Temperature, phenology, and plant defenses predict fitness near colder range limit

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Abstract

The space for time substitution posits that warmer locations can provide a source of genetic variation that could be adaptive for future climate change conditions. While this approximation might be useful for planning assisted gene flow, it relies on the importance of abiotic adaptations over biotic ones. Here I address this gap by assessing influence of anti-herbivore defenses, phenology, and morphology on the seed production of 146 populations of *Oenothera biennis* close to the plant's cold range limit. Genotypes from 2.1° South of the common garden produce more seeds than most northern lineages. Adaptations across space are a suitable substitute for climate change, but there is still substantial fitness variability. These differences were best explained by bolt date, flowering time, and greater defenses against herbivores. Given the impacts of climate change, plant defenses might already be of similar adaptive importance to phenology close to northern rage limits.

Key Words

Local adaptation, climate gradient, seed production, flowering time, herbivory, ellagitannins, *Oenothera bennis*.

Introduction

Climate change is exposing populations to climate extremes that are increasingly further from historical averages (IPCC 2014; Pörtner *et al.* 2022). These deviations from baseline climatic conditions are overwhelming abiotic and possibly biotic adaptations (Etterson 2004; Anderson *et al.* 2012; Franks *et al.* 2014) leading to mortality (Van Mantgem & Stephenson 2007), population decline, (Willis *et al.* 2008) and range contractions (Freeman *et al.* 2018). Many populations that were locally adapted to their abiotic environment prior to the onset of climate change (Leimu & Fischer 2008; Sanford & Kelly 2011) are becoming maladapted (Aitken *et al.* 2008; Wang *et al.* 2010; Wilczek *et al.* 2014; Anderson & Wadgymar 2020; Bontrager *et al.* 2020). These populations may have to undergo range shifts to more suitable conditions to survive (Parmesan & Yohe 2003; Parmesan 2006; Poloczanska *et al.* 2013; Fadrique *et al.* 2018), or rapidly evolve to reverse demographic decline (Bell & Gonzalez 2011; Bell 2017). Such rapid evolution can occur from standing genetic variation (Barrett & Schluter 2008), yet extreme climate change may require adaptations beyond locally available genetic variation.

The space-for-time substitution is a useful approximation in which spatial biodiversity patterns across abiotic gradients are used to predict future temporal changes (Pickett 1989; Blois *et al.* 2013a). Space-for-time has been used widely to study community composition (Walker *et al.* 2010; Blois *et al.* 2013a), phenology (Buyantuyev *et al.* 2012), range-shifts (Eskildsen *et al.* 2013), and landscape-level climate maladaptation (Fitzpatrick & Keller 2015; Exposito-Alonso *et al.* 2018; Dauphin *et al.* 2021). When invoking space-for-time in the context of climate change adaptation, missing adaptive genetic variation from higher latitudes are assumed to be present in warmer populations, such that space can substitute for adaptations needed across time (Wogan & Wang 2018). Indeed, climate change experiments suggest that genetic variation from warmer

52 localities can perform better in historically colder locations (Anderson & Wadgymar 2020;
53 Bontrager *et al.* 2020). However, space-for-time fails to consider non-analogue future climates
54 (Williams & Jackson 2007), unchanging landscape features such as photoperiod (Walker *et al.*
55 2019), and increasingly strong biotic interactions as higher latitude sites become warmer (Blois *et*
56 *al.* 2013b; HilleRisLambers *et al.* 2013).

57 How the space-for-time substitution is influenced by differences in biotic interactions
58 between populations remains largely untested (Bucharova 2017). Plant populations may be
59 particularly vulnerable to increases in biotic interactions such as herbivory, which have been
60 documented to increase at higher latitudes due to greater herbivore biomass and increased intensity
61 of herbivore outbreaks (de Sassi & Tylianakis 2012; DeLucia *et al.* 2012; Ju *et al.* 2015; Jactel *et*
62 *al.* 2019). Plants have evolved chemical and physical defenses to protect against herbivory (Ehrlich
63 & Raven 1964; Agrawal & Weber 2015) as well as herbivore avoidance strategies such as changes
64 in phenology (Carmona *et al.* 2011). However, biogeographic theory predicts that populations at
65 higher latitudes will have lower plant defenses (Dobzhansky 1950; MacArthur 1972; Schemske *et*
66 *al.* 2009; Baskett & Schemske 2018) (but see Moles *et al.* 2011; Anstett *et al.* 2016). This could
67 leave higher latitude populations poorly defended and vulnerable to more mobile insect herbivores
68 and may make plant defenses adaptive during climatic change.

69 Nevertheless, assuming genetic variation from lower latitudes will be effective in
70 mediating herbivory at higher latitude sites ignores the complex and variable nature of plant-
71 herbivore interactions. Latitudinal patterns in herbivory are notoriously variable across species
72 (Moles *et al.* 2011; Anstett *et al.* 2016), meaning that some systems might naturally have higher
73 herbivory in colder climates. Herbivore identity and importance can also vary latitudinally with
74 differences in herbivory varying across herbivore guild and specialization (Hiura & Nakamura

75 2013; Anstett *et al.* 2014). In these conditions, plant defenses that protect against one herbivore
76 species might be less useful on another (Krischik *et al.* 1991; Kursar *et al.* 2009; Ali & Agrawal
77 2012) or could even act as a feeding stimulant (Malcolm & Brower 1989; Holeski *et al.* 2013;
78 Singh *et al.* 2021). Thus, genetic variation from donor sites could introduce plant defense traits
79 which could be unhelpful, or even attract greater herbivore damage. Similar arguments could be
80 made about other biotic interactions, making it vital to test the space-for-time substitution using
81 common gardens that measure biotic interactions and traits that mediate them.

82 A common garden can establish what historical climate best predicts performance, and
83 what biotic and abiotic adaptations may underly this increased performance. Multiple common
84 garden experiments have been carried out in tree species, testing the performance of range-wide
85 genetic variation across the range of the species including close to colder range limits (Schreiber
86 *et al.* 2013; Aitken & Bemmels 2016; Risk *et al.* 2021). Yet less focus has been placed on testing
87 the performance of range-wide genetic variation in forbs (but see Anderson & Wadgymar 2020)
88 and comparing what traits provide the biotic and abiotic adaptations that cause performance
89 differences. Given their shorter generation time, forbs might be more able to rapidly evolve and
90 establish, yet their smaller size might make them more vulnerable to larger herbivore loads.

