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Drought Tolerance in Native and Invasive Populations of the Centaurea jacea Hybrid Complex

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DROUGHT TOLERANCE IN NATIVE AND INVASIVE POPULATIONS OF THE *CENTAUREA JACEA* HYBRID COMPLEX

A Thesis Presented

by

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of

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ABSTRACT

Introduced plants face many ecological and evolutionary challenges when establishing in a new range, such as strong abiotic stressors and potentially novel selective environments. One such abiotic stress is water availability, which is a strong selective force shaping physiological and phenological traits that enable plants to tolerate or avoid drought stress. Despite the challenges of establishing in a new range, thousands of species have become invasive in recent centuries. Two hypotheses that may explain how a species is able to withstand stress in its introduced range are preadaptation, which posits that species are adapted to similar environments in their native ranges before being introduced to a new range, and post-introduction adaptation, which posits that species are able to rapidly adapt after being introduced.

The *Centaurea jacea* hybrid complex is a complex of the species Brown Knapweed (*C. jacea*), Black Knapweed (*C. nigra*), and their fertile hybrid Meadow Knapweed (*C.* × *moncktonii*), which readily backcrosses with its parental species. This complex is native to western Europe and invasive in North America. In this study, I investigated differences in drought response between native European populations and invasive North American populations to test the hypotheses of preadaptation versus post-introduction adaptation. I grew individuals from 11 populations from the European range and 11 from the Pacific Northwest region of the invaded range in a greenhouse experiment, which included a control group and a drought treatment group. Over the 8-week drought treatment, I measured stress response physiology, phenology, size, and reproductive effort in order to discern if invasive populations of this species complex show evidence of (1) pre-adaptation to the environmental niche, (2) preadaptation as an ecological generalist, (3) post-introduction evolution of invasiveness, or (4) post-introduction evolution of clinal adaptation. I found evidence of post-adaptation evolution towards larger biomass and a higher number of capitula and in physiological traits related to stomatal conductance in invasive Pacific Northwest populations. There was also evidence of preadaptation in traits such as first year flowering, chlorophyll absorbance, and total seed production. These results suggest that both preadaptation and post-introduction evolution have contributed to the invasion of the *Centaurea jacea* hybrid complex in the Pacific Northwest of the United States.

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1.1. Introduction

Introduced species face many challenges establishing in a new range, such as potentially novel habitats and stressors. However, with the rise of globalization, thousands of species have successfully become invasive, a number that has significantly increased over the last 50 years (Seebens et al. 2017). There are several hypotheses for how introduced populations can become invasive despite the challenges of establishing in a new environment, often in relatively low numbers of individuals (Catford, Jansson, and Nilsson 2009; Colautti et al. 2014; van Kleunen et al. 2010). The ability of an invasive species to withstand abiotic stress may be key to a successful invasion of novel environments. Two hypotheses that may explain how invasive populations are able to achieve this are through preadaptation of those populations to stress within their native range prior to introduction or through post-introduction adaptation in the introduced range (reviewed in Bock et al. 2015; Colautti et al. 2017).

Pre-adaptation is the idea that a species was already adapted to withstand stress in its native range prior to being introduced to a habitat with similar stressors. One hypothesis that falls under the pre-adaptation umbrella is that a species is an ecological generalist (Baker 1965), consisting of populations that have the capacity to withstand a wide range of environmental stressors. This may be due to plasticity, allowing the species to respond to stress through changes in morphology or gene expression. For example, a reciprocal transplant study of *Reynoutri japonica* showed that there was no evidence of local adaptation in invaded populations, despite genetic differences between them, and that there was evidence for sufficient phenotypic plasticity to survive across the species' introduced range (VanWallendael, Hamann, and Franks 2018). An RNA-seq experiment in *Gypsophila paniculata* populations from climatically different parts of its invaded range found evidence for a high degree of plasticity in the form of many differentially expressed transcripts across the climate gradient, but few SNP differences between populations (Lamar, Beddows, and Partridge 2020). In these cases, plasticity in either phenotypic traits or gene expression appears to represent a preadaptive ecological generalist strategy that allows species to persist in a wide variety of environments across their invaded ranges.

Successful invasive species that are ecological generalists may also possess life history or ecophysiological traits that lead to fast growth and high demographic rates and allow them to successfully establish growing populations under different environments. Studies supporting this hypothesis in plants have found that traits such as the ability to maintain high growth rates under nutrient limited conditions and competition (Montesinos and Callaway 2018; Thébaud et al. 1996), higher biomass and faster germination (van Kleunen et al. 2011; Schlaepfer et al. 2010), higher photosynthetic capacity (Guo et al. 2014), and the ability to reproduce vegetatively (Reichard and Hamilton 1997) were found to be significantly different between invasive and non-invasive congeners. Traits such as these may be important pre-adaptations for invasion especially when species experience novel ecological environments within the introduced range, such as the loss of natural enemies.

A second form of the pre-adaptation hypothesis is that populations within the native range of an invasive species are genetically differentiated along an ecological gradient, and some subset of these populations already possess the traits necessary to thrive as an invader because they are preadapted to a similar environment in the introduced range. In a survey of 50 terrestrial plant invaders, fewer than 15% of species studied have more than 10% of their invaded range outside of their native climate niches, suggesting that invasive species tend to be successful in ranges where they are already pre-adapted to the introduced climate (Petitpierre et al. 2012). Pre-adaptation of certain native range populations may be particularly important for certain environmental stressors found in the native range and prevalent where the species invades, such as a tolerance for serpentine soils as seen in invasive *Aegilops triuncialis* populations in California (Meimberg et al. 2010).

Similar to environmental stressors, invasive species may also be preadapted to anthropogenic stressors in their introduced range. The anthropogenically induced adaptation to invade (AIAI) hypothesis suggests that invasive species may already have adapted to anthropogenic disturbance in their native range, which subsequently benefits them in their introduced ranges when competing with native species (Hufbauer et al.

2012). While human disturbances are often detrimental to native plant communities, invasive species often show higher tolerance to disturbances such as clipping and defoliation, as seen in both diploid and tetraploid *Centaurea stoebe* (Rosche, Hensen, and Lachmuth 2018). Researchers have also found that replaced native grassland in California was only reinvaded successfully by exotic annual grasses under treatments that included nitrogen and water limitation and disturbance regimes, suggesting that the invasive grasses are only superior competitors in this ecosystem in the presence of these disturbances (Seabloom et al. 2003).

In contrast to pre-adaptation, the hypothesis of post-introduction adaptation posits that invasive populations can rapidly adapt to new environments in the introduced range by responding to selection on existing genetic variation, perhaps augmented by polyploidy (te Beest et al. 2012) or via hybridization between closely related species and/or admixture following multiple introductions (Buswell, Moles, and Hartley 2011; Lavergne and Molofsky 2007). This may involve divergence in the mean phenotypic trait value between the native and introduced ranges, for example, evolution towards functional traits associated with fast growth and high reproductive output that facilitates rapid population increases. In a study that used 1900 herbarium specimens which represented 23 plant species introduced to Australia, researchers found that over 70% of the invasive species in the study showed evidence of evolution over a century in trait means such as stem height, leaf area, and leaf shape, which was a significantly higher proportion of species than seen in herbarium specimens of native species over the same time (Buswell et al. 2011). Another study utilizing herbarium samples of invasive plants of *Sisymbrium austriacum* subsp. *chrysanthum* found evidence of strong sequence divergence in flowering time genes during the establishment phase of the species after its initial introduction, but before beginning to rapidly spread, suggesting that evolutionary change in flowering time within the introduced range was key to becoming invasive (Vandepitte et al. 2014). Evidence of evolution towards smaller genome size was observed in invasive *Phalaris arundinacea* populations compared to native range populations of this species, and evolutionary models supported the hypothesis that this change in genome size was a response to selection that also benefitted plants through correlated traits such as stem growth rate (Lavergne, Muenke, and Molofsky 2010). Numerous other examples of post-introduction adaptation have been reported for invasive plants (reviewed in Colautti and Lau 2015; Keller and Taylor 2008; Prentis et al. 2008).

There is also evidence of adaptive differentiation of invasive populations within the introduced range as a response to heterogeneous selection. For example, locally adaptive clines along environmental gradients can be reestablished in a species' invaded range, parallel to clines seen in the species' native range. Oduor, Leimu, and van Kleunen (2016) found in a phylogenetically controlled meta-analysis of 134 plant species that invasive species are locally adapted just as frequently as native plant species. This has been seen in multiple studies of clines in *Ambrosia artemisiifolia* in its native North America and both invaded ranges in Australia and Europe in traits related to phenology, climate niche, growth and reproduction, and defense (van Boheemen, Bou-Assi, et al.

2019; van Boheemen, Atwater, and Hodgins 2019; van Boheemen and Hodgins 2020; Hodgins and Rieseberg 2011; McGoey, Hodgins, and Stinchcombe 2020). Locally adaptive clines have also been observed for flowering time in *Lythra salicaria* (Colautti and Barrett 2013; Montague, Barrett, and Eckert 2008) and *Medicago polymorpha* (Helliwell et al. 2018), flowering time and size in *Eschscholzia californica* (Leger and Rice 2007), and drought stress traits in *Brachypodium silvaticum* (Marchini, Arredondo, and Cruzan 2018; Marchini, Maraist, and Cruzan 2019). The reestablishment of life history trait clines in *Silene latifolia* and *Silene vulgaris* in their introduced North American ranges was observed even when controlling for expectations of neutral genetic drift due to introduction history, providing evidence that this reestablishment was a result of adaptive evolution within the introduced range (Keller et al. 2009).

