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 149 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 (Godfrey 2014; Johnson and Godfrey in review). Thus
109 populations are usually dominated by a clonal single genotype, and produce genetically identical
110 seeds (Levin 1975). This mating system in conjunction with a semelparous life history make it
111 possible to measure total lifetime male and female fitness in terms of the number of seeds produced
112 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 149 populations of *O. biennis,* including 137 from the main range and 12 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 149 populations. I previously characterized latitudinal gradients in physical and
138 chemical defense traits, resistance to local herbivores and phenological differences across 137
139 populations in the main range of *O. biennis*. Here I assess how geography and climate of origin of
140 *O. biennis* impacts seed production, and what traits explain performance when measured as total
141 lifetime fitness. The timing of the common garden captures the early effects of climate change in
142 North America with mean annual temperature being 1.6 °C warmer at the common garden across

143 the 2012 and 2013 period when compared to the historical baseline temperatures under which *O.*
144 *biennis* evolved.
145
146 *Climate & geography*
147 Historical climate data from 1960 to 1990 was downloaded from Climate NA for each population
148 (Wang *et al.* 2016), which is representative of pre-climate change conditions. I selected seven
149 temperature and four precipitation associated variables that could impact *O. biennis* fitness. Most
150 variables were highly correlated ($r > 0.7$; Table S1), hence only mean annual temperature (MAT),
151 mean summer precipitation (MSP), cumulative moisture deficit (CMD), and relative humidity
152 (RH) were included in downstream calculations. Monthly climate data was downloaded from
153 Climate NA for 2012 and 2013 for the Koffler Science Reserve (44.026479, -79.544686). MAT,
154 CMD, and RH was calculated as a combined metric for July 2012 to September 2013, by first
155 averaging July, August and September values across both years and then calculating a 12 month
156 mean. This calculation reflects the entire length of the common garden experiment. MSP for the
157 common garden was only included for 2013 since supplemental watering was provided in 2012
158 after seedling transplant. Climatic distance to the common garden was calculated for each climatic
159 variable by subtracting the common garden value from the 30-year mean.
160
161 *Trait measurement*
162 I measured 23 traits including phenological (flowering date, bolt date, growth rate); morphological
163 (leaf toughness, trichome number, specific leaf area (SLA); % water content), secondary chemistry
164 across leaf, flower and fruit tissues (total phenolics, oxidative capacity; concentration of
165 ellagitannin compounds oenothein A and oenothein B); and herbivore resistance traits (generalist

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

189 S2). Quadratic predictors were assessed separately from first order predictors using the I(Trait^2)
190 function in R. Regression models were reduced by using stepwise AIC, selecting the model with
191 the lowest AIC score. Significance of this model was assessed using a type 2 anova using the
192 *Anova* command in the CAR package in R (Fox *et al.* 2012). Results were visualized using visreg
193 in the *visreg* package in R (Breheny *et al.* 2020), with scale= "response", which applies an inverse
194 log-link function scaling the plots in seed number units.

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

208 To understand the major axes of variation across these predictive variables, I also carried
209 out principal component analysis using *prcomp()* in R and associated the most important axes with
210 seed production. Biplots were visualized using *autoplot()* in the ggfortify package in R (Tang *et*
211 *al.* 2016). To further establish which variables are most important in predicting *O. biennis* seed

212 number, I compared the relative importance of all variables that had evidence of predictive seed
213 number (flowering date, bolt date, water content, growth rate, and number of trichomes as well as
214 leaf, flower and fruit total phenolics and oenothein A). I carried out this comparison with the
215 machine learning algorithm, *randomFores*t in R (Liaw & Wiener 2002) using 1000 trees and
216 plotted relative variable importance. I further visualized the relationship between latitude, seed
217 number and six of the most important traits by plotting trait change over latitude, while color
218 coding points for seed number using ggplot2 in R (Wickham 2011).

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

222

223 **Results**

224 *Geography & climate*

225 Seed production increased with latitude, peaking at 41.9° N, 2.1° south of the common garden
226 before declining (Fig. 2A, Table 1; linear P = 0.001, quadratic P = 0.002). Indeed, five of the top
227 six genotypes with the most fitness were from 41.9°N or further south. Models with longitude had
228 higher AIC and were not selected. Seed production decreased at distances further from the
229 common garden (P = 0.044; Fig. 2B), although the highest performing genotypes were from 100's
230 or 1000's of km from the common garden.