91 To address these gaps, I investigate the performance of genotypes from across the range of
92 *Oenothera biennis* in a northern common garden, extending prior work on genetically-based clines
93 in anti-herbivore defenses and plant phenology (Anstett *et al.* 2015). Specifically, I ask: (1) Does
94 geography and environment of origin of populations from across the range of *O. biennis* predict
95 seed production when grown close to the northern range limit? (2) Does increased herbivory
96 predict decreased plant performance? (3) What phenological, morphological, and defensive traits
97 predict performance? This approach establishes that plant defenses are as important as abiotic

98 adaptations in predicting plant performance at higher latitudes and in explaining the traits
99 underlying space-for-time substitution.
100
101 **Methods**
102 *Study system*
103 Common evening primrose, *Oenothera biennis* L., Onagraceae, is a facultatively biennial,
104 semelparous species found along disturbed habitats such as roadsides and along bodies of water
105 (Dietrich *et al.* 1997). This herbaceous forb is found throughout Eastern North America with
106 limited populations in Western North America (Fig. 1), spanning a subtropical to temperate climate
107 gradient. Due to permanent translocation heterozygosity (PTH), *O. biennis* is functionally asexual
108 (Cleland 1972) and has near 100% selfing (Maron *et al.* 2018; Johnson and Godfrey in review).
109 Thus populations are usually dominated by a clonal single genotype, and produce genetically
110 identical seeds (Levin 1975). This mating system in conjunction with a semelparous life history
111 make it possible to measure total lifetime male and female fitness in terms of the number of seeds
112 produced by each plant.
113 *Oenothera biennis* is an interesting case study for considering biotic adaptation during
114 climate change. It has substantial heritable genetic variation in morphological, physiological, life
115 history and herbivore resistance traits (Johnson *et al.* 2009) and established latitudinal gradients
116 across many of these traits (Anstett *et al.* 2015). A diverse community of herbivores attacks *O.*
117 *biennis*, including chewing herbivores, xylem feeders, and multiple specialized seed predators
118 (Johnson & Agrawal 2005). Attacks by seed predators target the plant's direct link to seed
119 production and fitness and have been shown to drive rapid evolution (Agrawal *et al.* 2012). These
120 seed predators are known to impact plants more at higher latitudes, while leaf chewing herbivory

121 shows no association with latitude (Anstett *et al.* 2014). Phenolics and specifically the ellagitannin
122 compound oenothein A have been associated with decreased seed predation and increased seed
123 production (Johnson *et al.* 2009; Agrawal *et al.* 2012). Total phenolics from *O. biennis* and a
124 mixture of oenothein A and structurally similar polymers decrease survival of both generalist and
125 specialist insect herbivores of *O. biennis* (Anstett *et al.* 2019). Ellagitannins such as oenothein A
126 are highly reactive in alkaline conditions (Salminen & Karonen 2011), such as those found in
127 lepidopteran insect guts (Appel 1993), making this compound valuable to anti-herbivore defense.
128

129 *Experimental design*

130 Seeds from 146 populations of *O. biennis,* including 132 from the main range and 14 from Western
131 North America (Fig. 1), were sourced from established seed collections provided by Marc T.J.
132 Johnson. These seed lines were collected one to three decades before the start of this experiment
133 and kept in dry conditions in a -20 freezer until germination. Five replicates per population were
134 germinated and grown for three weeks, and then planted in a common garden at the Koffler
135 Scientific Reserve (New Market, Ontario, Canada) and grown during 2012 and 2013 as outlined
136 in Anstett *et al.* (2015). Because of the PTH genetic system, these replicates are a single clone
137 from each of the 146 populations. I previously characterized latitudinal gradients in physical and
138 chemical defense traits, resistance to local herbivores and phenological differences across the
139 range of *O. biennis*. Here I assess how geography and climate of origin of *O. biennis* impacts seed
140 production, and what traits explain performance when measured as total lifetime fitness. The
141 timing of the common garden captures the early effects of climate change in North America with
142 mean annual temperature being 1.6 °C warmer at the common garden across the 2012 and 2013
143 period when compared to the historical baseline temperatures under which *O. biennis* evolved.

144

145 *Climate & geography*

146 Historical climate data from 1960 to 1990 was downloaded from Climate NA for each population
147 (Wang *et al.* 2016), which is representative of pre-climate change conditions. I selected seven
148 temperature and four precipitation associated variables that could impact *O. biennis* fitness. Most
149 variables were highly correlated ($r > 0.7$; Table S1), hence only mean annual temperature (MAT),
150 mean summer precipitation (MSP), cumulative moisture deficit (CMD), and relative humidity
151 (RH) were included in downstream calculations. Monthly climate data was downloaded from
152 Climate NA for 2012 and 2013 for the Koffler Science Reserve (44.026479, -79.544686). MAT,
153 CMD, and RH was calculated as a combined metric for July 2012 to September 2013, by first
154 averaging July, August and September values across both years and then calculating a 12 month
155 mean. This calculation reflects the entire length of the common garden experiment. MSP for the
156 common garden was only included for 2013 since supplemental watering was provided in 2012
157 after seedling transplant. Climatic distance to the common garden was calculated for each climatic
158 variable by subtracting the common garden value from the 30-year mean.

159

160 *Trait measurement*

161 I measured 23 traits including phenological (flowering date, bolt date, growth rate); morphological
162 (leaf toughness, trichome number, specific leaf area (SLA); % water content), secondary chemistry
163 across leaf, flower and fruit tissues (total phenolics, oxidative capacity; concentration of
164 ellagitannin compounds oenothein A and oenothein B); and herbivore resistance traits (generalist
165 leaf herbivory, number of *Philaenus spumarius* L. Hemiptera: Aphrophoridae, fruits damaged by
166 specialist *Schinia florida* G. Lepidoptera: Noctuidae, fruits damaged by specialist *Mompha*

167 *brevivitella* C. Lepidoptera: Momphidae). The detailed methodology for the estimation of 22 of
168 these traits is found in Anstett *et al.* (2015). Additionally, I monitored bolt date throughout the
169 season, defined as the first day the stem length was greater than 5 cm. The total number of fruits
170 and the length and width of 5 fruits produced by each plant was recorded. Seed number was
171 estimated by the following formula: seeds number = 2.33*Average_Fruit_Length *
172 Average_Fruit_Width - 102.47 * Damaged_Fruits. This calculation has been established to
173 accurately estimate fruit number in prior work conducted on *O. biennis* (Agrawal *et al.* 2012). The
174 damaging effects of specialist seed predators were also taken into account in the final estimation
175 of seed production by subtracting the total number of *S. florida* and 0.18**M. brevivitella* damaged
176 fruits. The latter represents average known amount of seed loss per pod due to *M. brevivitella*
177 damage (M.T.J. Johnson personal communication).
178
179 *Statistical analyses*
180 All analyses were performed using R (R Development Core Team 2020). Means of each
181 population were used for all traits ensuring a comparable design across all traits, since dried,
182 ground plant issue was averaged across genotypes prior to chemical analysis. I predicted seed
183 number using geographic, climatic, herbivory and plant traits. Seed number had inflated zeros and
184 was overdispersed ($P < 0.001$) using *dispertiontest* in the *AER* package in R (Kleiber *et al.* 2020).
185 Hence, all regressions predicting seed number were performed using a negative binomial
186 generalized linear model in the *MASS* package in R (Ripley *et al.* 2013). For each model, I tested
187 first order patterns, and quadratic patterns (if this pattern appeared possible in the raw data) (Table
188 S2). Quadratic predictors were assessed separately from first order predictors using the I(Trait^2)
189 function in R. Regression models were reduced by using stepwise AIC, selecting the model with

190 the lowest AIC score. Significance of this model was assessed using a type 2 anova using the
191 *Anova* command in the CAR package in R (Fox *et al.* 2012). Results were visualized using visreg
192 in the *visreg* package in R (Breheny *et al.* 2020), with scale= "response", which applies an inverse
193 log-link function scaling the plots in seed number units.

