1, [Table X](#)<sup>4</sup>). At each of these natural populations we collected seeds. Some of the  
92 seeds of *E. virginicus* were heat treated to produce endophyte negative plants ( $E^-$ ) . To do so, we  
93 placed these seeds in a drying oven set at 60°C for approximately five days (120 hours). While  
94 this method eliminates endophytes from all individuals, it does not affect seed viability. All  
95 seeds (both heat-treated and non-heat-treated) were planted in the Rice University greenhouse.  
96 Seedlings were regularly fertilized every two weeks. The seedlings were then vegetatively prop-  
97 agated to produce enough individuals for your experiment (N = 840). Before planting in the  
98 field, we confirmed the endophyte status ( $E^+$  or  $E^-$ ) of all seedlings using either microscopy or  
99 an immunoblot assay. This was necessary due to the varying success of the heat treatment and  
100 differences in the prevalence of endophytes between the natural populations. Leaf tissues were  
101 stained with aniline blue lactic acid and viewed under a compound microscope at 200x-400x  
102 to identify fungal hyphae. The immunoblot assay (Phytoscreen field tiller endophyte detection  
103 kit, Agrinostics Ltd. Co.) uses monoclonal antibodies that target proteins of *Epichloë* spp. and  
104 chromagen to visually indicate presence or absence. Both methods yield similar detection rates.

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<sup>4</sup>[We need this table in the Appendix](#)

## Climatic data

106 To characterize the stress gradient with respect to climatic conditions, we collected the hourly  
 107 temperature and soil moisture at each site using the HOBO MX2307 data loggers. We used this  
 108 hourly variable to calculate the daily mean temperature ( $^{\circ}\text{C}$ ) and soil moisture (%) (fig. 3). **We**  
 109 **calculated the mean soil moisture and the coefficient of variation from the time the plants were**  
 110 **placed on the ground to the time we collected demographic data**<sup>5</sup>. The coefficient of variation of  
 111 soil moisture was estimated to capture season variability in climatic data (Medvigy and Beaulieu,  
 112 2012; Meshram et al., 2017).