Black and brown knapweed (*Centaurea nigra* and *jacea*, respectively) are two closely related weedy plant species in the Asteraceae family (Garcia-Jacas et al. 2000, 2001, 2006; Gardou 1972). These species hybridize to form meadow knapweed (*C.* × *moncktonii*) which, because it is fertile and readily backcrosses with both of its parent species, forms a hybrid swarm referred to here as the *Centaurea jacea* hybrid complex. This hybrid complex is native to Europe and invasive in North America. The complex is found as both a diploid and a tetraploid in its native range, however only tetraploids have been observed in its introduced range (Lachmuth et al. 2019). Observations of the complex have been reported in the Pacific Northwest of the United States and Canada as early as the first half of the 19th century, after being introduced through ship ballast and possibly as forage for honeybees, although it was not reported as invasive in the Pacific Northwest until the early 20th century (Roche and Roche 1991). Other early observations of *C. jacea* include the northeastern US and southeastern Canada beginning in the 1860s, and it was recognized as invasive in this part of its North American range in the first half of the 20th century (Zenkert and Zander 1934).

Water availability is an environmental gradient that may pose a selective filter on introduced populations of the *C. jacea* hybrid complex in the Pacific Northwest, and for which pre-adaptation may exist within the native range. Water availability is necessary for plants to survive and reproduce, as it is needed for photosynthesis, maintenance of turgor pressure, and transport of nutrients (Hetherington and Woodward 2003; Tezara et al. 1999). Because of this importance, water availability is a strong selective force shaping physiological and phenological traits that enable plants to tolerate or avoid drought stress, and water availability plays an important role in determining species distributions and ecological niche (Svenning and Sandel 2013). While yearly precipitation increased over the last century in the *C. jacea* hybrid complex's introduced range in the Pacific Northwest (Mote 2003), seasonal precipitation regimes have become more extreme, with lower precipitation in summer months during the growing season, and seasonal precipitation differences in this region are predicted to become more extreme over coming decades (Abatzoglou, Rupp, and Mote 2014; Mote and Salathé 2010; Tohver, Hamlet, and Lee 2014). While these precipitation changes likely represent an important stressor on plants in these communities, the disturbance arising from drier or more variable precipitation regimes may be beneficial to plant invaders that are able to tolerate higher drought stress through pre-adaptation, plasticity, or post-introduction adaptation to conditions of limited water availability. In studies focusing on its native European range, this hybrid complex has been shown to be drought tolerant. In a study looking at the effects of site biodiversity on drought tolerance, *C. jacea* was one of only two plants of the eight species native to Europe included which did not experience loss of performance in dry years, regardless of the species diversity in plots in which they were grown (Wright et al. 2021). In a study which combined experimental drought and nitrogen loading, *C. jacea* was also able to tolerate lower leaf water potentials during drought treatments than the other two species studied (Kübert et al. 2021).

In this study, I investigated differences in drought response between native European and invasive North American populations of the *Centaurea jacea* hybrid complex. The goal of the study was to discern if variation in ecological niche with respect to precipitation across the hybrid complex's native and introduced ranges is due to pre-adaptation or to post-introduction adaptation. To investigate this, I performed a greenhouse common garden experiment in which individuals from populations across the complex's native European (EU) and introduced Pacific Northwest (US) ranges were subjected to a drought treatment, and functional traits associated with stress response physiology, phenology, plant size, and reproductive effort were measured.

I used this experiment to test for genetic differentiation in traits between regions (EU vs. US) and in the plastic response to stress. In addition, I evaluated model support among two hypotheses of preadaptation (hypotheses 1 and 2) and two hypotheses of post-introduction adaptation (hypotheses 3 and 4): (1) pre-adaptation to the environmental niche, (2) ecological generalism, (3) post-introduction evolution of invasiveness, and (4) post-introduction evolution of clinal adaptation. I formulated predictions for each of these hypotheses based on linear models relating drought responsiveness (e.g., trait response in drought relative to control) to the source climate of populations within each range (EU and US). In the case of preadaptation (1), EU populations would be locally adapted to different source climates, resulting in a cline along the environmental gradient, while US populations would be similar to a subset of EU populations that were preadapted to the introduced environment (Figure 1A). In the case of ecological generalism (2), EU and US populations would show similar broad variation in drought response, with no regional differentiation (Figure 1B). In the case of post-introduction adaptation for invasion (3), the mean drought response in EU populations would be genetically differentiated from US populations but without the specific case of a locally adaptive cline (Figure 1C), Lastly, in the case of post-introduction evolution of clinal adaptation (4), there would be a cline over the environmental gradient in both the EU and US populations (Figure 1D).

1.2. Methods

Population Sampling and Climate Niche Characterization

Populations from the Pacific Northwest region of North America (US) in the introduced range and from western Europe (EU) in the native range were used in the experiment. In 2017, 34 EU populations were sampled, and in 2019, 23 US populations were sampled from Washington and Oregon (Figure 2). To characterize the climatic niche in each range, I performed principal components analysis (PCA) using 19 Bioclim climate variables from the Wordclim global gridded climate data at 2.5 arc-minutes resolution (Fick and Hijmans 2017) using the FactoMineR package in R (Le, Josse, and Husson 2008). The 19 variables included annual mean temperature (bio1), mean diurnal range (bio2), Isothermality (bio3), temperature seasonality (bio4), maximum temperature of the warmest month (bio5), minimum temperature of the coldest month (bio6), temperature annual range (bio7), mean temperature of the wettest quarter (bio8), mean temperature of the driest quarter (bio9), mean temperature of the warmest quarter (bio10), mean temperature of the coldest quarter (bio11), annual precipitation (bio12), precipitation of the wettest month (bio13), precipitation of the driest month (bio14), precipitation seasonality (bio15), precipitation of the wettest quarter (bio16), precipitation of the driest quarter (bio17), precipitation of the warmest quarter (bio18), and precipitation of the coldest quarter (bio19). Bioclim variables that contributed strongly to PC1 were predominately related to moisture availability, such as precipitation seasonality (bio15), precipitation of the warmest quarter (bio18), and precipitation of the driest month (bio14), as well as temperature variables that likely interact with precipitation to affect plant water availability during the growing season, such as mean temperature of the driest quarter (bio9). Based on the strong moisture gradient represented by PC1, I selected 11 US populations and 11 EU populations (Table 1) from the initial collections to sample across the gradient in climatic niche.

Greenhouse Experiment

From each of the 11 EU and 11 US populations, 4 maternal families were chosen. From each maternal family, 8-10 seeds were germinated in petri dishes on wetted filter paper and placed upright in trays under low intensity fluorescent lighting. Dishes were sealed with parafilm and checked daily to refill water as needed to prevent seeds from drying out.

I transplanted germinating seeds into 60 cu. in. pots containing Promix BX soil mix. Plants were randomly placed in blocks of 15 plants spread in a checkerboard pattern over two trays. Plants were grown in the greenhouse at University of Vermont under 16-hour days with temperatures at 20 – 24 °C during the day and 15 – 18 °C during the night. During weeks 1 through 4 of the experiment post-transplanting, the plants were watered regularly and fertilized (Jack's Professional 15-16-17 Peat-Lite at 200 ppm) once a week to allow for establishment.

Blocks were assigned to either treatment or control and randomized on the bench every week to avoid bench effects. There were 329 individuals from 22 populations grown in the greenhouse experiment (Table 1). Of these, 11 were removed from the analysis because their morphology after flowering was more consistent with a related species *C. nigrescens*, which is not part of the *C. jacea* hybrid complex. This left 318 individuals in the data analysis.

At week 6 post-transplanting, I began the experimental treatment by withholding water from the drought group while the control group remained well-watered. To determine when plants needed to be watered in both controls and treatments, I monitored soil volumetric water content (VWC) daily using a Fieldscout Soil Sensor Reader and Waterscout SMEC 300 Soil Moisture Temperature Sensor (Spectrum Technologies). VWC was measured on 10 haphazardly chosen pots from each treatment group from different places on the greenhouse bench. Based on preliminary measurements on pilot plants of similar size and developmental stage, field capacity was determined to be about 25% VWC. Pilot plants were also used to test drought conditions and determine the intensity of the drought treatment based on VWC that induced turgor loss and how well pilot plants recovered after watering. Based on these pilot observations, control plants were watered once the soil dried to an average 40% of field capacity (10% VWC). Treatment plants were allowed to dry down to 4-6% field capacity (1-2% VWC) and then 48 hours later, were watered. Whenever water was applied to either control or treatment groups, plants were watered enough to reach at least 80% of field capacity (20% VWC).

During weeks 6 – 12 of the experiment, I conducted daily phenological observations for bolting and flowering. This species complex is protandrous, and flowering was defined as when the flower was open enough for anthers to be available. To assess reproductive effort, flowers were hand pollinated as they opened. Every day once flowers began to open, available pollen was collected from open flowers into a bulk pollen sample to avoid incompatibilities between populations or individuals of this self-incompatible species complex, and the pollen was applied onto receptive flowers in female phase using a camelhair brush.