194 Seed number was modeled as a function of latitude and longitude, distance to common
195 garden, climate present at each population, and climate distance to the common garden (Table S2).
196 Then to determine what types of traits underly the geographical and climatic patterns, resistance
197 to herbivores, phenology, morphology, and plant secondary chemistry were each used to predict
198 seed number. I used multiple regression within each class of traits whenever possible (see Table
199 S2 for every tested model). Leaf toughness was highly correlated with other morphological
200 variables (r>0.7) and was not included in the morphology multiple regression. For resistance to
201 herbivores, I ran separate regressions for seed predators. For secondary compounds, I ran
202 regressions separately for each tissue. Total phenolics was assessed separately from individual
203 compounds. Oxidative capacity was highly correlated with total phenolics (r>0.7); oenothein B
204 was highly correlated with oenothein A. Hence, I selected only total phenolics and oenothein A
205 based on prior knowledge of what traits more strongly predict resistance to herbivores (Agrawal
206 *et al.* 2012; Anstett *et al.* 2015).

207 To understand the major axes of variation across these predictive variables, I also carried
208 out principal component analysis using *prcomp()* in R and associated the most important axes with
209 seed production. Biplots were visualized using *autoplot()* in the ggfortify package in R (Tang *et*
210 *al.* 2016). To further establish which variables are most important in predicting *O. biennis* seed
211 number, I compared the relative importance of all variables that had evidence of predictive seed
212 number (flowering date, bolt date, water content, growth rate, and number of trichomes as well as

213 leaf, flower and fruit total phenolics and oenothein A). I carried out this comparison with the
214 machine learning algorithm, *randomFores*t in R (Liaw & Wiener 2002) using 1000 trees and
215 plotted relative variable importance. I further visualized the relationship between latitude, seed
216 number and six of the most important traits by plotting trait change over latitude, while color
217 coding points for seed number using ggplot2 in R (Wickham 2011).

218 All analyses were repeated using undamaged fruits as a response instead of the estimated
219 seed number. No major differences were detected in trends and only minor difference in
220 significance (data not shown); hence I present only the seed number results.

221

222 **Results**

223 *Geography & climate*

224 Seed production increased with latitude, peaking at 41.9° N, 2.1° south of the common garden
225 before declining (Fig. 2A, Table S3; linear P = 0.001, quadratic P = 0.001). Indeed, five of the top
226 six genotypes with the most fitness were from 41.9°N or further south. Models with longitude had
227 higher AIC and were not selected. Seed production decreased at distances further from the
228 common garden (P = 0.03; Fig. 2B), although the highest performing genotypes were from 100's
229 or 1000's of km from the common garden.

230 Temperature and relative humidity explained plant fitness. Seed production increased
231 towards genotypes from warmer temperatures, peaking at 9.1°C, 1.2°C warmer than the common
232 garden before declining at higher temperatures of origin (Fig. 2C; linear P = 0.004, quadratic P =
233 0.001). Genotypes from locations with greater relative humidity had higher seed production up to
234 62.4% relative humidity, 6.6% higher than the common garden, before declining at higher relative
235 humidity (Fig. 2D). However, this effect was only weakly supported (linear P = 0.057, quadratic

236 P = 0.055). Models with May to September precipitation and Hargreaves climatic moisture deficit
237 had higher AIC and were not selected. Temperature distance from common garden has a similar
238 effect with genotypes from locations with greater temperature distance peaking 1.4°C warmer from
239 the common garden before declining (Fig. S1, Table S3, P < 0.002). There was also weak support
240 for mean summer precipitation distance predicting seed number (P = 0.083; Table S3). Models
241 with relative humidity distance and Hargreaves climatic moisture deficit distance were not
242 selected.
243
244 *Resistance to herbivory*
245 Herbivory had variable relationships with seed production (Table S3). Seed production increased
246 with *Philaenus spumarius* number of to 14 individuals and then declined (Fig. 3A; linear P <0.001,
247 quadratic P = 0.01). The model that included leaf herbivory had higher AIC and was not selected.
248 Seed predators had contrasting relationships with seed production. Seed number increased with
249 higher *S. florida* damage or *S. florida* simply attacked more fecund plants (Fig. 3B; P = 0.003).
250 Seed number decreased with greater *M. brevivitella* damage although there was considerable
251 variability (P = 0.058; Fig. 3C).
252
253 *Plant phenotype*
254 All phenology variables predicted *O. biennis* seed number in some way (Table 3). Plants with early
255 or late flowering date performed poorly, with peak flowering date occurring at an intermediate
256 flowering date (Fig. 3D; P < 0.001). Genotypes with later bolt date had low seed production, while
257 plants with earlier bolt date showed higher seed production (on average) but with higher variability
258 (Fig. 3E; P < 0.001). Plants with intermediate growth rate had higher seed production although

259 this relationship was weak (linear P = 0.049, quadratic P = 0.071; Fig. 4F). Most plant morphology
260 variables also predicted seed number (Table S3). Seed production increased with trichome number
261 up to 110 per 5 mm^2, before decreasing at higher trichome numbers (Fig. 3G; P < 0.001). Fitness
262 also peaked at intermediate water content (P = 0.03), with seeds increasing with water content up
263 to 74.3% before decreasing (Fig. 3H). Finally, seed number was not associated with SLA (Fig. 3I;
264 P = 0.11).

265 Plant defense chemistry metrics also predicted seed production but only for some tissues
266 (Table S4). Higher leaf total phenolics weakly predicted greater seed production (Fig. S2A; P =
267 0.069), while leaf total oenothein A does not predict seed number (linear P = 0.44; quadratic P =
268 0.76; Fig. S2B). Higher flower total phenolics predicted greater seed number up to 54.7 mg/g and
269 then declined (Fig. 4A; linear P = 0.045, quadratic P =0.02). Seed number increased with flower
270 oenothein A (Fig. 4C; linear P = 0.01) and declined slightly at higher values (quadratic P = 0.01).
271 Greater fruit total phenolics predicted greater seed production (Fig. 4B; P = 0.03) with poor
272 evidence of decline at high fruit total phenolics (P = 0.12). Seed number also increases with greater
273 fruit oenothein A (P = 0.006; Fig. 4D).