231 Temperature and relative humidity explained plant fitness. Seed production increased
232 towards genotypes from warmer temperatures, peaking at 9.1°C, 1.2°C warmer than the common
233 garden before declining at higher temperatures of origin (Fig. 2C; linear P = 0.004, quadratic P =
234 0.001). Genotypes from locations with greater relative humidity had higher seed production up to

235 62.4% relative humidity, 6.6% higher than the common garden, before declining at higher relative
236 humidity (Fig. 2D). However, this effect was only weakly supported (linear P = 0.063, quadratic
237 P = 0.062). Models with May to September precipitation and Hargreaves climatic moisture deficit
238 had higher AIC and were not selected. Temperature distance from common garden has a similar
239 effect with genotypes from locations with greater temperature distance peaking 1.4°C warmer from
240 the common garden before declining (Fig. S1, Table S1, P < 0.001). There was also weak support
241 for mean summer precipitation distance predicting seed number (P = 0.083; Table S1). Models
242 with relative humidity distance and Hargreaves climatic moisture deficit distance were not
243 selected.
244
245 *Resistance to herbivory*
246 Herbivory had variable relationships with seed production (Table 1). Seed production increased
247 with *Philaenus spumarius* number of to 14 individuals and then declined (Fig. 3A; linear P = 0.004,
248 quadratic P = 0.063). The model that included leaf herbivory had higher AIC and was not selected.
249 Seed predators had contrasting relationships with seed production. Seed number increased with
250 higher *S. florida* damage or *S. florida* simply attacked more fecund plants (Fig. 3B; P = 0.004).
251 Seed number decreased with greater *M. brevivitella* damage although there was a lot of variability
252 (P = 0.051; Fig. 3C).
253
254 *Plant phenotype*
255 All phenology variables predicted *O. biennis* seed number in some way (Table 1). Plants with early
256 or late flowering date performed poorly, with peak flowering date occurring at an intermediate
257 flowering date (Fig. 3D; P < 0.001). Genotypes with later bolt date had low seed production, while

258 plants with earlier bolt date showed higher seed production (on average) but with higher variability
259 (Fig. 3E; $P < 0.001$). Plants with intermediate growth rate had higher seed production although
260 this relationship was weak (linear $P = 0.05$, quadratic $P = 0.073$; Fig. 4F). Most plant morphology
261 variables also predicted seed number (Table 1). Seed production increased with trichome number
262 up to 110 per 5 mm^2, before decreasing at higher trichome numbers (Fig. 3G; $P < 0.001$). Fitness
263 also peaked at intermediate water content (linear $P = 0.029$; $P = 0.03$), with seeds increasing with
264 water content up to 74.3% before decreasing (Fig. 3H). Finally, seed number was not associated
265 with SLA (Fig. 3I; $P = 0.12$).

266 Plant defense chemistry metrics also predicted seed production but only for some tissues
267 (Table 2). Higher leaf total phenolics weakly predicted greater seed production (Fig. S2A; $P =$
268 0.84), while leaf total oenothein A does not predict seed number (linear $P = 0.44$; quadratic $P =$
269 0.76; Fig. S2B). Higher flower total phenolics predicted greater seed number up to 55 mg/g and
270 then declined (Fig. 4A; linear $P = 0.12$, quadratic $P =0.03$). Seed number increased with flower
271 oenothein A (Fig. 4C; linear $P = 0.01$) and declined slightly at only very high values ($P = 0.01$).
272 Greater fruit total phenolics predicted greater seed production (Fig. 4B; $P = 0.03$) with poor
273 evidence of decline at high fruit total phenolics ($P = 0.11$). Seed number also increases with greater
274 fruit oenothein A ($P = 0.002$; Fig. 4D).

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

281 greater seed number (P=0.02). Smaller values of PC2, associated with decreased SLA, growth,
282 rate, and water content predicted greater seed number (P=0.004), but with much variation and with
283 the highest seed producers having intermediate seed numbers.

284 Gradient forest analysis showed that, out of the variables predictive of seed number, bolt
285 date, flower oenothein A, fruit oenothein A, fruit total phenolics, and flowering date were the five
286 most important (Fig. 6). After this there is a larger gap in importance, with growth rate, flower
287 total phenolics, trichome number and water content being the least important. Latitude-trait plots
288 with color-coded seed production emphasized flowering time just south of the common garden
289 leads to increased seed production (Fig. S4A), while later bolt date from further south leads to
290 massively decreased seed production (Fig. S4B). Populations from directly south of the common
291 garden that have greater fruit defenses perform considerably better (Fig. S5). Flower defenses
292 south of the common garden are also somewhat predictive of greater seed production (Fig. S6).
293

294 **Discussion**

295 This study shows that anti-herbivore defenses can at least partially explain the space-for-time
296 substitution that genotypes from warmer latitudes are more adapted to climate change conditions.
297 A 1.6 degree increase from historical baseline temperatures has made *O. biennis* genotypes from
298 2.1° South of the common garden perform better than local more northern genotypes. This
299 performance difference is best explained by both phenology and plant phenolics, suggesting both
300 biotic and abiotic factors impact performance during the early effects of climate change. The
301 importance of phenology as a climate change adaptation is already well established (Hamann *et*
302 *al.* 2018; Wadgymar *et al.* 2018). Here I extend our understanding of climate adaptation by