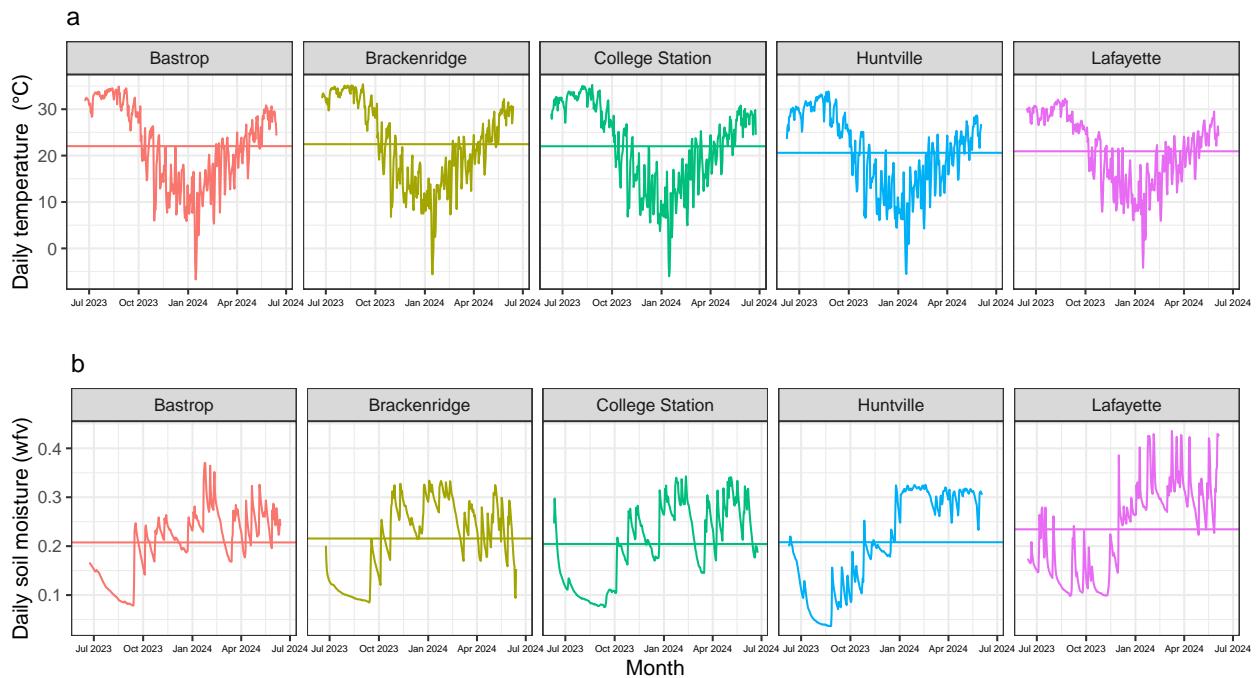


Figure 2: Climate variation across common garden sites. (a) Daily temperature, estimated from average hourly data collected by HOBO data loggers. (b) Daily soil moisture, estimated from average hourly data collected by HOBO data loggers.

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<sup>5</sup> I will change this later

113

## Demographic data

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

122

## Models building and models selection

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

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

130 We modeled survival using a Bernoulli distribution, growth with a Gaussian distribution,  
 131 flower with a negative binomial, fertility (number of spikelet) with a negative binomial distri-  
 132 bution. To check whether the fitted models are compatible with the observed data, we used the

<sup>133</sup> posterior predictive checks (Berkhof et al., 2000; Gelman et al., 2000). All models make sense to  
<sup>134</sup> explain the observed data (fig.A1, fig.A2, fig.A3, fig.A4).

<sup>135</sup> To select the best model for each vital rate, we compared the four models using the leave-  
<sup>136</sup> one-out cross-validation (LOOCV) (Vehtari et al., 2017). LOOCV combines both validation and  
<sup>137</sup> training methods. In this approach, one observation is used for validation while the training  
<sup>138</sup> set consists of n-1 observations. This process is repeated for each observation, resulting in n  
<sup>139</sup> estimated models (Silva and Zanella, 2024). The estimate of test error from LOOCV is calculated  
<sup>140</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>141</sup> All models were performed in R (R Core Team, 2023) and Stan (Stan Development Team,  
<sup>142</sup> 2024).

## Results

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

Vital rate	Model	$\Delta\text{elpd}$	$\Delta\text{se}$
<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>Spikelet</b>	<b>model2</b>	<b>0.0</b>	<b>0.0</b>
Spikelet	model3	-0.5	0.6
Spikelet	model1	-0.7	1.2
Spikelet	model4	-1.2	1.2