274 Principle component analyses revealed that most of *O. biennis* trait variation was predicted
275 by the first two principal components (Fig. S3; Table S5), with PC1 explaining 33.65% of the
276 variation and PC2 explaining 13.8%. PC1 is primarily associated with flowering date, fruit
277 oenothein A, fruit phenolics, flower oenothein A, leaf oenothein A, leaf phenolics, and bolt date,
278 while PC2 is primarily associated with growth rate, SLA, and water content (Fig. 5A, Table S6).
279 Smaller values of PCA 1, associated with later phenology and higher plant defenses predicted
280 greater seed number (P=0.02; Table S4). Smaller values of PC2, associated with decreased SLA,

growth, rate, and water content predicted greater seed number (P=0.004), but with much variation and with the highest seed producers having intermediate seed numbers.

Gradient forest analysis showed that, out of the variables predictive of seed number, bolt date, flower oenothein A, and fruit oenothein A were the most important (Fig. 6). Fruit total phenolics, and flowering date were next in variable importance. Then growth rate, flower total phenolics, water content, and trichome number were the least important. Latitude-trait plots with color-coded seed production emphasized flowering time just south of the common garden leads to increased seed production (Fig. S4A), while later bolt date from further south leads to massively decreased seed production (Fig. S4B). Populations from directly south of the common garden that have greater fruit defenses perform considerably better (Fig. S5). Flower defenses south of the common garden are also somewhat predictive of greater seed production (Fig. S6).

Discussion

This study shows that anti-herbivore defenses can at least partially explain the space-for-time substitution that genotypes from warmer latitudes are more adapted to climate change conditions. A 1.6 degree increase from historical baseline temperatures has made *O. biennis* genotypes from 2.1° South of the common garden perform better than local more northern genotypes. This performance difference is best explained by both phenology and plant phenolics, suggesting both biotic and abiotic factors impact performance during the early effects of climate change. The importance of phenology as a climate change adaptation is already well established (Hamann *et al.* 2018; Wadgymar *et al.* 2018). Here I extend our understanding of climate adaptation by implicating the benefit of increased plant chemical traits typically associated with plant defense as an important part of climate change adaptation.

304

305 *Climate change adaptation close to cooler range limit*

306 Plant defenses can be an important adaptation at higher latitude sites and could even be more
307 important than abiotic adaptations. Here I show that genotypes from across the range of *O.*
308 *biennis* with greater fruit total phenolics, greater fruit oenothein A and intermediate values of
309 flower oenothein A (mostly higher than the local population) had greater seed production when
310 grown close to the cold range limit of *O. biennis* (Fig. 4-5, Fig. S5-6). Additionally, *O. biennis*
311 chemical traits ranked higher in variable importance than flowering time (Fig. 6), which is
312 usually more commonly associated with the impacts of climate change. This importance of plant
313 defenses at higher latitudes in *O. biennis* is supported by prior work showing that greater
314 oenothein A explains decreased seed predator damage in this common garden (Fig. S4A; Anstett
315 *et al.* 2015) and during experimental evolution (Agrawal *et al.* 2012). Seed predator damage,
316 especially by *M. brevivitella*, is also known to lead to decreased seed production (Fig. 3C;
317 Agrawal *et al.* 2012), pointing to the importance of greater plant defenses at higher latitudes in
318 this system.

319 Flowering time and bolt date were also both predictive of greater seed production,
320 with earlier bolt date and an intermediate date of first flower being optimal. Some plants failed to
321 bolt until much later in the season, thus producing many fewer seeds (Fig. S3B) and making bolt
322 date the most important predictor of success for this small subset of populations (Fig. 6).
323 *Oenothera biennis* populations from the southern part of the range of the plant showed a
324 suboptimal, much later flowering time when grown in the northern common garden (Anstett *et*
325 *al.* 2015) likely due to photoperiod and/or temperature cue issues, which are often raised when
326 considering phenotypic mismatch during latitudinal translocations (Wadgymar & Weis 2017).

327 Indeed, these plants would have less time to acquire resources and produce seeds before the end
328 of the growing season (Inouye 2008; Franks 2015). Thus, plants from higher latitudes that are
329 just South of the common garden were better able to produce more seeds because they likely had
330 a photoperiod closer to the garden.

331 This begs the question as to why an intermediate flowering date leads to greater seed
332 production in *O. biennis* rather than the earliest possible flowering date. One possibility is that
333 these early flowering plants developed too early and cooler weather damaged the plants. Another
334 explanation is that plants that flower earlier are available for longer in the season and are thus
335 more susceptible to herbivory. Indeed, prior work in this system suggests that genotypes with
336 earlier flowering have greater damage by generalist and specialist herbivores (Agrawal *et al.*
337 2013; Anstett *et al.* 2015). This pattern is not surprising given that life history traits have been
338 found to be consistent predictors of resistance to herbivores (Pilson 2000; Kawagoe & Kudoh
339 2010), and at times more reliable than traditional chemical or physical defenses (Carmona *et al.*
340 2011). Overall, this suggests that even phenological adaptations at northern range limits may be
341 adaptations to biotic interactions.

342

343 *Implications for space-time substitution*

344 Using space as a proxy for time during translocations takes into account the effects of
345 temperature adaptation on average, but ignores how genetics within a region may impact the
346 realized fitness of a randomly selected individual. On average, genotypes from south of the
347 common garden with slightly higher mean annual temperature perform better (Fig. 2A,C), while
348 other climate traits were less important. This matches previous findings pointing to temperature
349 as a key factor in field transplant experiments (Bontrager *et al.* 2020). However, average

350 temperature or latitude may hide considerable genetic variability within a particular temperature
351 band. Multiple genotypes from latitudes at or slightly above the common garden still had above
352 average seed number (Fig. 2A), while some genotypes from across the entire range had low
353 fitness. This variability points to the importance of testing on-the-ground fitness at future
354 introduction sites or at least considering the variety of abiotic and biotic adaptations that might
355 be needed and where this genetic variation might be sourced.