303 implicating the benefit of increased plant chemical traits typically associated with plant defense
304 as an important part of climate change adaptation.
305
306 *Climate change adaptation close to cooler range limit*
307 Plant defenses can be an important adaptation at higher latitude sites and could even be more
308 important than abiotic adaptations. Here I show that genotypes from across the range of *O.*
309 *biennis* with greater fruit total phenolics, greater fruit oenothein A and intermediate values of
310 flower oenothein A (mostly higher than the local population) had greater seed production when
311 grown close to the cold range limit of *O. biennis* (Fig. 4-5, Fig. S5-6). Additionally, *O. biennis*
312 chemical traits ranked higher in variable importance than flowering time (Fig. 6), which is
313 usually more commonly associated with the impacts of climate change. This importance of plant
314 defenses at higher latitudes in *O. biennis* is supported by prior work showing that greater
315 oenothein A explains decreased seed predator damage in this common garden (Fig. S4A; Anstett
316 *et al.* 2015) and during experimental evolution (Agrawal *et al.* 2012). Seed predator damage,
317 especially by *M. brevivitella*, is also known to lead to decreased seed production (Fig. 3C;
318 Agrawal *et al.* 2012), pointing to the importance of greater plant defenses at higher latitudes in
319 this system.
320 Flowering time and bolt date were also both predictive of greater seed production,
321 with earlier bolt date and an intermediate date of first flower being optimal. Some plants failed to
322 bolt until much later in the season, thus producing many fewer seeds (Fig. S3B) and making bolt
323 date the most important predictor of success for this small subset of populations (Fig. 6).
324 *Oenothera biennis* populations from the southern part of the range of the plant showed a
325 suboptimal, much later flowering time when grown in the northern common garden (Anstett *et*

326 *al.* 2015) likely due to photoperiod and/or temperature cue issues, which are often raised when
327 considering phenotypic mismatch during latitudinal translocations (Wadgymar & Weis 2017).
328 Indeed, these plants would have less time to acquire resources and produce seeds before the end
329 of the growing season (Inouye 2008; Franks 2015). Thus, plants from higher latitudes that are
330 just South of the common garden were better able to produce more seeds because they likely had
331 a photoperiod closer to the garden.

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

343

344 *Implications for space-time substitution*

345 Using space as a proxy for time during translocations takes into account the effects of
346 temperature adaptation on average, but ignores how genetics within a region may impact the
347 realized fitness of a randomly selected individual. On average, genotypes from south of the
348 common garden with slightly higher mean annual temperature perform better (Fig. 2A,C), while

349 other climate traits were less important. This matches previous findings pointing to temperature
350 as a key factor in field transplant experiments (Bontrager *et al.* 2020). However, average
351 temperature or latitude may hide considerable genetic variability within a particular temperature
352 band. Multiple genotypes from latitudes at or slightly above the common garden still had above
353 average seed number (Fig. 2A), while some genotypes from across the entire range had low
354 fitness. This variability points to the importance of testing on-the-ground fitness at future
355 introduction sites or at least considering the variety of abiotic and biotic adaptations that might
356 be needed and where this genetic variation might be sourced.

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

372

373 *Implications for biogeographic hypotheses*

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

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

395 biotic interactions in colder climates. Climate change is changing this reality and is likely going
396 to pose new biotic challenges on historically cooler sites that may lack adaptations for greater
397 mean interaction intensity (de Sassi & Tylianakis 2012; Ju *et al.* 2015) and greater variability in
398 that intensity (Wetzel *et al.* 2023). Greater biotic adaptations such as plant defenses against
399 herbivores may become an increasingly important part of plant adaptation at higher latitudes
400 leading to the weakening of latitudinal gradients in biotic interactions and the traits that mediate
401 them. Lack of these adaptations at northern range limits could even slow plant range expansions
402 that are so often evoked when studying climate change.
403

404 **Data Availability**

405 All code and data for this paper is available on GitHub https://github.com/anstettd/KSR_North.
406

407 **Conflict of Interest**

408 The author has no conflicts of interest.
409

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419

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642 **Table 1. P-values and model coefficients for geographic, climatic, climate distance, resistance**
643 **to herbivores, phenological, and morphological models predicting seed number.** All variables
644 within horizontal lines are within the same model. Bold indicates P < 0.05; underlined indicates P
645 < 0.10.