I also conducted weekly measurements of leaf chlorophyll absorbance as an index of stress tolerance using a SPAD meter (Konica Chlorophyll Meter SPAD-502Plus) throughout the experiment. Beginning in week 5 and extending throughout the rest of the experiment, chlorophyll absorbance was measured by taking the average reading across 3 young, fully expanded basal leaves per plant, avoiding large veins.

During week 13, the physiological traits stomatal conductance (g_{sw}), chlorophyll fluorescence (Φ-PSII), and leaf temperature (T_{leaf}) were measured using a LiCor Li-600 porometer. Physiological measurements were taken 1 day after the drought treatment concluded and plants were watered and allowed to fully rehydrate. This allowed direct comparison of the long-term effects of the drought treatment on physiological adjustment across plants of different size experiencing similar (full) turgor. Measurements were done on one young, fully expanded basal leaf per plant. Measurements were taken on the flat part of the leaf, avoiding large veins and in the same orientation for all leaves. Three measurements were taken with the porometer per leaf and averaged.

After completing LiCor measurements, the same leaf was sampled to determine leaf Relative Water Content (RWC). Following sampling, the fresh weight (W) of the leaf was immediately measured. Leaves were then put into 50 mL screwcap tubes and placed into the cooler with ice packs to maintain freshness. A few cm of water was added to the tubes to fully hydrate the leaves through their petioles, and after 3-4 hours, the outside of the leaves was dried and the fully turgid weight (TW) of the samples was measured. Leaves were then placed in dried in a drying oven for 24 hours at 60 °C followed by determination of dry weight (DW). RWC was calculated using equation (Turner 1981):

$$\boldsymbol{eq\ 1}: RWC\ (\%) = \left[\frac{(W - DW)}{TW - DW}\right] X\ 100\%$$

During week 14 (1 week after treatment ended), the plants were harvested. The height of the tallest stem (from the base of the stem to the base of the tallest capitula), the width of the tallest stem was recorded, and the number of opened and unopened capitula were recorded. Two mature capitula per plant were collected for seed counts to use as a measure of reproductive fitness. The total seed count was estimated by multiplying the average seed/capitula by the total number of capitula. Above-ground biomass was estimated by harvesting all aboveground parts, placing them in a drying oven at 60 °C for 48 hours, and measuring dry weight.

Statistical Analysis

All statistical analyses were done in R (4.2.1). Plotting and data manipulation were done using *ggplot2* (Wickham 2016) and *dplyr* (Wickham et al. 2022) packages. Principal components analysis (PCA) was performed using the R packages *FactoMineR* (Le et al. 2008) and *factoextra* (Kassambara and Mundt 2020) using all 19 Bioclim variables and retrieved using the *raster* package (Fick and Hijmans 2017; Hijmans 2022). The packages *lme4* (Bates et al. 2015), *sjPlot* (Lüdecke 2021), *sjmisc* (Lüdecke 2018), and *glmmTMB* (Brooks et al. 2017) were used for linear mixed models.

Dry weight, stem width, and leaf temperature were log transformed and stem height, total capitula, and stomatal conductance (g_{sw}) were square root transformed to improve normality. Extreme individuals were removed as outliers.

Linear mixed models (LMMs) or Generalized Linear Mixed Models (GLMMs) were used on the data to assess the effects of treatment (Control or Treatment), region (native EU or invasive US), and their interactions on the traits measured. Treatment (trt), region, and their interactions were treated as fixed effects to reflect the crossed design of the experiment, while population (pop), maternal family (mat) and block (blockNum) were modeled as random effects (equation 2).

$$\boldsymbol{eq.2}: trait \sim trt * region + (1|pop) + (1|mat) + (1|blockNum)$$

LMMs were performed on the growth traits (dry weight, stem height, and stem width), physiology traits (RWC, g_{sw}, Φ-PSII, and T_{leaf}), and reproduction traits (total capitula, bolting day, flowering day, and days since bolting to flowering). A binomial generalized linear mixed model (GLMM) was performed on the logical trait bolted (i.e., whether individuals bolted or not during the experiment). A zero-inflated GLMM was performed on the trait estimated seed. Plants that did not bolt or that bolted but did not set seed were assigned values of 0 seeds, leading to a zero-inflated count distribution that was modeled with a mixture of binomial and negative binomial distributions in the GLMM.

Because the chlorophyll absorbance data was taken on individuals weekly, a repeated measures ANOVA was performed on this trait. Because I was interested in the effects of the treatment over the time course of the experiment, week was included as a fixed effect, including its interactions with region and treatment. Because of the weekly measurements, the data for individuals each week were not independent from one another, and therefore individual (indID) was included as an additional random effect in the model. The model was performed with equation 3:

$$\boldsymbol{eq.\,3}: trait \sim week * trt * region + (1|pop) + (1|mat) + (1|blockNum) + (1|indID)$$

In order to differentiate between hypotheses for pre- and post-introduction adaptation, I performed a second round of linear models to relate population-level drought response to the source climate. Best linear unbiased predictions (BLUPs) were obtained for the populations from a LMM using treatment as the fixed effect and population, maternal family, and block number as random effects. The estimates for each population's trait value in the drought treatment was divided by the estimated trait value in the control to obtain the Drought Ratio (Treatment:Control) as an index of population-level drought response. For each trait, five linear models were then performed with Drought Ratio predicted by PC1, region and/or their interaction. PC1 values were used as a proxy variable for historic water availability based on the contribution of Bioclim variables related to precipitation to PC1.

$$A. preadaptation: Drought\ Ratio \sim region * PC1$$

$$B. generalist: Drought\ Ratio \sim 1$$

$$C. post - introduction\ evolution\ of\ invasiveness: Drought\ Ratio \sim region$$

$$D1. post - introduction\ adaptation\ (clinal\ adaptation): Drought\ Ratio \sim PC1$$

$$D2. post - introduction\ adaptation\ (clinal\ adaptation): Drought\ Ratio \sim PC1 + region$$

To evaluate support for the different hypotheses, the Akaike Information Criterion (AIC) scores were calculated and support for a given hypothesis was based on the model with the lowest AIC score (e.g., the best fitting model). As a measure of how strongly supported the hypothesis was, we calculated the difference in AIC (ΔAIC) between the best fitting model and the other competing models, with ΔAIC < 2 corresponding to models with similar levels of support (Anderson and Burnham 2004).

1.3. Results

EU and US populations of the *Centaurea jacea* hybrid complex occupy divergent but partially overlapping ecological niches based on the PCA of the 18 Bioclim variables. The regions primarily separated along climate PC1, which explained 55.1% of the among-population variation in source climate (Figure 3). The Bioclim variables with the strongest variable contributions were isothermality (bio3), precipitation seasonality (coefficient of variance, bio15), precipitation of the warmest quarter (bio18), mean temperature of the warmest month (bio9), mean temperature of the coldest quarter (bio11). Thus, PC1 effectively captured a gradient of water availability primarily associated with the warm growing season months. While there was an overall shift from wetter to drier growing seasons between the native and introduced ranges, there was also a gradient of precipitation availability within each range (Figure 3).

The greenhouse experiment designed to test for variation in drought responsiveness between EU and US regions included populations sampled from across the precipitation and temperature gradient captured by PC1. Over the course of the experiment, individuals in the treatment group experienced 6 dry-down cycles in which mean VWC across 10 sampled plants was allowed to drop to 0% for 48 hours before plants in the treatment were watered; in contrast, individuals in the control group were watered 14 times over the course of the experiment to maintain VWC above stress levels (Figure 4).

There was a significant effect of drought treatment on growth traits (Table 2), including dry weight ($p < 0.001$; Figure 5A), stem width ($p = 0.015$; Figure 5B), and stem height ($p < 0.001$; Figure 5C). Dry weight, stem width, and stem height were all higher in the control group than the treatment group (Figure 5). In addition, region and the region*treatment interaction had significant effects on dry weight (Table 2). Dry weight was higher in the US populations than EU populations overall, although the negative effect of treatment was higher for US populations than EU populations (Figure 5A).

In contrast to growth traits, physiological traits differed mostly by region or the interaction between region and treatment (Table 2). There was a significant effect of region and region*treatment on stomatal conductance, Φ-PSII fluorescence, and leaf temperature (Figure 6). Stomatal conductance and Φ-PSII fluorescence were lower in US populations than EU populations in the control group and higher in US populations than EU populations in response to drought (Figure 6B; Figure 6C). Leaf temperature was higher in US populations than EU populations in control but lower in US populations than EU populations in the treatment group (Figure 6D). There was no significance in any of the fixed effects for RWC, although there was a slight trend towards higher RWC in response to drought (Table 2; Figure 6A). In the repeated measures ANOVA performed on chlorophyll absorbance, there was a significant effect of treatment ($p = 0.004$) and the interaction of week and treatment ($p < 0.001$; Table 3). The average absorbance measurements were higher in the treatment group which rose significantly over the 8 weeks of the treatment compared to the control group, which remained relatively constant (Figure 7). Unlike most other physiological traits, chlorophyll absorbance showed no significant difference between EU and US populations (Table 3).

Reproduction traits were primarily differentiated by region, with US plants generally showing higher reproductive capacity compared to EU plants across treatments (Table 2). There was a significant effect of region in the binomial GLMM for the probability of bolting (p = 0.023, Figure 8). US populations had a higher probability of bolting than the EU populations in both the treatment and control groups (Odds Ratio = 32.98). There was no significant effect of any of the fixed effects for bolting day, flowering day, or bolting to flowering day (Figures 9A-C). There was a significant effect of region on total capitula (p = 0.002; Table 2). Total capitula was higher in US populations than EU populations in both the control and treatment groups (Figure 7D).