356 This system suggests how possible trade-offs between biotic and abiotic adaptation may
357 be an important consideration for assisted gene flow. The benefits of assisted gene flow as a way
358 to rescue populations from climate change has frequently been weighed against risks of
359 introducing maladapted genetic variation (Vitt *et al.* 2010; Aitken & Whitlock 2013). Space-for-
360 time substitutions might serve as a way to source genetic material for assisted gene flow while
361 mitigating the risks of introducing further maladaptation in an already vulnerable species. This
362 study suggests there are risks of introducing genotypes that may be well suited for the new
363 abiotic environment but not the biotic one (or vice versa). These trade-offs are possible in *O.*
364 *biennis* especially given that multiple traits explain fitness and that these types of traits are
365 known to covary (Johnson *et al.* 2009). Given these findings, studies focusing on species of
366 immediate conservation concern should investigate the possible greater importance of biotic
367 adaptations close to northern range limits and how these may interact with abiotic adaptation
368 (Blois *et al.* 2013b; HilleRisLambers *et al.* 2013). This may be particularly true for translocations
369 across latitudinal gradients (Fielding *et al.* 1999), where adaptation to photoperiod may lead to
370 poor match with phenology (Walker *et al.* 2019).

371

372 *Implications for biogeographic hypotheses*

373 Greater plant defenses at higher latitudes may provide needed adaptations to increased herbivory.
374 Herbivore outbreaks at higher latitude sites are being attributed directly or indirectly to climate
375 change (de Sassi & Tylianakis 2012; DeLucia *et al.* 2012; Ju *et al.* 2015; Jactel *et al.* 2019),
376 raising the possibility that selection for greater defenses at higher latitudes might already be
377 occurring. Indeed, I previously documented that damage by seed predators is greater at higher
378 latitudes in *O. biennis* (Anstett *et al.* 2014), possibly due to early effects of climate change. This
379 idea falls against the established pre-climate change paradigm that greater biotic interactions at
380 warmer, lower latitudes leads to selection for greater traits that mediate these interactions
381 (Dobzhansky 1950; MacArthur 1972; Coley & Barone 1996; Schemske *et al.* 2009). However,
382 selection for greater plant defenses at higher latitudes due to climate change might explain why
383 more studies are finding greater plant defenses at higher latitudes (Moles *et al.* 2011; Anstett *et*
384 *al.* 2016).

385 As the impacts of the Anthropocene become more acute, long-held biogeographic
386 hypotheses may require a more complex and updated view. The causes and drivers of range
387 limits has been a key question throughout the history of evolutionary biology as well as a driver
388 of biogeographic patterns (Darwin 1859; MacArthur 1972; Sexton *et al.* 2009). Biotic
389 interactions are viewed as being less important for determining colder range limits (Louthan *et*
390 *al.* 2015; Paquette & Hargreaves 2021). Biotic interactions and the traits that mediate them are
391 also expected to be less intense and important at higher latitude sites (MacArthur 1972; Coley &
392 Aide 1991; Coley & Barone 1996; Schemske *et al.* 2009; Johnson & Rasmann 2011; Anstett *et*
393 *al.* 2016). Both of these ideas share the common viewpoint of diminishing the importance of
394 biotic interactions in colder climates. Climate change is changing this reality and is likely going
395 to pose new biotic challenges on historically cooler sites that may lack adaptations for greater

396 mean interaction intensity (de Sassi & Tylianakis 2012; Ju *et al.* 2015) and greater variability in
397 that intensity (Wetzel *et al.* 2023). Greater biotic adaptations such as plant defenses against
398 herbivores may become an increasingly important part of plant adaptation at higher latitudes
399 leading to the weakening of latitudinal gradients in biotic interactions and the traits that mediate
400 them. Lack of these adaptations at northern range limits could even slow plant range expansions
401 that are so often evoked when studying climate change.
402
403 **Data Availability**
404 All code and data for this paper is available on GitHub https://github.com/anstettd/KSR_North.
405
406 **Conflict of Interest**
407 The author has no conflicts of interest.
408
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418

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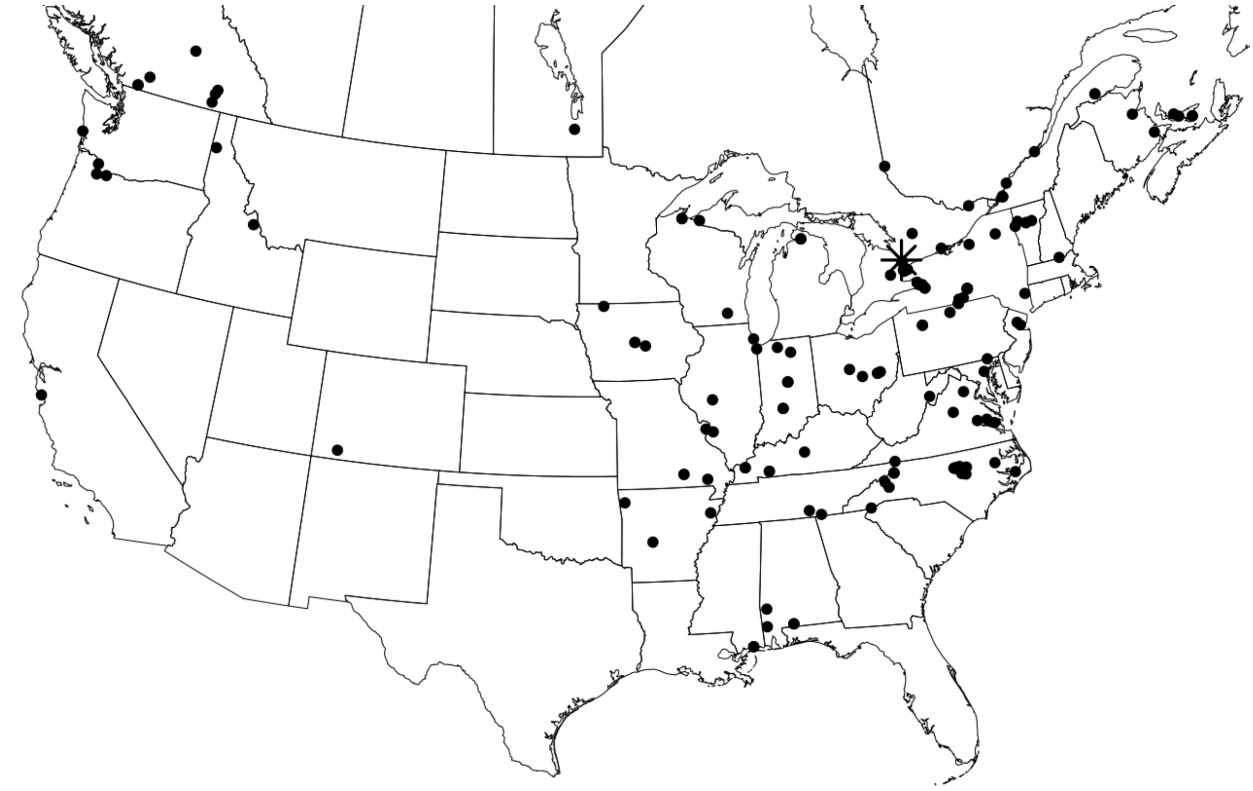


Figure 1. Locations of 146 sampled populations of *O. biennis* across North America. Each black dot represents one sampled population. The star represents the Koffler Scientific Reserve, the location of the common garden.