	Coefficient	P-value
Geography		
Latitude	12544.58	**0.002**
Latitude^2	-149.73	**0.002**
Distance to Common Garden	-0.0003	**0.04**
Climate		
Mean Annual Temperature	0.49	**0.004**
Mean Annual Temperature^2	-0.03	**0.001**
Relative Humidity	0.91	0.063
Relative Humidity^2	-0.01	0.062
Climate Distance		
Mean Summer Precipitation Distance	0.002	0.083
Mean Annual Temperature Distance^2	-0.02	**<0.001**
Resistance to Herbivores		
P. spumarius Number	0.29	**<0.001**
P. spumarius Number^2	-0.01	**0.01**
Fruits Impacted by *S. florida*	0.02	**0.003**
Fruits Impacted by M. brevivitella	-0.05	0.051
Phenology		
Flowering Date	0.28	**<0.001**
Flowering Date^2	-0.001	**<0.001**
Bolt Date	-0.04	**<0.001**
Growth Rate	64.39	0.0503
Growth Rate^2	-314.2	0.073
Morphology		
SLA	-0.02	0.12
Water Content	2.36	**0.029**
Water Content^2	-0.02	**0.032**
Trichome Number	0.04	**<0.001**

Trichome Number^2	-0.0002	**<0.001**

646

647 **Table 2. P-values and model coefficients for chemical defenses and principal components**

648 **predicting seed number.** Bold indicates P < 0.05; underlined indicates P < 0.10.

	Coefficient	P-value
Total Phenolics		
Leaf Total Phenolics	0.02	0.084
Flower Total Phenolics	0.05	0.12
Flower Total Phenolics^2	-0.0005	0.079
Fruit Total Phenolics	0.04	**0.03**
Fruit Total Phenolics^2	-0.0001	0.11
Ellagitannins		
Leaf Oenothein A	0.02	0.44
Leaf Oenothein A^2	-0.0002	0.76
Flower Oenothein A	0.07	**0.01**
Flower Oenothein A^2	-0.002	**0.01**
Fruit Oenothein A	0.01	**0.002**
Principal Components		
PC1	-0.09	**0.02**
PC2	0.2	**0.004**

649

650

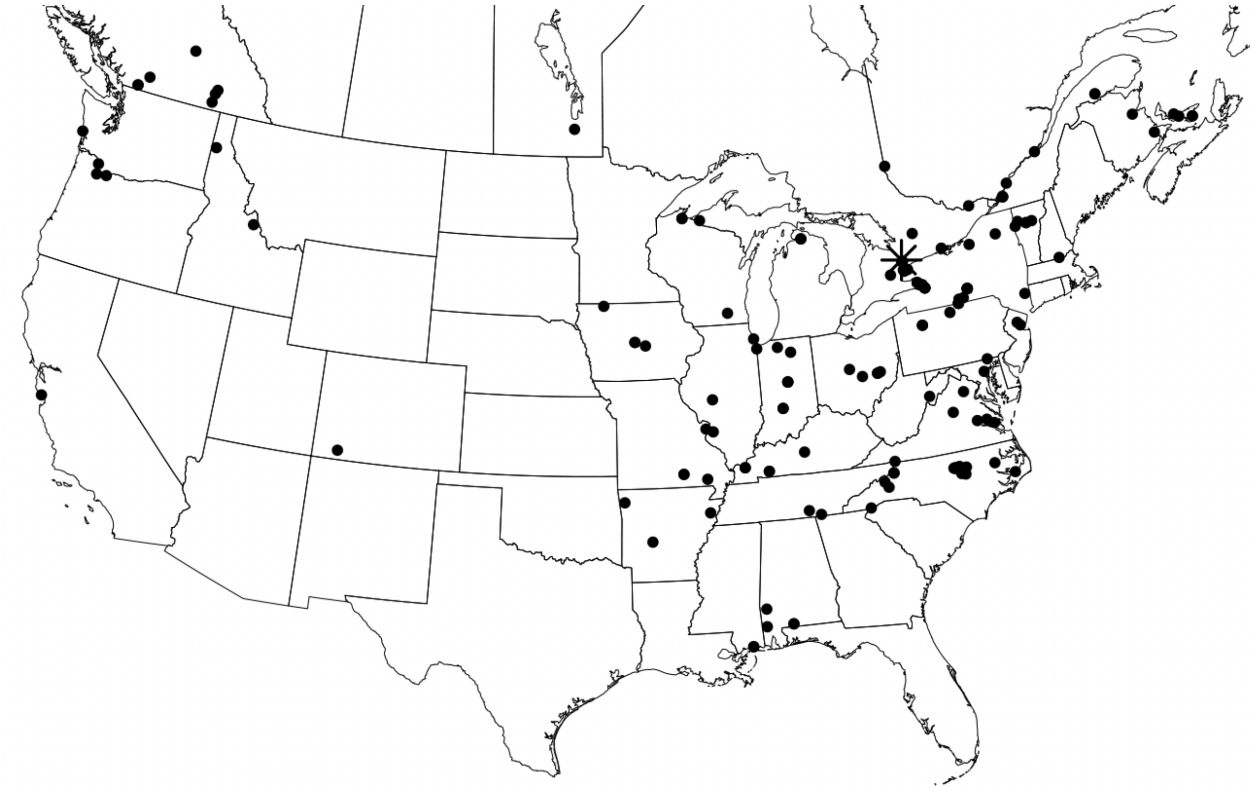


Figure 1. Locations of 149 sampled populations of *O. biennis* across North America. Each black dot represents one sampled population. The star represents the Koffler Scientific Reserve, the location of the common garden.