Total estimated seed count, our best proxy for fitness, showed a significant effect of treatment (p = 0.018; Table 2). The estimated seed was higher in the control group than the treatment group (Figure 9E). Although the US populations had slightly higher estimated seed than EU populations, it was not significant (p = 0.118; Table 2).

All three of the growth traits (dry weight, stem width, and stem height) were best supported by the linear model representing post-introduction adaptation with locally adapted clines in both the EU and US populations (model D1; Figure 10). The second-best fitting model for stem height also supported clinal evolution on a regional basis (model D2; Table 4; ΔAIC = 1.2802). The second-best fitting models for dry weight and stem width represented post-introduction adaptation without a cline (model C; Table 4; dry weight ΔAIC = 1.325 and stem width ΔAIC = 1.854).

Of the physiological traits, the model representing an ecological generalist (model B) best explained RWC (Table 4; Figure 11A), Φ-PSII fluorescence (Table 4; Figure 11C), and chlorophyll absorbance (Table 4; Figure 11E). Stomatal conductance (Figure 11B) and leaf temperature (Figure 11D) were both best explained by the model representing post-introduction adaptation with no cline (model C; Table 4).

Both bolting day (Figure 12A), flowering day (Figure 12B), and bolting probability (Figure 12E) were best fit by the model representing preadaptation by an ecological generalist (model B; Table 4). Days from bolting to flowering (Figure 12C) was best fit by the model representing post-introduction adaptation with clines (model D1; Table 4) and total capitula (Figure 12D) was best fit by the model representing post-introduction adaptation without clines (model C; Table 4). Estimated seed (Figure 12F) was the only Drought Ratio best fit by the model representing preadaptation from clinally adapted populations in the native range (model A; table 4).

1.4. Discussion

Invasive plants may benefit from both preadaptation and post-introduction adaptation when establishing and spreading in an introduced range. This study shows trait-specific evidence in the *Centaurea jacea* hybrid complex in drought response. The results of this study suggest that the *Centaurea jacea* hybrid complex was physiologically preadapted to drought stress in its native range, which may have facilitated the invasion in the Pacific Northwest region of its introduced range, but also that the complex adaptively evolved post-introduction towards higher dry weights and reproduction through higher capitula number and probability of bolting during the first year.

Even though US populations achieved higher growth and reproduction overall, they also appeared to be more sensitive to drought and were proportionately more negatively affected by the drought treatment than EU populations. This suggests US populations may have evolved a strategy of prioritizing early reproduction over long term survival, which may benefit invasive populations by leading to overall faster demographic rates and population growth. This potentially risky strategy of investing into early reproduction and larger size appears to pay off even under drought stress and would also allow invasive populations to achieve very high reproductive rates under optimal conditions when not under drought stress. This experiment was not designed to assess a potential tradeoff in survival from investing in earlier reproduction, so it is not clear if the US populations are prioritizing reproduction at the expense of longer-term survival. However, Hodgins et al. (2020) found that there was no evidence of a trade-off between performance and survivorship in invasive populations of Canada thistle, and found that even under stressful conditions, invasive populations maintain as good as or superior size and performance compared to native individuals, and the *C. jacea* hybrid complex may be benefitting from a similar lack of trade-off.

The drought treatment in this study had a negative effect on biomass, resulting in lower dry weight, stem height, and stem width in both US and EU populations. This is consistent with what has been seen in other *Centaurea* species, such as *C. stoebe* (Mraz et al. 2014). A study of invasive populations of *C. nigra* from Canada found that there was a decrease in both above and below-ground biomass in response to drought stress (Qaderi et al. 2014). However, another study that included 3 *Centaurea* species found that below-ground biomass was stable in *C. solstitialis* and *C. melitensis* and increased in *C. cyanus* under drought stress (Muth and Pigliucci 2007). While below-ground biomass was not measured in the current experiment, it is possible that below-ground biomass was also negatively affected by the drought treatment or showed population- or region-specific patterns of allocation in response to drought. Thus, another possible explanation for the lower dry weight under drought stress observed here may be that plants were responding by investing more into below-ground biomass.

Despite the negative effects of the drought treatment on growth traits, invasive populations were larger than native populations, which has been seen for overall biomass in *C. solstitialis* (Eriksen et al. 2012; Widmer et al. 2007) and in early accumulation of biomass in *C. stoebe* (Henery et al. 2010; Mráz, Tarbush, and Müller-Schärer 2014).

Invasive populations were also able to maintain a higher Drought Ratio in both stem width and height compared to native populations. Juvenile plants in this species complex consist of a basal rosette and only produce stems when bolting, so the proportionately larger decrease in dry weight in invasive populations compared to stem height and width may be due to invasive populations allocating more resources into reproductive shoot growth under drought conditions instead of into vegetative growth in order to prioritize reproduction. EU populations that did not bolt also had the highest Drought Ratio of dry weight, suggesting that not bolting allowed them to maintain very similar above-ground dry weights under drought stress relative to control conditions.

Stomatal conductance and Φ-PSII fluorescence were overall lower in US populations compared to EU populations, but as indicated by the significant positive interaction of region and treatment on these traits, US populations had a proportionately larger response to the drought treatment than EU populations. Under control conditions, US populations had lower stomatal conductance, which may suggest that the stomata were partially closed, or that the density of stomata per leaf area was lower. The measurements of these traits were taken 24 hours after the end of the drought treatment, so higher stomatal conductance in drought treated plants may be due to US populations opening stomata and increasing PSII productivity more quickly in response to increased water availability after closing them to respond to drought, or due to plants in the drought treatment producing leaves with higher density of stomata. This is also reflected in the drop of leaf temperature in US populations, which is likely due to transpirational cooling once stomata are reopened, as has been seen in other species such as maize and common bean (Deva et al. 2020; Liu et al. 2011).

It has also been shown in previous research that native populations of *C. stoebe* had higher stomatal conductance than invasive populations even under drought stress but appeared to be slower to close stomata in response to drought than invasive populations. It is also possible that partially closing stomata is a strategy to tolerate keeping them open longer under drought stress, but stomatal conductance was not measured over the course of the drought treatment, so this study does not provide evidence that US populations were able to keep stomata open for longer during the drought cycle. However, previous research has shown that *C. jacea* was more tolerant of very low leaf water potentials than grasses compared in the study (Kübert et al. 2021), possibly as part of an anisohydric strategy in which a plant takes the risk of tolerating more variable leaf water potential in exchange for keeping photosynthetic rates higher for longer under drought stress. Whether the post-introduction evolution in stomatal conductance and leaf temperature is reflective of a more drought tolerant and anisohydric strategy or a more drought avoidant and responsive strategy in US populations, this less conservative behavior in response to drought may be further evidence that invasive populations prioritize traits that maximize reproduction, even when risky.

Relative Water Content of the leaves (RWC) was not significant for any effects, which my in part reflect a higher overall measurement variance for this trait. While not significant, RWC did appear to be higher in both invasive and native populations under drought stress, which has been shown in other species such as Jerusalem artichoke (Chaimala et al. 2021; Puangbut, Jogloy, and Vorasoot 2017). This overall pattern might indicate some degree of osmotic adjustment occurring in response to repeated drought stress exposure. While region also was not significant, the slightly lower RWC in US populations may be further evidence that invasive populations of the species complex have a more anisohydric strategy, as anisohydric plants regulate RWC more strictly than water potential (Sade, Gebremedhin, and Moshelion 2012).

Chlorophyll absorbance (SPAD) stayed relatively constant over the course of the experiment in control plants but rose in treatment plants. Because this occurred gradually over the experiment and did not rise and fall along with water availability, this is likely an induced response to drought in which the plants increase chlorophyll concentration to mitigate loss of photosynthesis productivity.

Nearly all US populations in both treatment groups bolted, however only about half bolted in EU populations in either treatment. However, the Drought Ratio was around 1 for most EU and US populations, which suggests that bolting probability during the first year was not affected by drought specifically, which is why the linear models representing post-introduction adaptation of drought tolerance were not supported. The difference between EU and US populations is therefore likely another way that the invasive populations are prioritizing reproduction, in this case regardless of water availability, through a rapid life history that increases demographic growth rates. The estimated seed also supports this, suggesting that EU populations that were preadapted to first year bolting and flowering, and realized high seed production during the first year, may have been better preadapted for invasion.

The drought experiment did not affect the timing of bolting or flowering and there was no evidence of evolution in these reproductive phenological traits in invasive populations, in contrast with other studies that have seen earlier bolting in invasive populations of *C. solstitialis* (Eriksen et al. 2012). Although there was no significant effect of region on the days between bolting and flowering, there was an apparent pattern of US populations taking longer to flower after initiation of bolting than EU populations, especially under drought conditions. The Drought Ratio analysis suggested post-introduction adaptation in this trait, where drought stressed US populations spent slightly longer time after bolting before flowering. However, this difference is very small, and may be due to the smaller number of EU populations included, due to those that had no bolting being excluded.