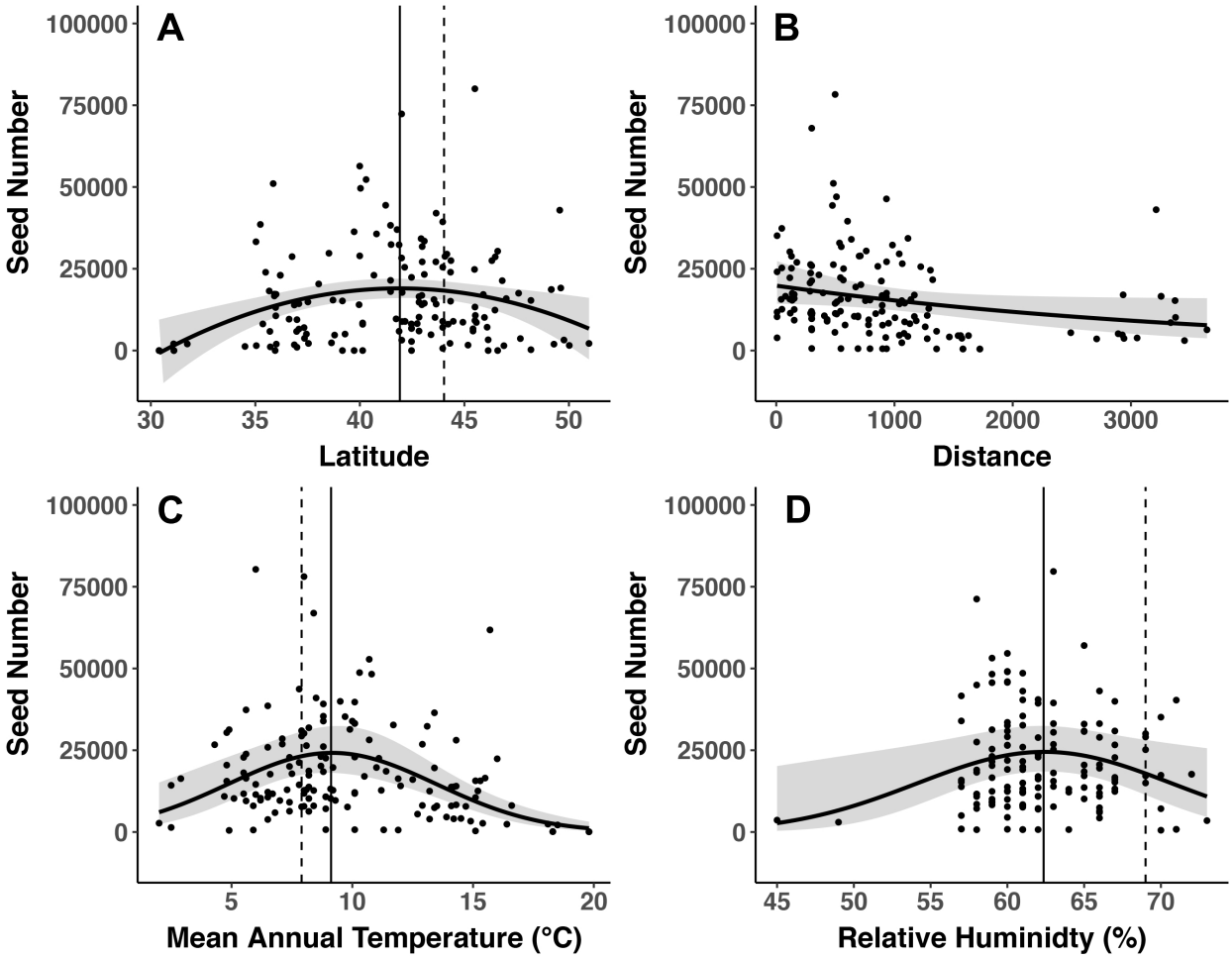
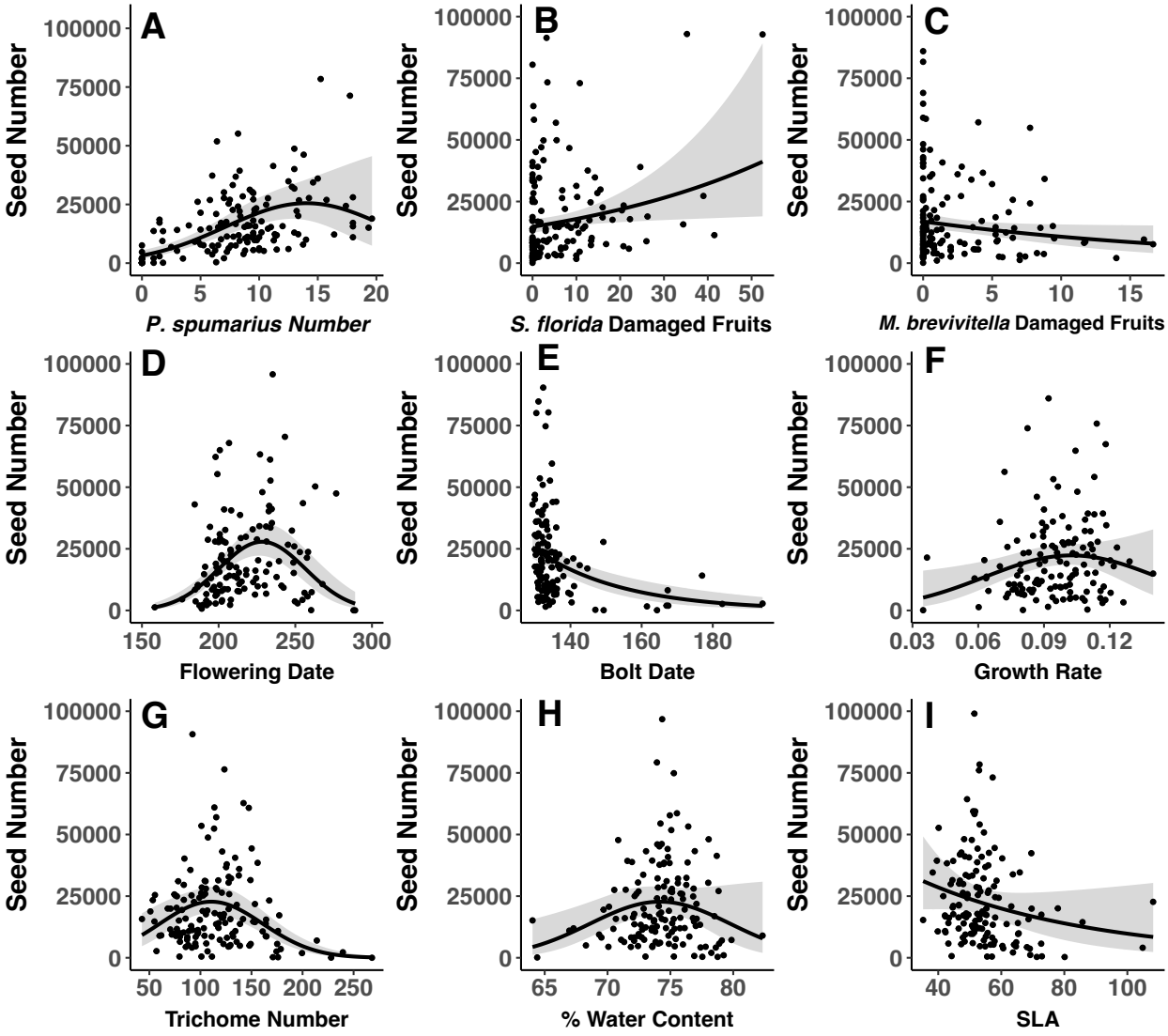