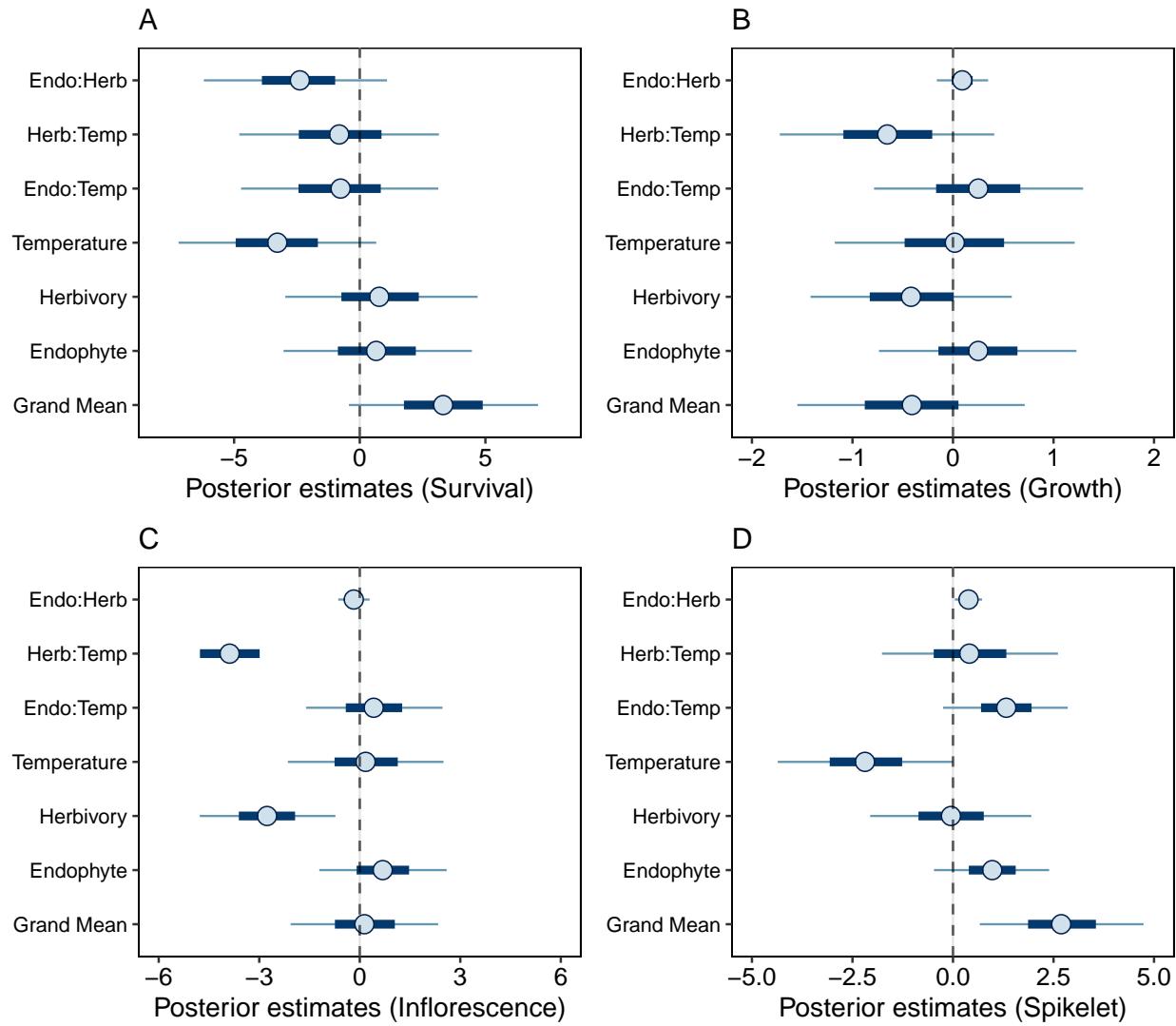


Figure 3: Posterior mean estimates for each vital rate. (a) Daily temperature, estimated from average hourly data collected by HOBO data loggers. (b) Daily soil moisture, estimated from average hourly data collected by HOBO data loggers.

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## Acknowledgments

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## Statement of Authorship

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## Data and Code Availability