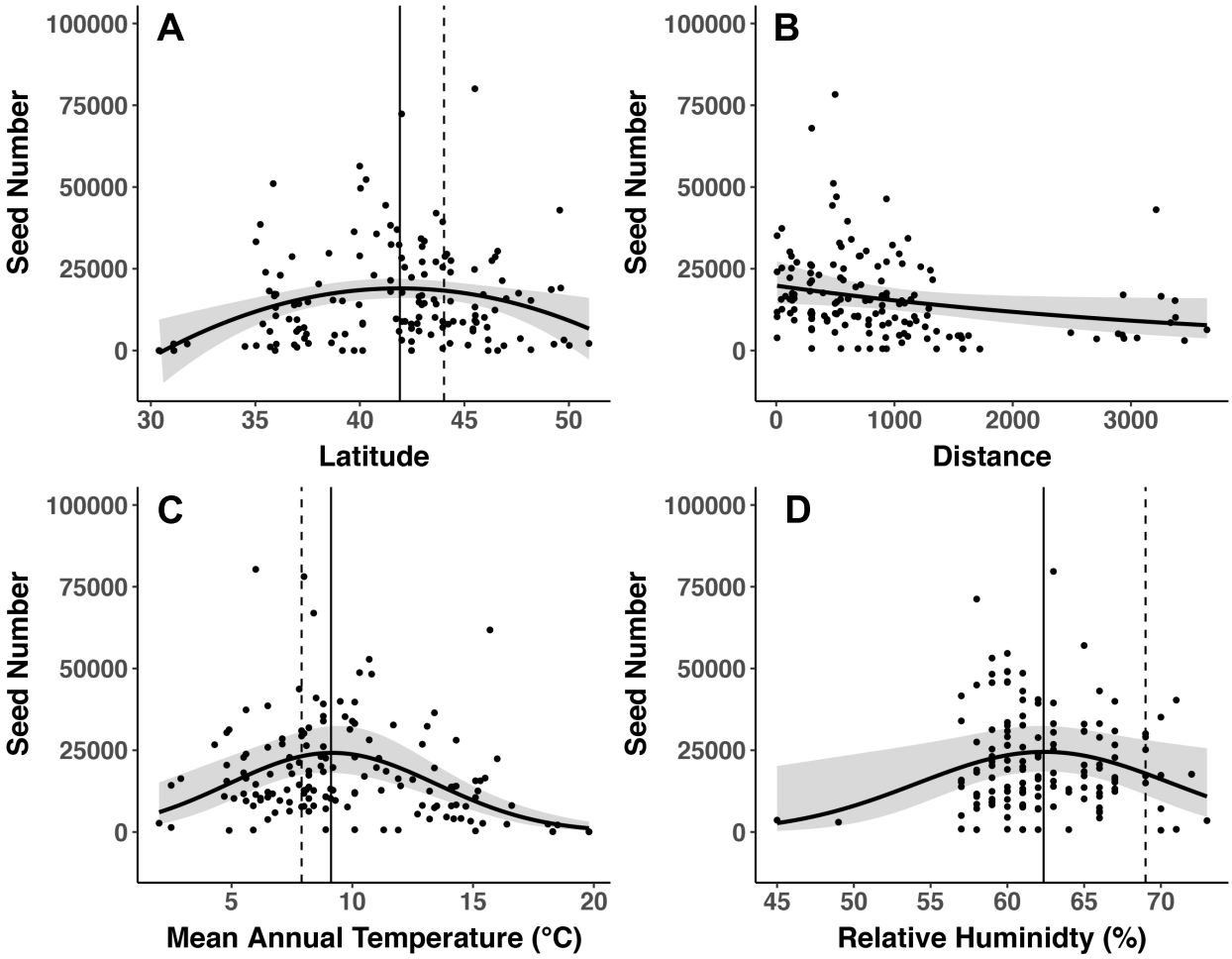


Figure 2. Seed production of *O. biennis* in a Northern common garden as predicted by (A) latitude of origin, (B) Euclidian distance to the common garden in km, (C) mean annual temperature at collected location, and (D) relative humidity at collected location. The solid vertical line gives the latitude 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.