Total capitula was significantly affected by region, and US populations produced a much larger number of capitula than EU populations. There was also support in the Drought Ratio analysis for post-introduction adaptation, suggesting that US populations have evolved towards producing more capitula, but also experienced a larger drop in the number of capitula produced in the drought treatment compared to EU populations. This may be evidence that US populations are able to capitalize on optimal environments to maximize reproductive output, but under drought stress produce fewer capitula to invest limited resources towards seed production more efficiently. Post-introduction evolution favoring reproduction has also been seen in higher seed production and in earlier flowering in *C. stoebe* invasive populations compared to native populations (Hahn, Buckley, and Müller-Schärer 2012; Henery et al. 2010; Mráz et al. 2014).

There are some caveats in interpreting this work. First, there were only 22 populations included in the experiment. Although the populations represented a wide climatic gradient in relevant precipitation and temperatures and a large latitudinal gradient across the species complex’s native and invaded ranges, it is true that more populations would provide a more complete picture of the environmental variation in the complex’s ranges. Some traits measured may have been influenced by latitude and Norwegian EU populations sampled were from latitudes 10° farther north than the farthest north US populations. However, there did not seem to be a pattern in phenology traits such as timing of bolting and flowering over latitude in the native range. This study also included US populations from only the Pacific Northwest region of the introduced range, and further work can use populations from other regions, such as the Northeast US, to look for evidence of pre- and post-introduction adaptation.

Second, the number of populations included also means that some caution should be used in interpreting the Drought Ratio models, however when post-introduction adaptation was the best fitting model, such as model D1 in the cases of growth traits dry weight, stem width, and stem height, the next best supported models with ΔAIC below 2, indicating similar levels of support (Anderson and Burnham 2004), were other models representing post-introduction adaptation (i.e., models C and D2 in the cases of growth traits).

While this work presents evidence of post-introduction adaptation, there are different modes through which the *C. jacea* hybrid complex has been able to quickly respond to selection in its introduced range. Post-introduction adaptation may be facilitated by hybridization as there may be species-specific traits (between *C. jacea* and *C. nigra*) that may contribute the genetic basis of beneficial traits to the hybrid complex for selection to act on. Multiple introductions are also extremely likely in this invasion, so it follows that admixture from populations across the native range may be providing variation for selection to act upon in the introduced range of the species. Therefore, to further understand the nature of preadaptation and post-introduction adaptation in the *C. jacea* hybrid complex, future work should address questions surrounding the demographic history of the complex, especially with respect to source populations contributing to the invasion and the nature of interspecific hybridization, which so far appears to have happened in its native EU range and to be ongoing in its introduced North American range, similar to work related species, such as *C. diffusa* and *C. stoebe* (Blair and Hufbauer 2010; Hufbauer and Sforza 2008) and *C. solstitialis* (Barker et al. 2017).

My results provide evidence for both preadaptation and post-introduction adaptation facilitating the invasion of the *C. jacea* hybrid complex in its Pacific Northwest introduced North American range. The hybrid complex appears to have been preadapted to drought tolerance in its native EU range in some traits, such as chlorophyll absorbance and fluorescence, and some populations in the native EU range appear to have been preadapted for invasion to early reproduction through bolting during the first year. However, this study also provides evidence that the *C. jacea* hybrid complex has experienced evolution towards larger biomass and stem sizes, early reproduction even under stress, and further drought tolerance. My research increases knowledge of how evolution of drought response and reproduction has proceeded in this invasive species complex both before and after introduction in its introduced range.

1.5. Figures and Tables

1.5.1. Tables

Table 1. Populations used in the greenhouse experiment, ordered by range and latitude.

Population Name	*Pop Code*	*Region*	*US State or Country*	*PC1 Score*	*Latitude*	*Longitude*
Norway 1	NOR1	EU	Norway	-3.20	61.18	7.23
Norway 8	NOR8	EU	Norway	-3.69	59.55	10.44
Norway 5	NOR5	EU	Norway	-3.48	59.44	10.49
Norway 2	NOR2	EU	Norway	-3.41	59.21	10.38
Luxembourg	LUX1	EU	Luxembourg	-1.85	49.62	6.14
Germany 4	GER4	EU	Germany	-2.59	48.62	8.64
France 2	FR2	EU	France	-1.87	45.49	5.38
France 3	FR3	EU	France	-1.62	45.41	5.42
Spain 2	SP2	EU	Spain	2.20	43.54	-6.52
Spain 4	SP4	EU	Spain	0.55	43.03	-5.83
Spain 5	SP5	EU	Spain	-0.04	42.94	-5.27
Trapline Rd	TR	US	Washington	1.26	48.95	-122.35
Bakerview James	BJ	US	Washington	1.02	48.79	-122.46
Johnson Creek	JC	US	Washington	1.35	48.16	-123.30
Williams Creek	WC	US	Washington	-0.01	47.24	-120.70
Isabella Lake	IL	US	Washington	3.96	47.17	-123.11
Highland	HL	US	Washington	3.55	45.91	-122.62
Appleton	AP	US	Washington	1.66	45.81	-121.28
Hood River	HR	US	Oregon	3.02	45.66	-121.59
Horton	HO	US	Oregon	4.40	44.22	-123.51
Drain	DR	US	Oregon	4.45	43.66	-123.33
Selma	SE	US	Oregon	4.95	42.28	-123.64

Table 2. Predictor estimates ± standard error for the fixed effects of the LMMs performed for growth, physiology, and reproduction traits. Farthest right column shows Marginal R^2/Conditional R^2. Bolded values are significant (* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

Trait Category	*Trait*	*Predictors*				
		Intercept	Trt [T]	Region [US]	Trt [T] * region [US]	R^2
Growth	Dry Weight	**1.3488 ±0.0082 *****	**-0.0438 ±0.0077 *****	**0.0363 ±0.0102 *****	**-0.0195 ±0.0081 ***	0.37/0.58
	Stem Width	**0.5758 ±0.0244 *****	**-0.0487 ±0.0199 ***	-0.0087 ±0.0300	-0.0199 ±0.0215	0.12/0.50
	Stem Height	**6.639 ±0.216 *****	**-1.210 ±0.225 *****	0.377 ±0.258	0.185 ±0.249	0.28/0.43
Physiology	RWC	**88.953 ±1.653 *****	2.674 ±2.246	-2.546 ±1.717	1.571 ±2.316	0.04/NA
	Stomatal Conductance	**0.658 ±0.0563 *****	-0.0481 ±0.0715	**-0.108 ±0.0348 ****	**0.127 ±0.0323 *****	0.02/0.44
	Fluorescence	**0.694 ±0.0271 *****	-0.0297 ±0.0354	**-0.0423 ±0.0158 ****	**0.0542 ±0.0165 *****	0.01/0.35

	Leaf Temperature	**1.388 ±0.00569** ***	-0.00734 ±0.00752	**0.00983 ±0.00221** ***	**-0.0126 ±0.00199** ***	0.18/0.68
Reproduction	Total Capitula	**3.631 ±0.221** ***	-0.500 ±0.269	**0.835 ±0.264** **	-0.326 ±0.308	0.18/NA
	Bolting Day	**11.481 ±2.44** ***	-2.082 ±2.415	-0.579 ±2.961	2.329 ±2.681	0.003/0.29
	Flowering Day	**36.578 ±1.92** ***	1.48 ±1.993	1.804 ±2.25	1.138 ±2.168	0.05/0.34
	Bolting to Flowering	**30.855 ±1.22** ***	-1.072 ±1.385	1.538 ±1.461	1.884 ±1.596	0.07/NA
	Estimated Seed[a]	**5.6527 ±0.2141** ***	**-0.7020 ±0.2961** *	0.3909 ±0.2500	0.0428 ±0.3427	0.14/NA
	Probability of Bolting[b]	0.2090 ±1.0206	-0.8926 ±0.6978	**3.4958 ±1.5328** *	1.2891 ±0.8919	0.27/0.79

[a] Zero inflated GLMM, [b] Binomial GLMM

Table 3. Repeated measures ANOVA performed on chlorophyll absorbance index (SPAD) measured weekly over the 8 week period of the drought treatment.

Fixed effect	*Estimate ±Std error*
Intercept	45.039 ±1.163 ***
Week	0.00496 ±0.102
Trt[T]	-3.412 ±1.180 ***
Region[US]	-0.849 ±1.439
Trt[T]:region[US]	0.387 ±1.298
Week:trt[T]	0.976 ±0.139 ***
Week:region[US]	0.0797 ±0.127
Week:trt[T]:region[US]	0.147 ±0.173

Table 4. Delta AIC scores for the Drought Ratio linear models compared to the best fitting model. A = preadaptation, B = generalist, C = post-introduction adaptation without a cline, D1/D2 = post-introduction adaptation with a cline.