## Appendix A

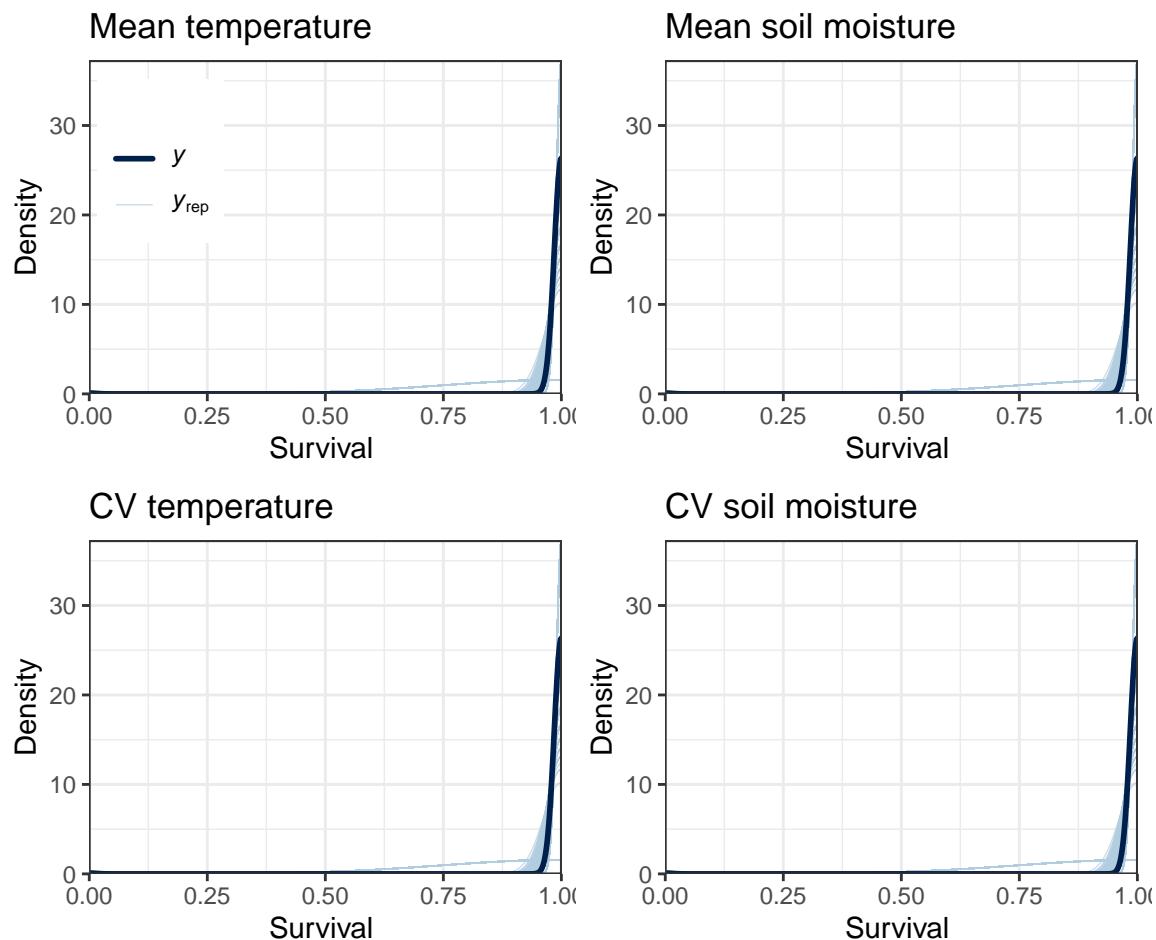


Figure A1: Comparison of the observed survival data with the posterior predictions from the survival models for each climate variable