Figure 2. Seed production of *O. biennis* in a Northern common garden as predicted by (A) latitude of origin, (B) Euclidian distance to the common garden in km, (C) mean annual temperature at collected location, and (D) relative humidity at collected location. The solid vertical line gives the latitude or environmental condition at origin of population with the highest predicted seed production. The dashed vertical line gives the latitude or environmental condition at the common garden location. Each point represents one population.



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653 Figure 3. Herbivory, phenology and plant morphology predicts seed production in *O. biennis* in a
654 northern common garden. Plots are given for (A) number of *Philaenus spumarius*, (B) number of
655 *Schinia florida* damaged fruits, (C) number of *Mompha brevivitella* damaged fruits, (D) flowering
656 date (in Julian days), (E) bolt date (in Julian days), (F) growth rate (cm/d), (G) trichome number
657 in a 5 mm diameter disk, (H) % water content, and (I) specific leaf area (SLA). Each point
658 represents one population.

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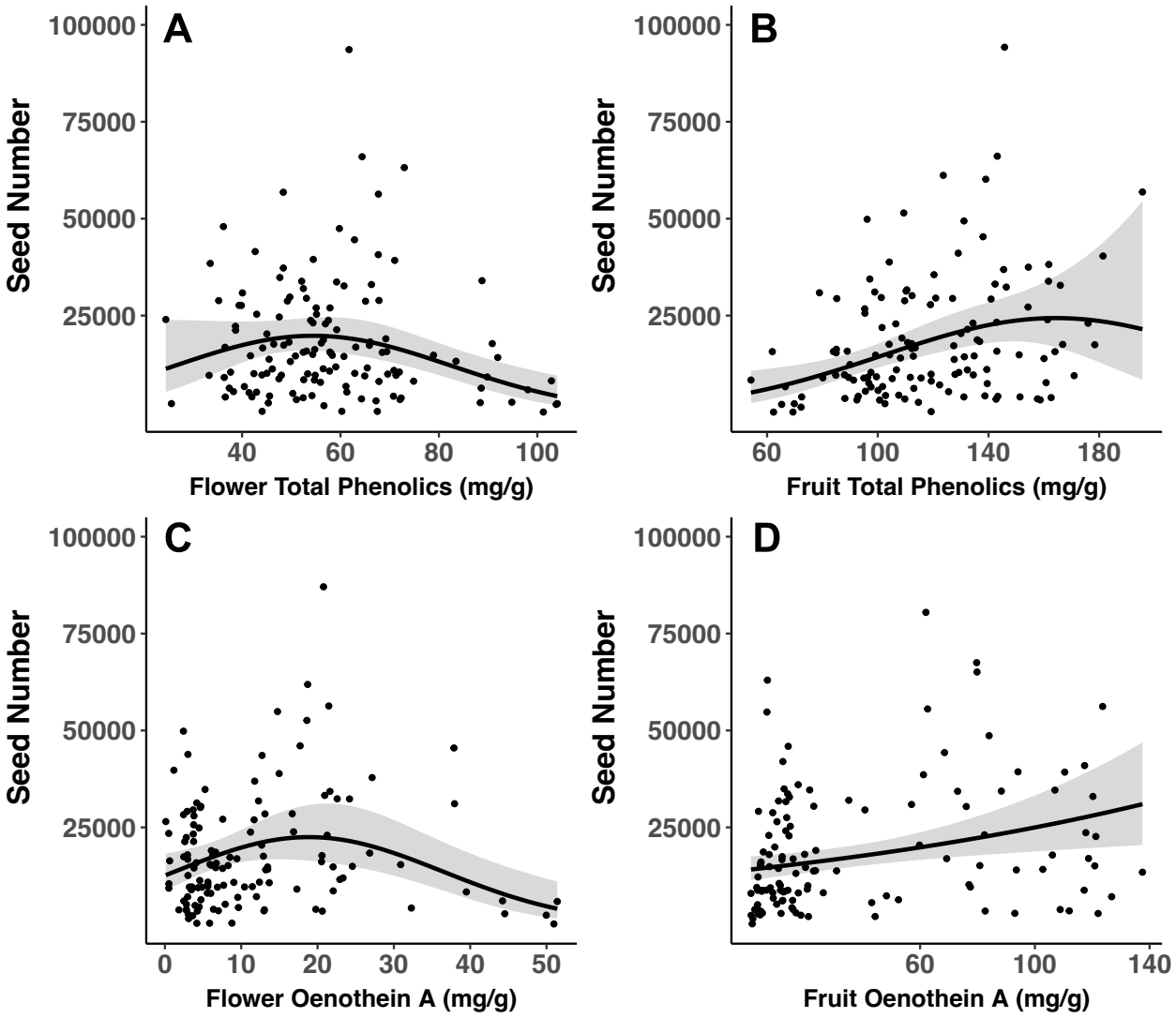

Figure 4. Fruit chemical defenses predict seed production of *O. biennis* in a common garden. Plots are given for (A) flower total phenolics, (B) fruit total phenolics, (C) flower oenothein A, and (D) fruit oenothein A. Units are in mg of given secondary metabolite(s) per g of dry mass. Each point represents one population.