Trait Type	*Trait*	*ΔAIC*				
		Pre-adaptation Clinal	Pre-adaptation Generalist	Post-Introduction Adaptation	Post-Introduction Clinal Adaptation	
		A	B	C	D1	D2
Growth	Dry Weight	3.4782	3.5905	1.3152	**0**	1.8758
	Stem Width	2.85922	3.81118	1.8542	**0**	1.8690
	Stem Height	2.7224	9.0227	3.2153	**0**	1.2802
Physiology	RWC	5.6112	**0**	1.9276	1.9907	3.6806
	Stomatal Conductance	3.00056	6.17214	**0**	2.32272	1.8579
	Fluorescence	4.47842	**0**	0.73296	1.67301	2.4793
	Leaf Temperature	2.3137	8.928	**0**	7.6097	0.4583
	SPAD	5.0986	**0**	1.9996	1.6552	3.0987
Reproduction	Total Capitula	3.07927	3.95298	**0**	1.39745	1.6901
	Bolting Day	5.045061	**0**	1.605292	1.999754	3.081286
	Flowering Day	4.4945	**0**	0.7238	1.7499	2.4947
	Bolting to Flowering	3.99332	1.77197	1.76748	**0**	1.9995
	Estimated Seed	**0**	21.10641	16.6133	9.814538	11.718
	Bolting Probability	2.94142	**0**	0.83448	1.31716	2.8339

1.5.2. Figures

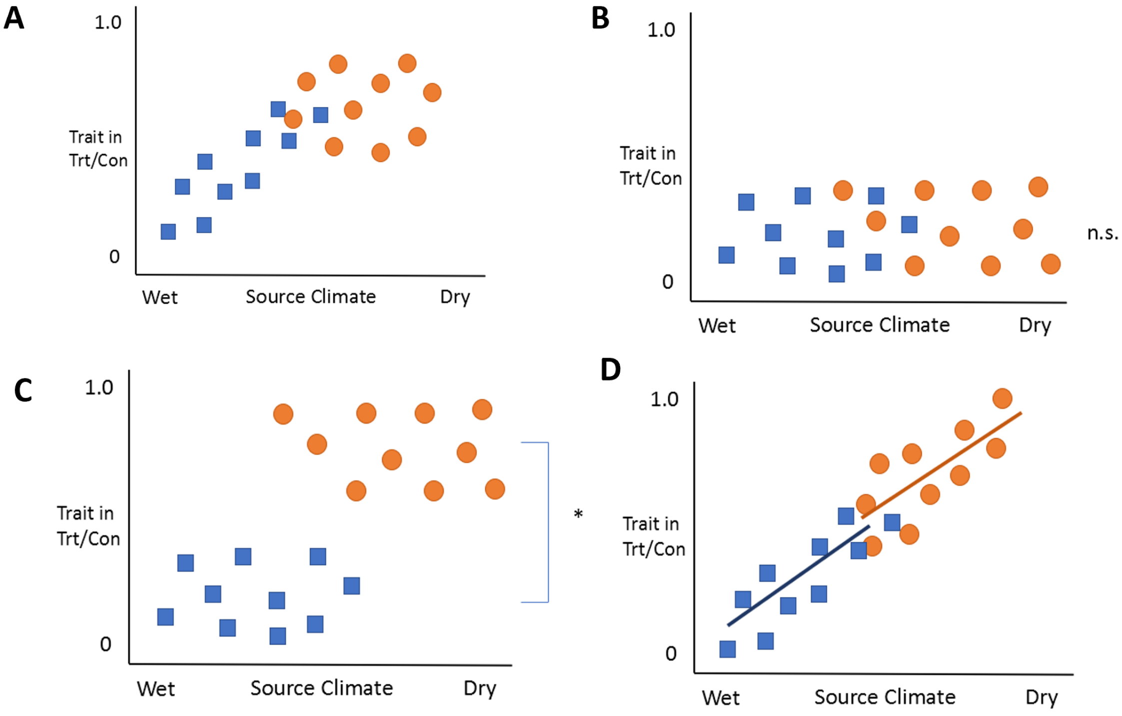

Figure 1 Predictions of relationship of Drought Ratio (population average trait value in the treatment divided by the average trait value in the control) over PC1, the variable used as a proxy for source climate in the cases of A. preadaptation with local adaptation in the native range, B. ecological generalist, C. post-introduction adaptation with no clines, and D. post-introduction adaptation with the establishment of clines for local adaptation. Orange circles represent invasive US populations and blue squares represent native EU populations.

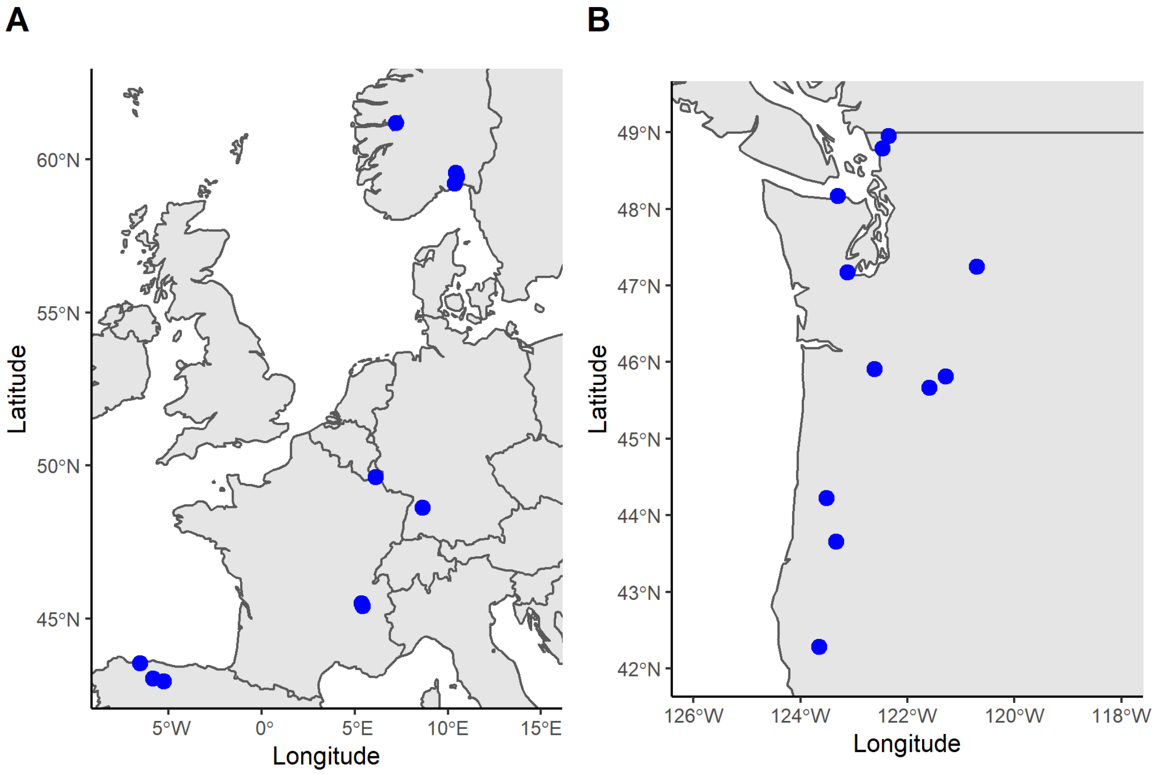

Figure 2 Map of sample populations used in greenhouse experiment in A. the native European range collected in 2017 and B. the invasive Pacific Northwest range in North America collected in 2019.