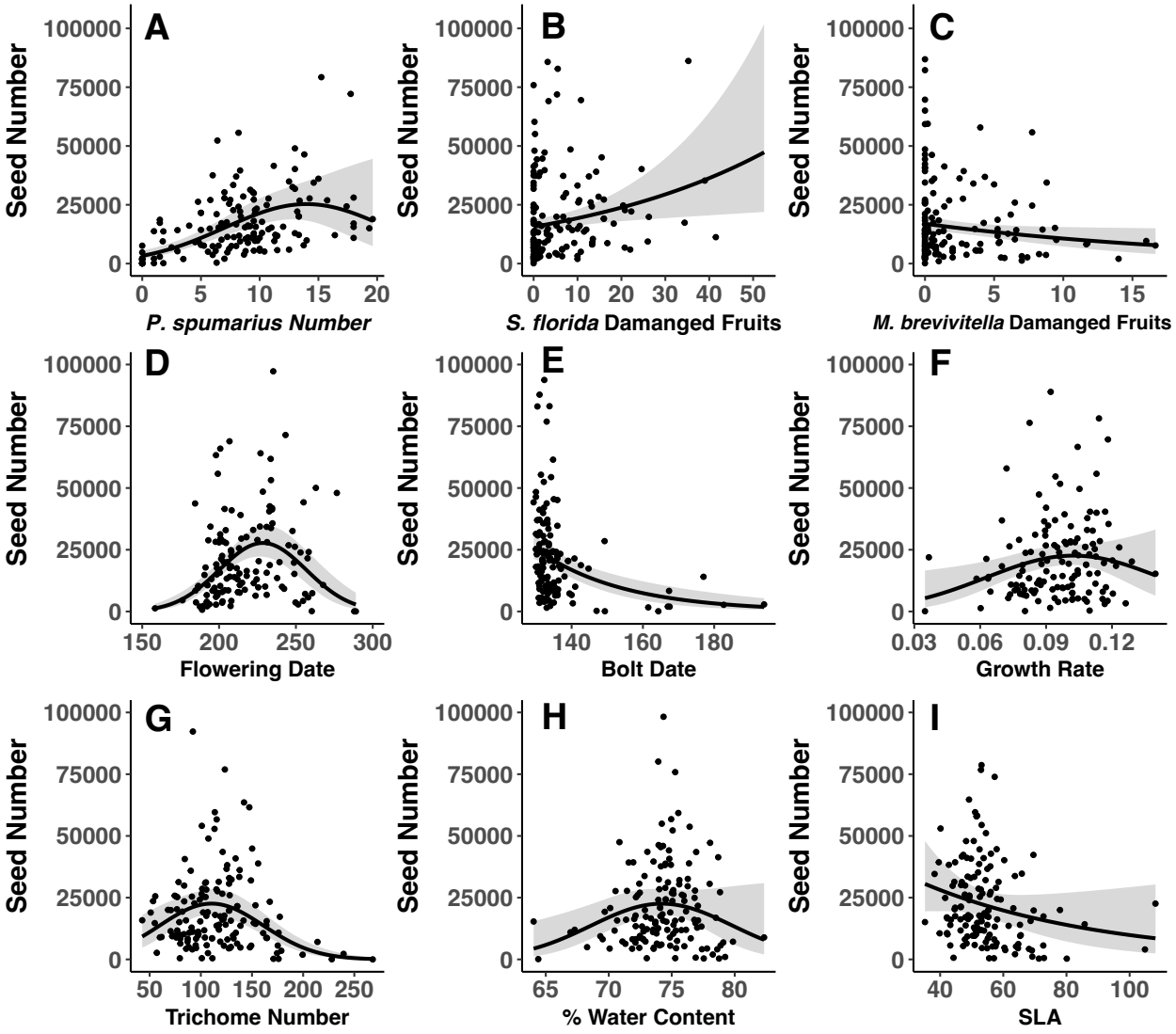


Figure 3. Herbivory, phenology and plant morphology predicts seed production in *O. biennis* in a northern common garden. Plots are given for (A) number of *Philaenus spumarius*, (B) number of *Schinia florida* damaged fruits, (C) number of *Mompha brevivitella* damaged fruits, (D) flowering date (in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