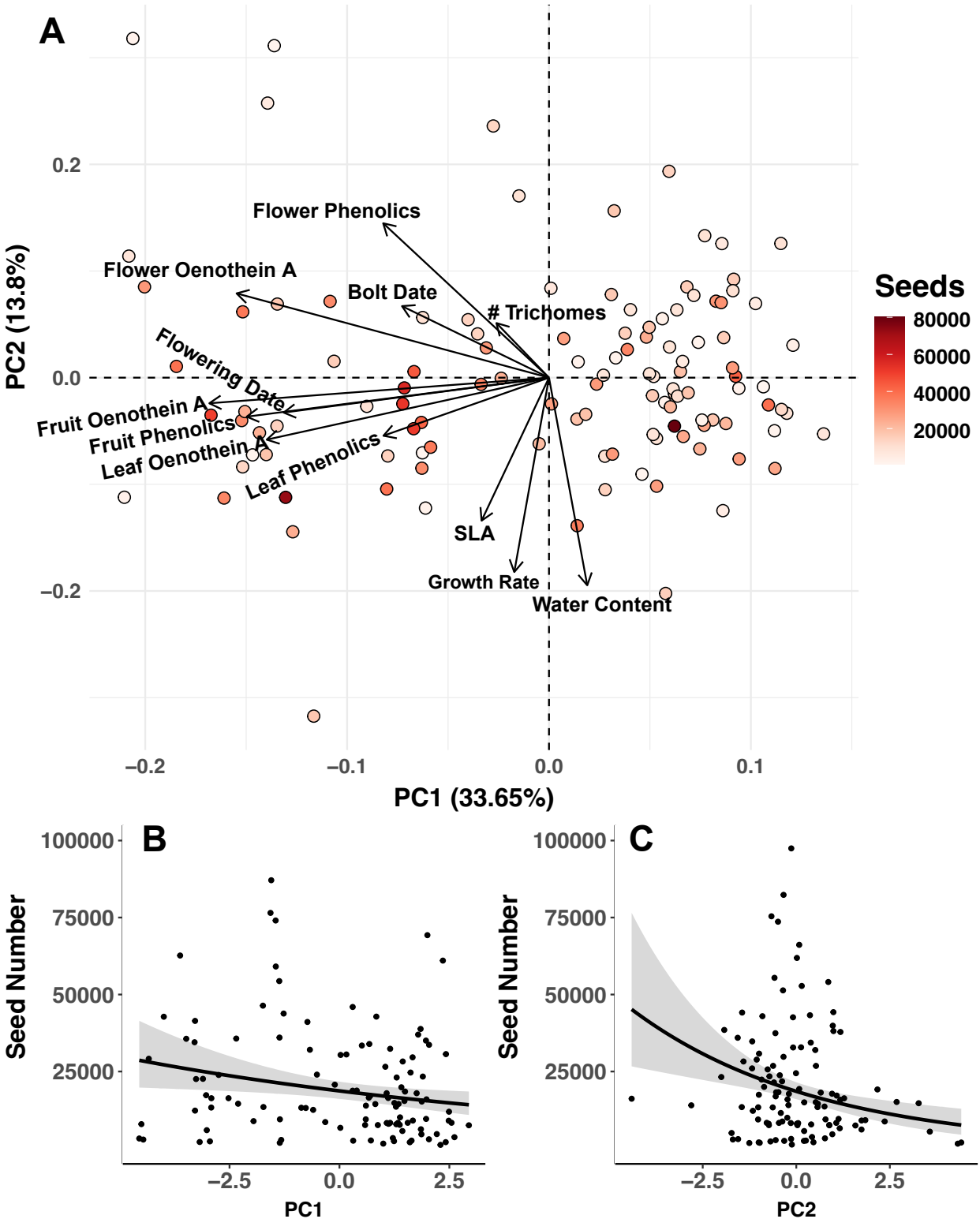

Figure 5 Principal component analyses of chemical, phenological and morphological traits of *O. biennis*. (A) PCA bioplot showing first and second principal component. PC1 describes mostly

chemical and phenological traits, while PC2 describes primarily morphological traits and growth rate. Each dot represents one population. Number of seeds per plant is color coded for each point. Seeds are predicted by (A) PC1 and (B) PC2. Each point represents one population.

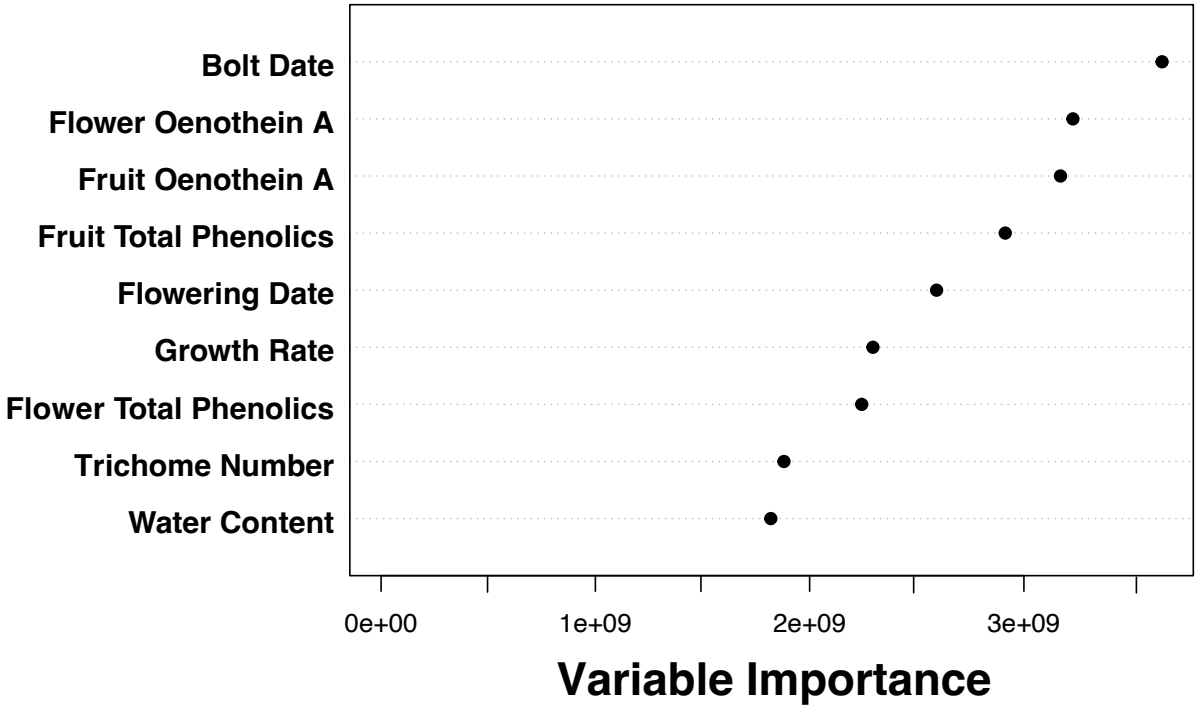

Figure 6. Variable importance for *O. biennis* traits predicting seed production in a Northern common garden. Values were generated using gradient forest.