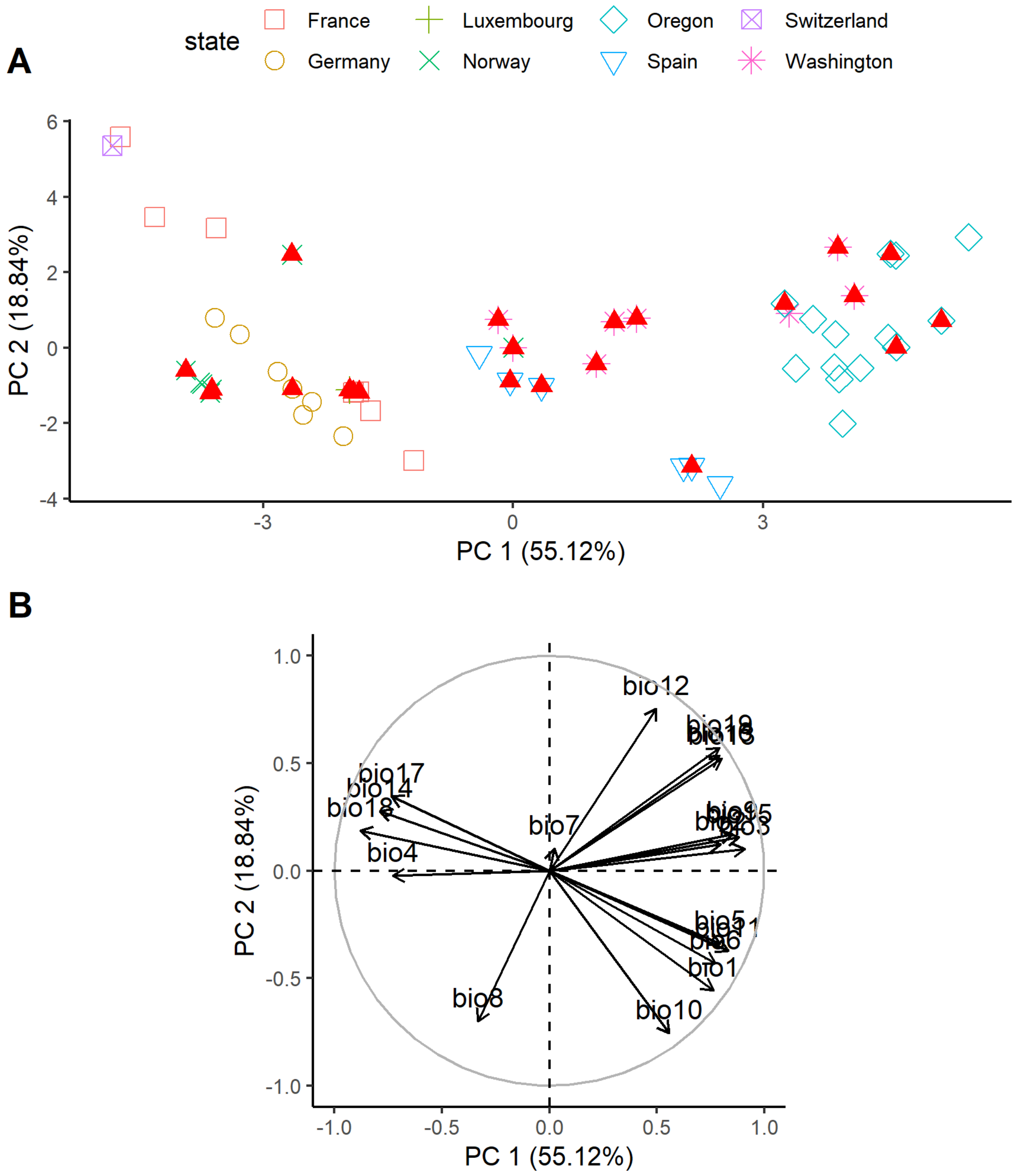

Figure 3 A. Principal components analysis of BIOCLIM variables for all collected PNW and EU populations, with colors corresponding to state or country and the percent of explained variance on the x and y axis. Populations selected for the greenhouse experiment are overlaid with a red triangle. B. Loadings for the 19 BIOCLIM variables used in principal components analysis. The Bioclim variables with the strongest variable contributions were isothermality (bio3), precipitation seasonality (coefficient of variance, bio15), precipitation of the warmest quarter (bio18), mean temperature of the warmest month (bio9), mean temperature of the coldest quarter (bio11).

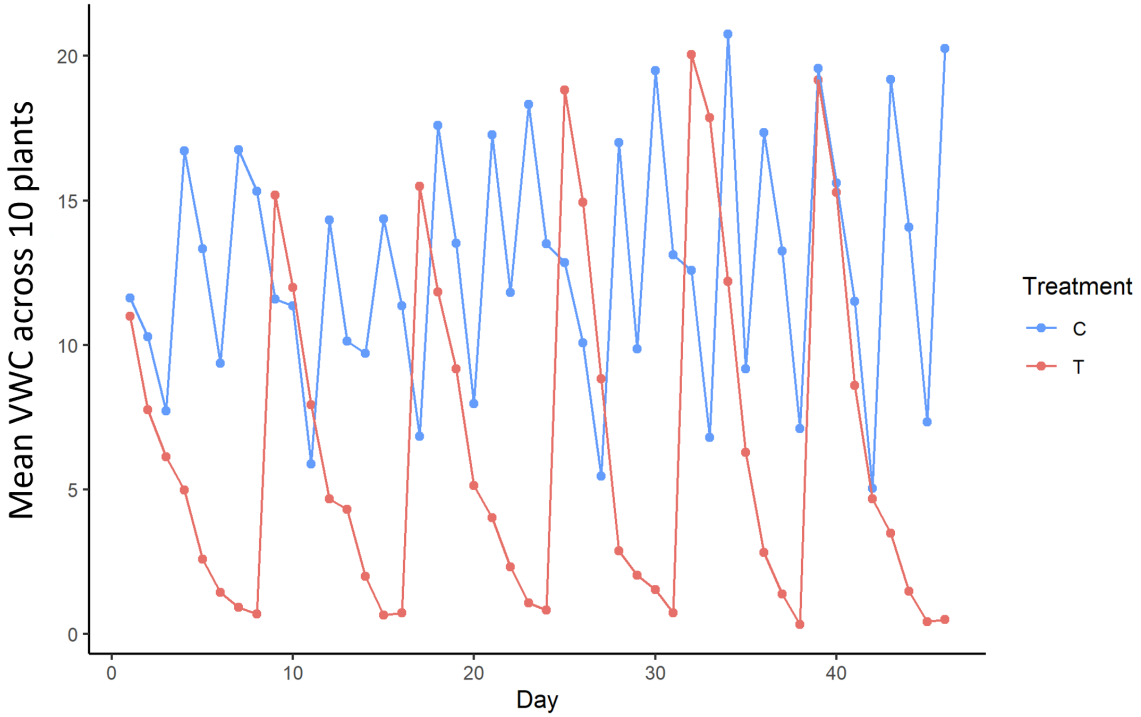

Figure 4 Mean volumetric water content (VWC) of 10 random plants per treatment group sampled over the course of the experiment.

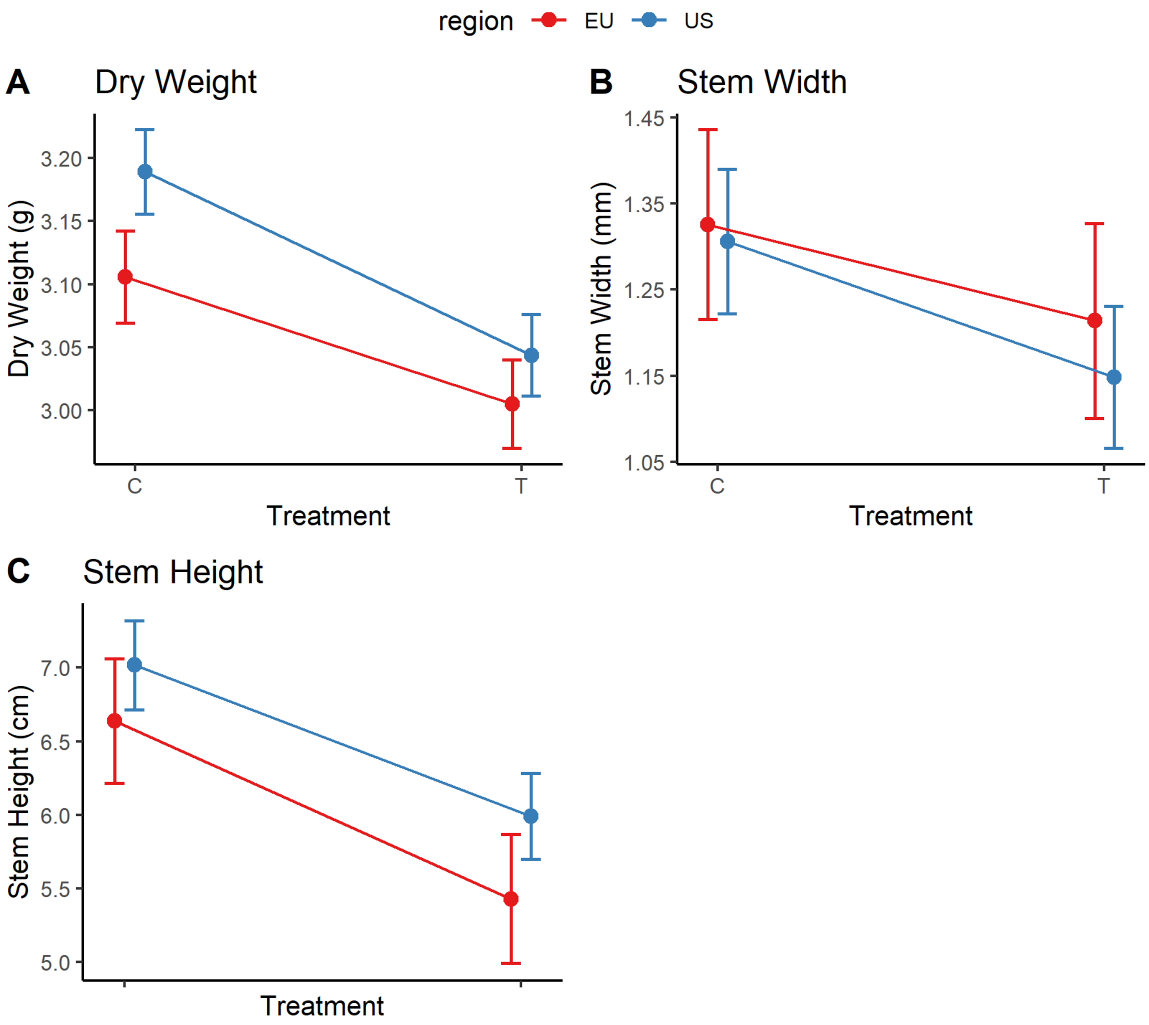

Figure 5 Growth traits LMM predicted values across region in each treatment.