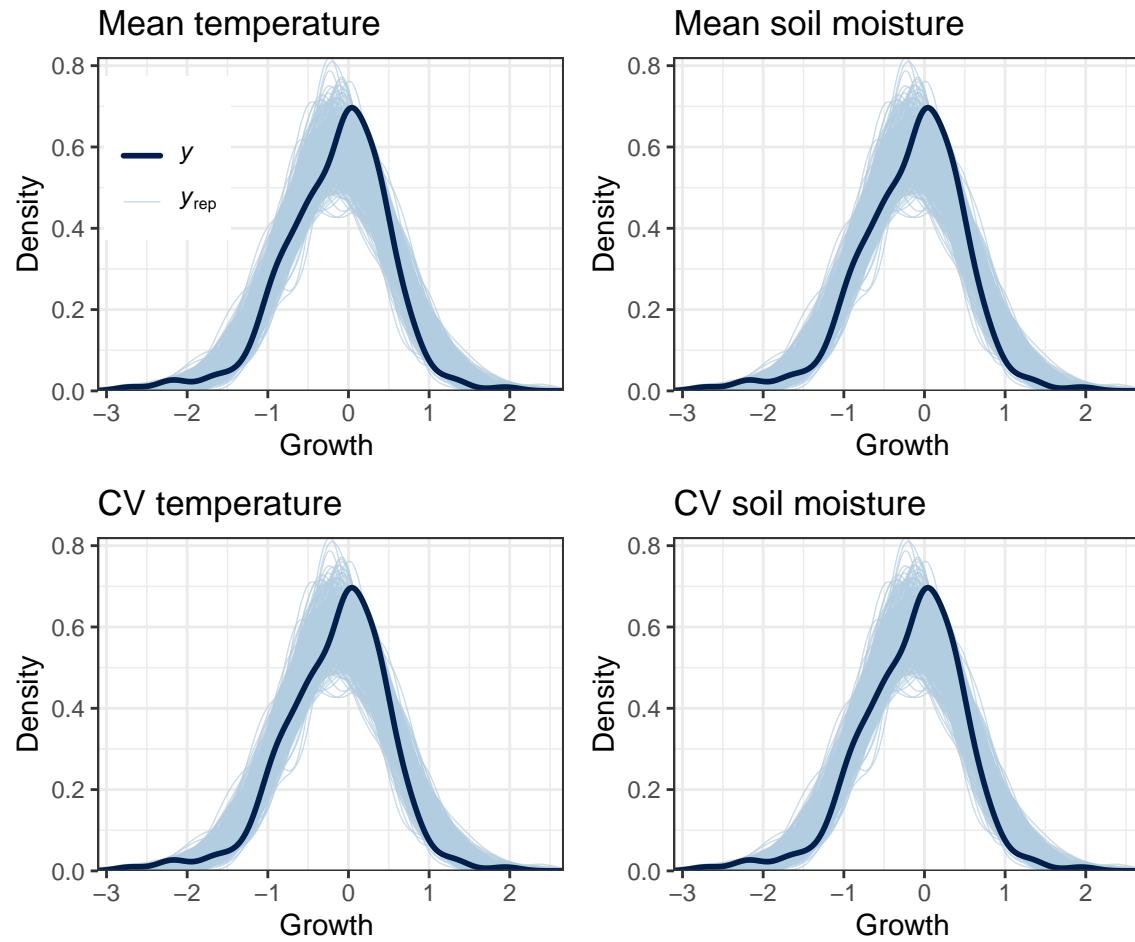


Figure A2: Comparison of the observed growth data with the posterior predictions from the growth models for each climate variable

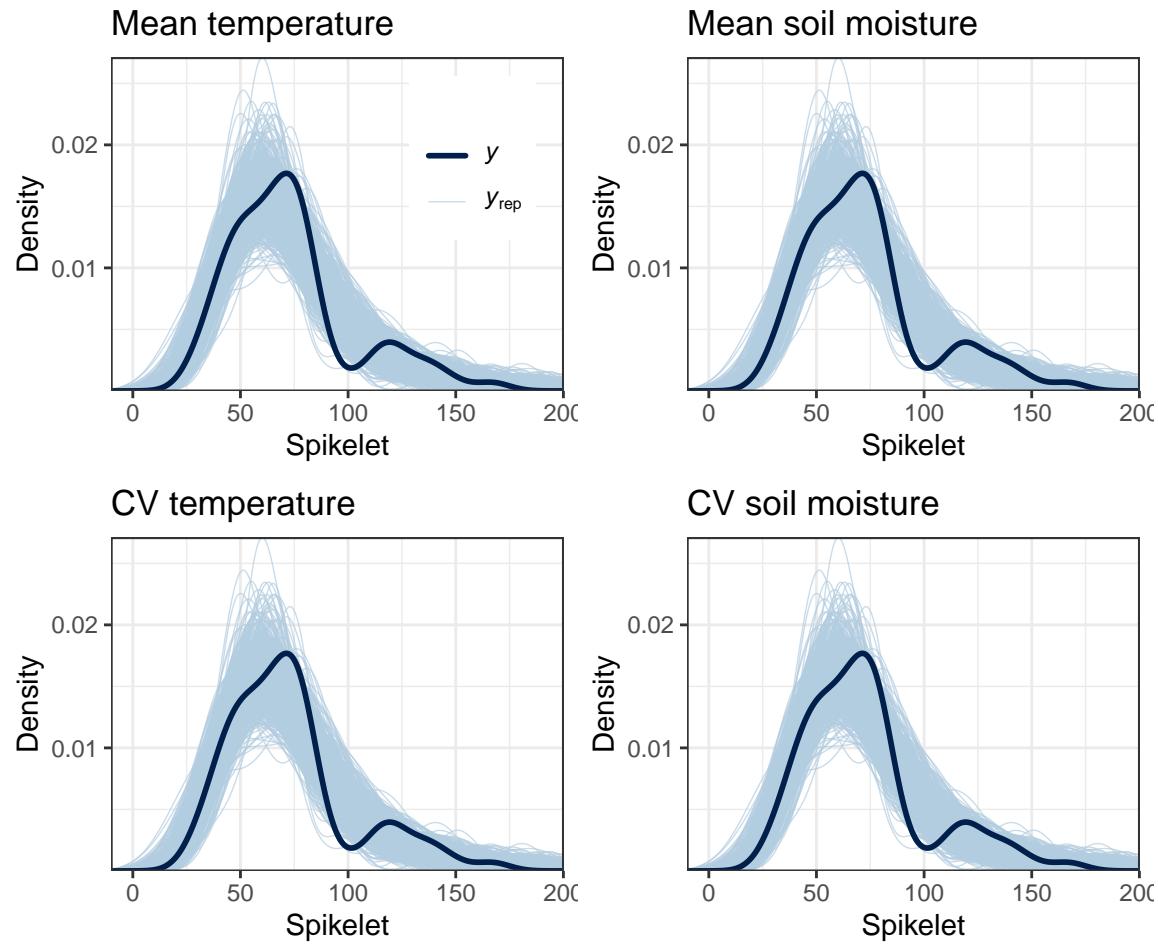


Figure A3: Comparison of the observed spikelet data with the posterior predictions from the spikelet models for each climate variable