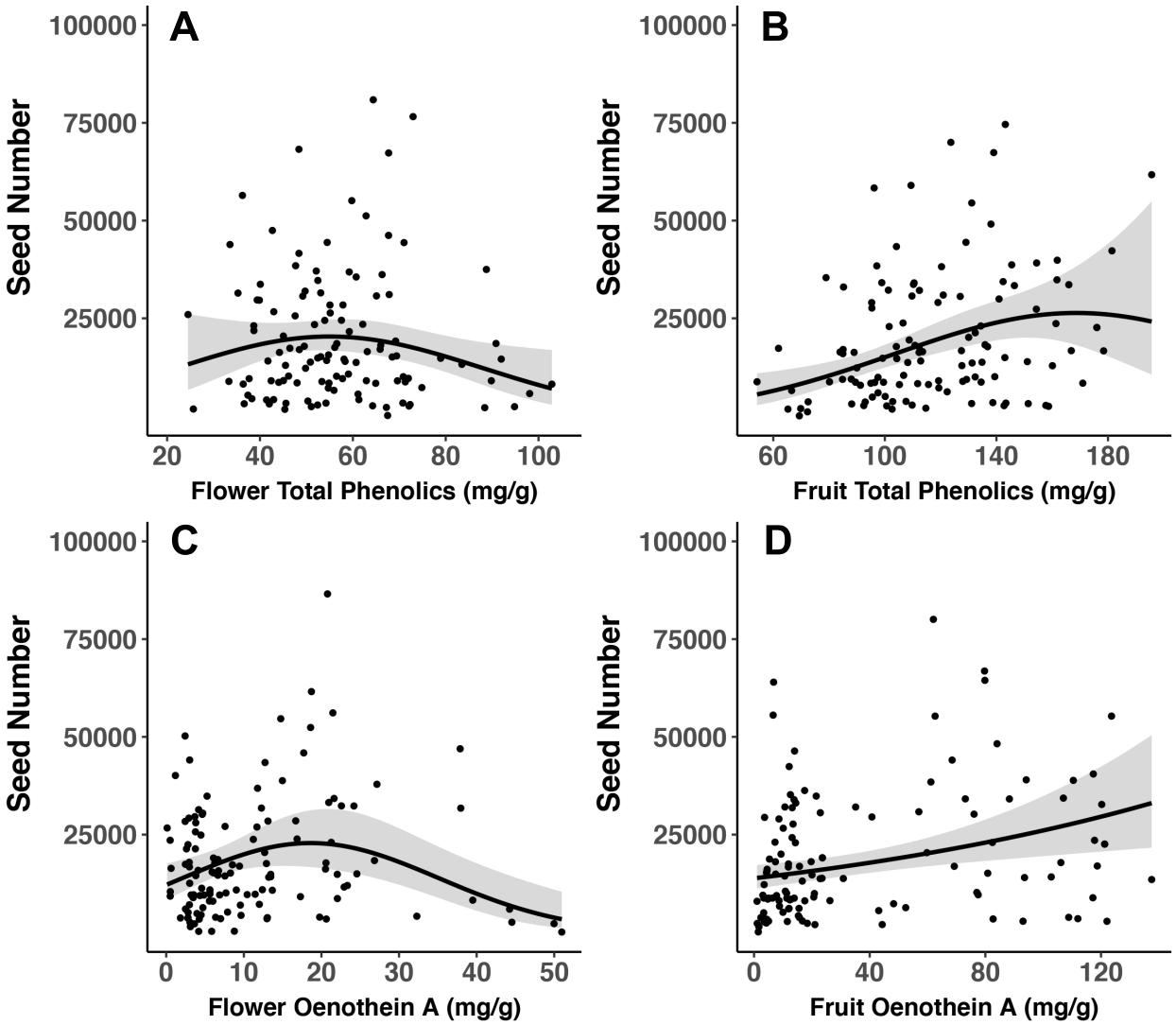
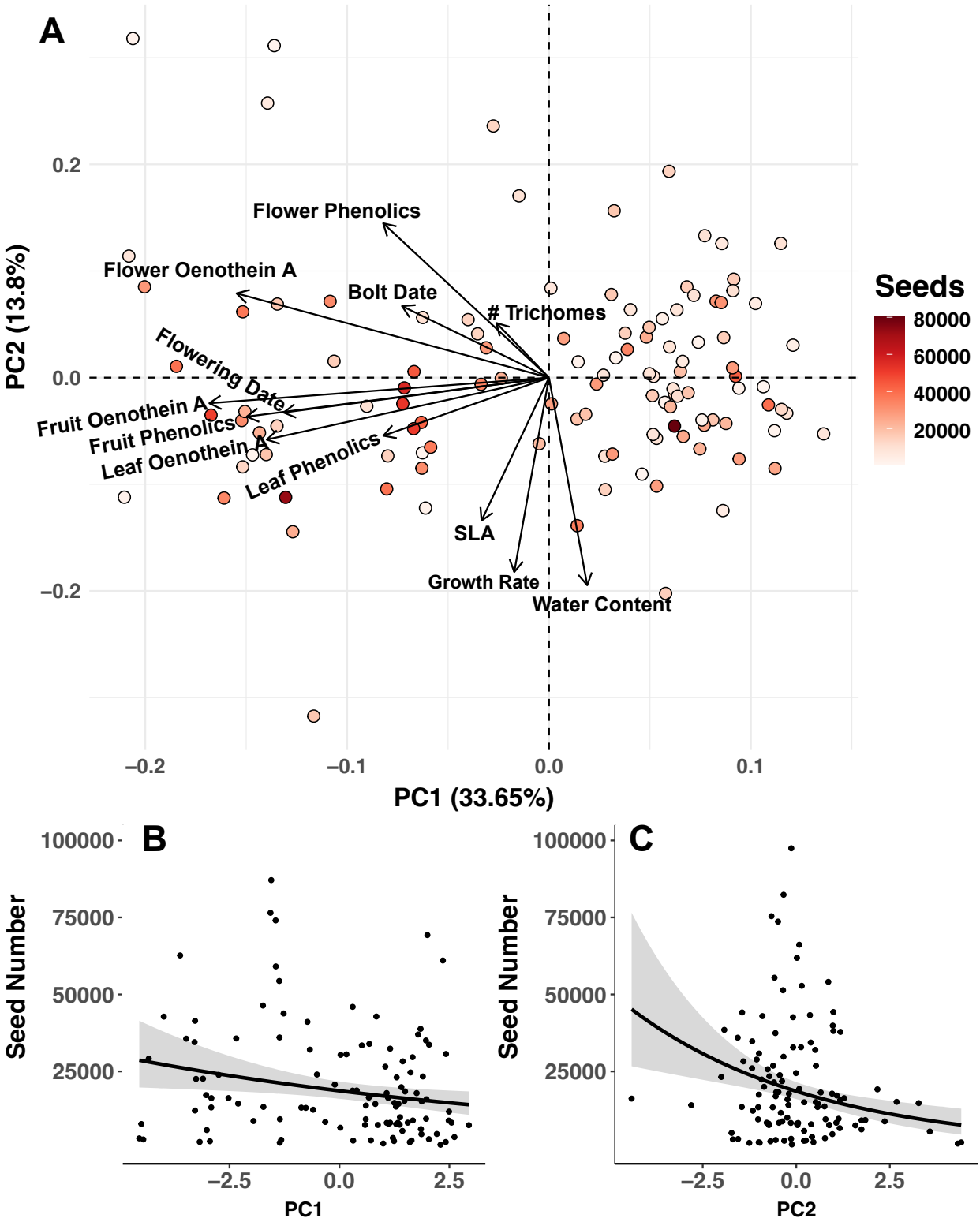