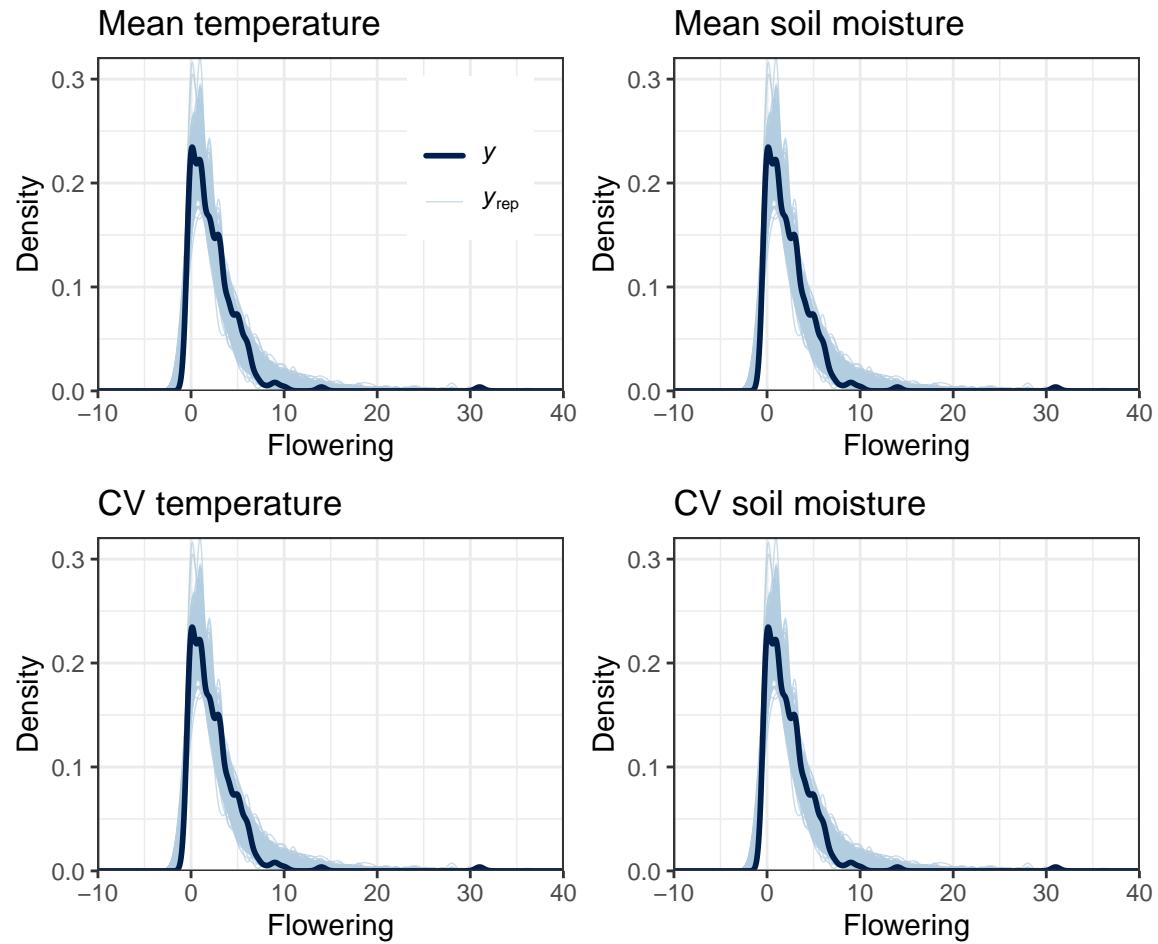


Figure A4: Comparison of the observed flowering data with the posterior predictions from the flowering models for each climate variable

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