Figure 4. Fruit chemical defenses predict seed production of *O. biennis* in a common garden. Plots are given for (A) flower total phenolics, (B) fruit total phenolics, (C) flower oenothein A, and (D) fruit oenothein A. Units are in mg of given secondary metabolite(s) per g of dry mass. Each point represents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



676

677 Figure 5 Principle component analyses of chemical, phenological and morphological traits of *O.*

678 *biennis*. (A) PCA bioplot showing first and second principle 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.

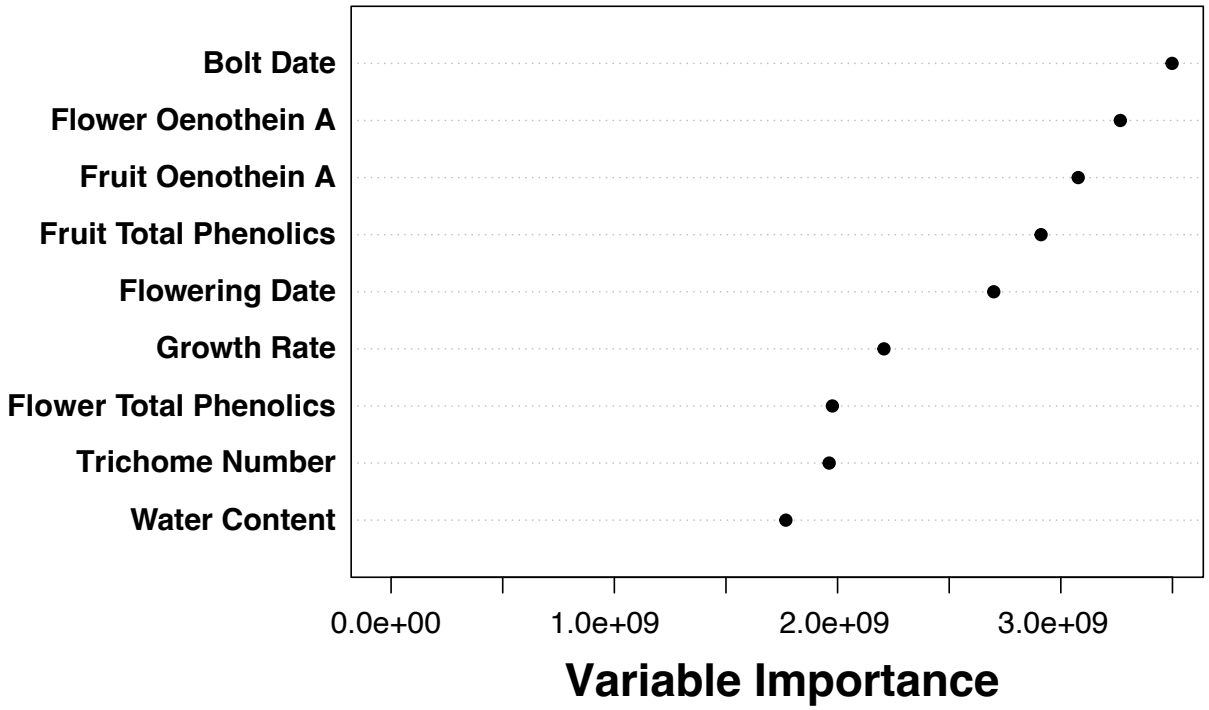


Figure 6. Variable importance for *O. biennis* traits predicting seed production in a Northern common garden. Values were generated using gradient forest.