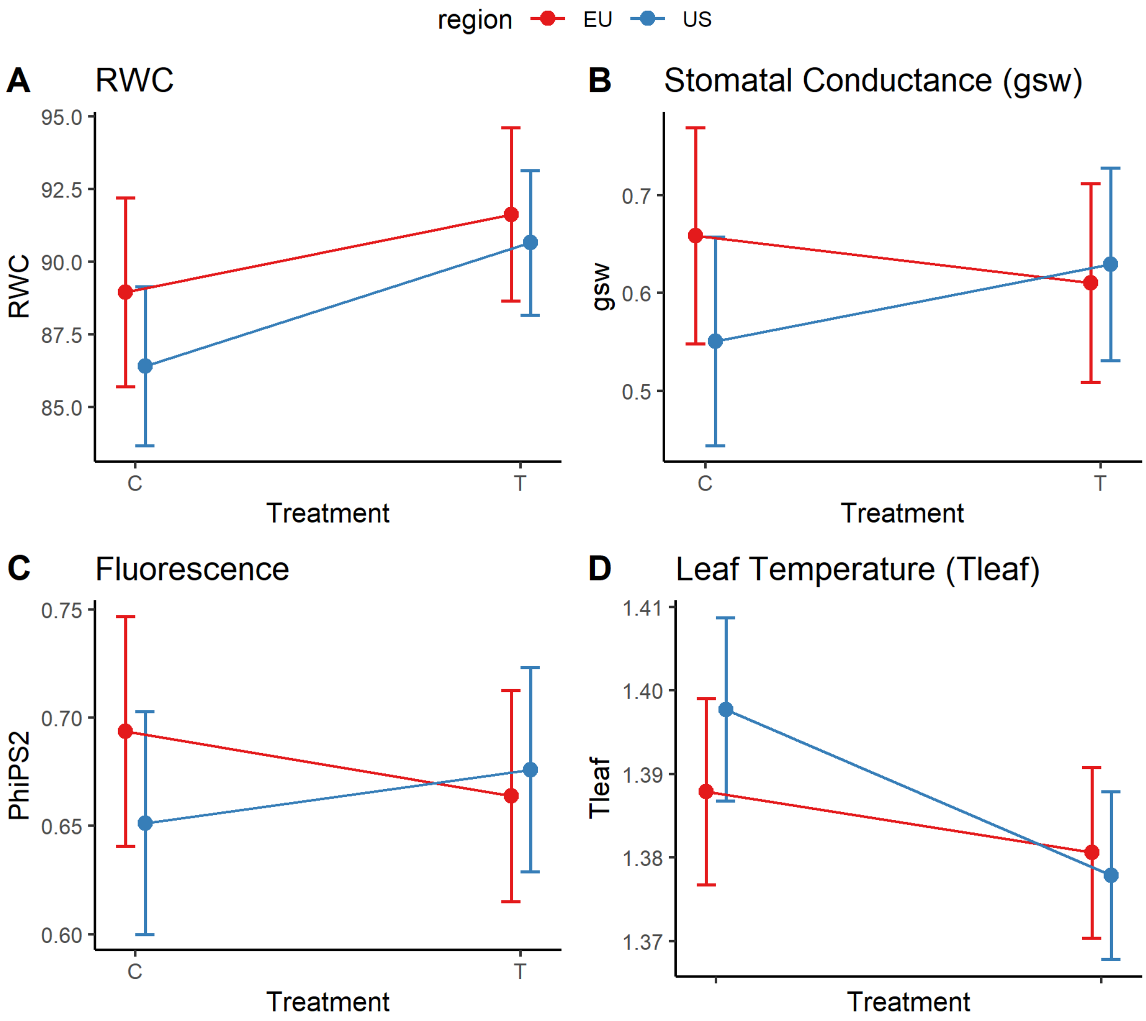

Figure 6 Physiological traits LMM predicted values across region in each treatment.

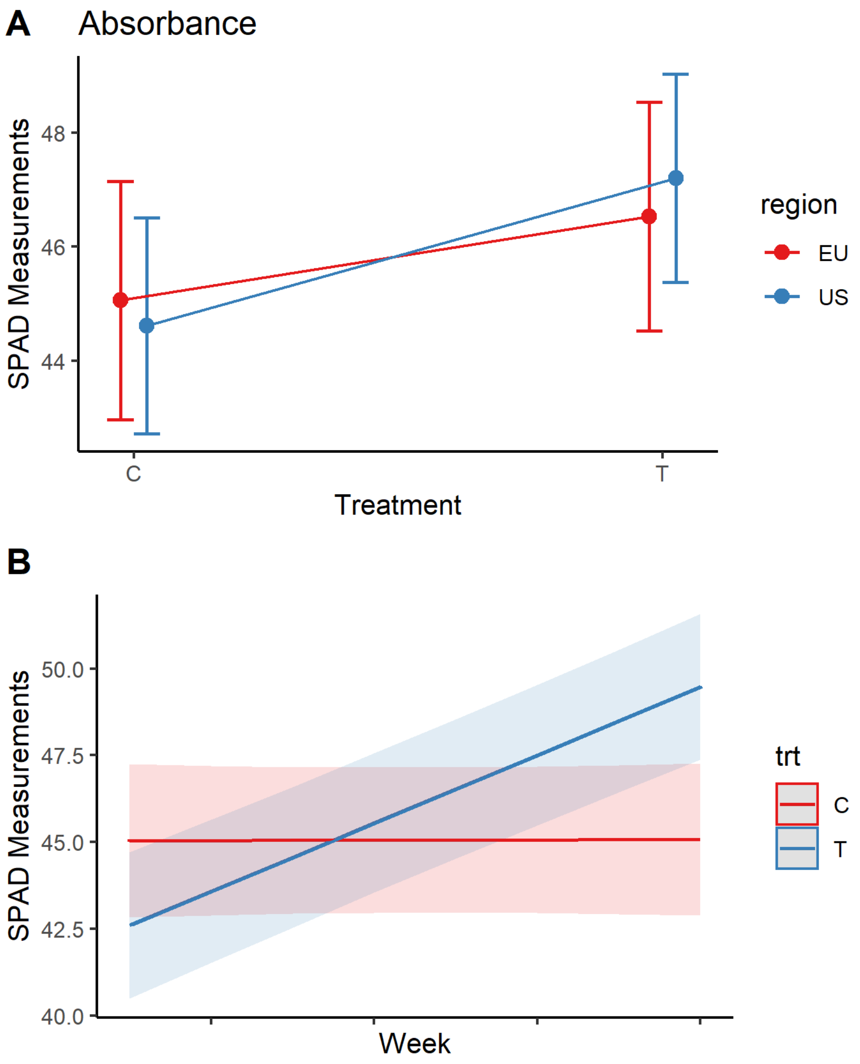

Figure 7 A. Chlorophyll absorbance (SPAD) measurement over treatment with lines for US and EU, B. SPAD measurements over week with lines for control and treatment.

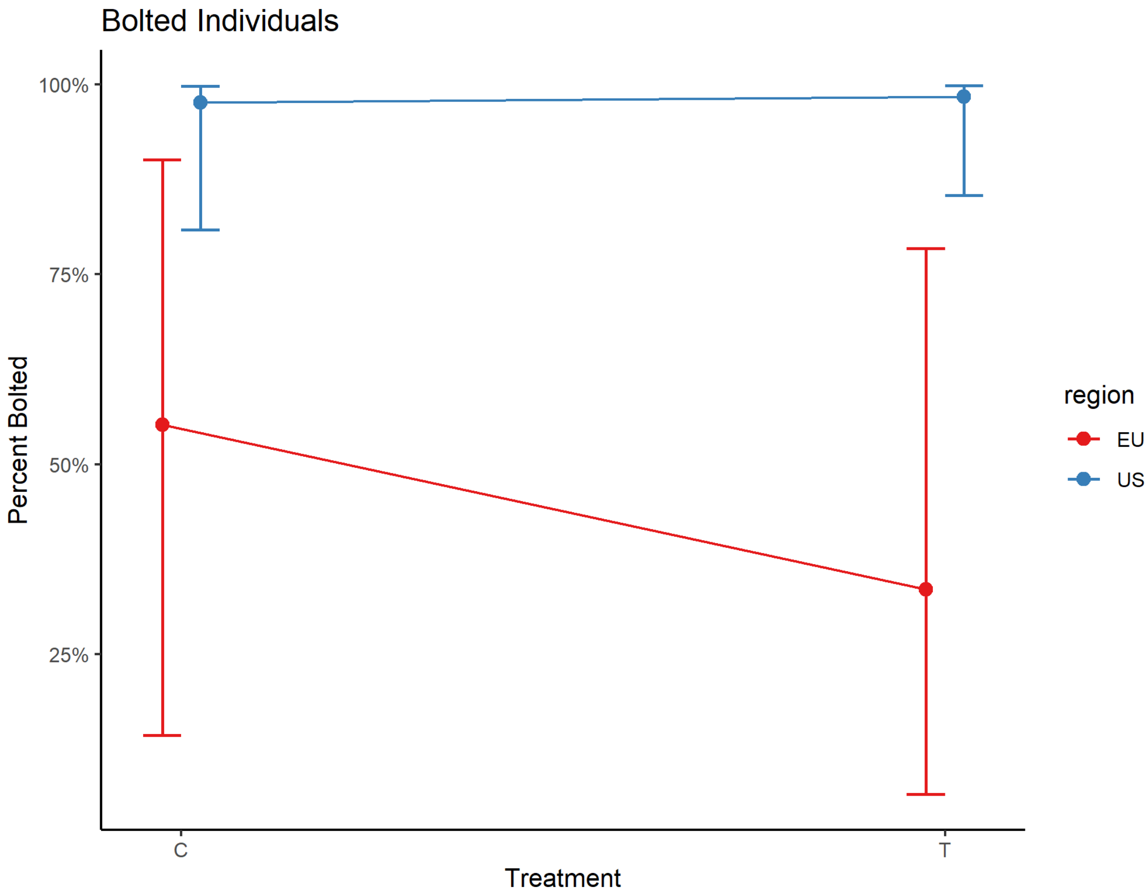

Figure 8 Binomial GLMM predicted probability of bolting vs non-bolting across region in each treatment.

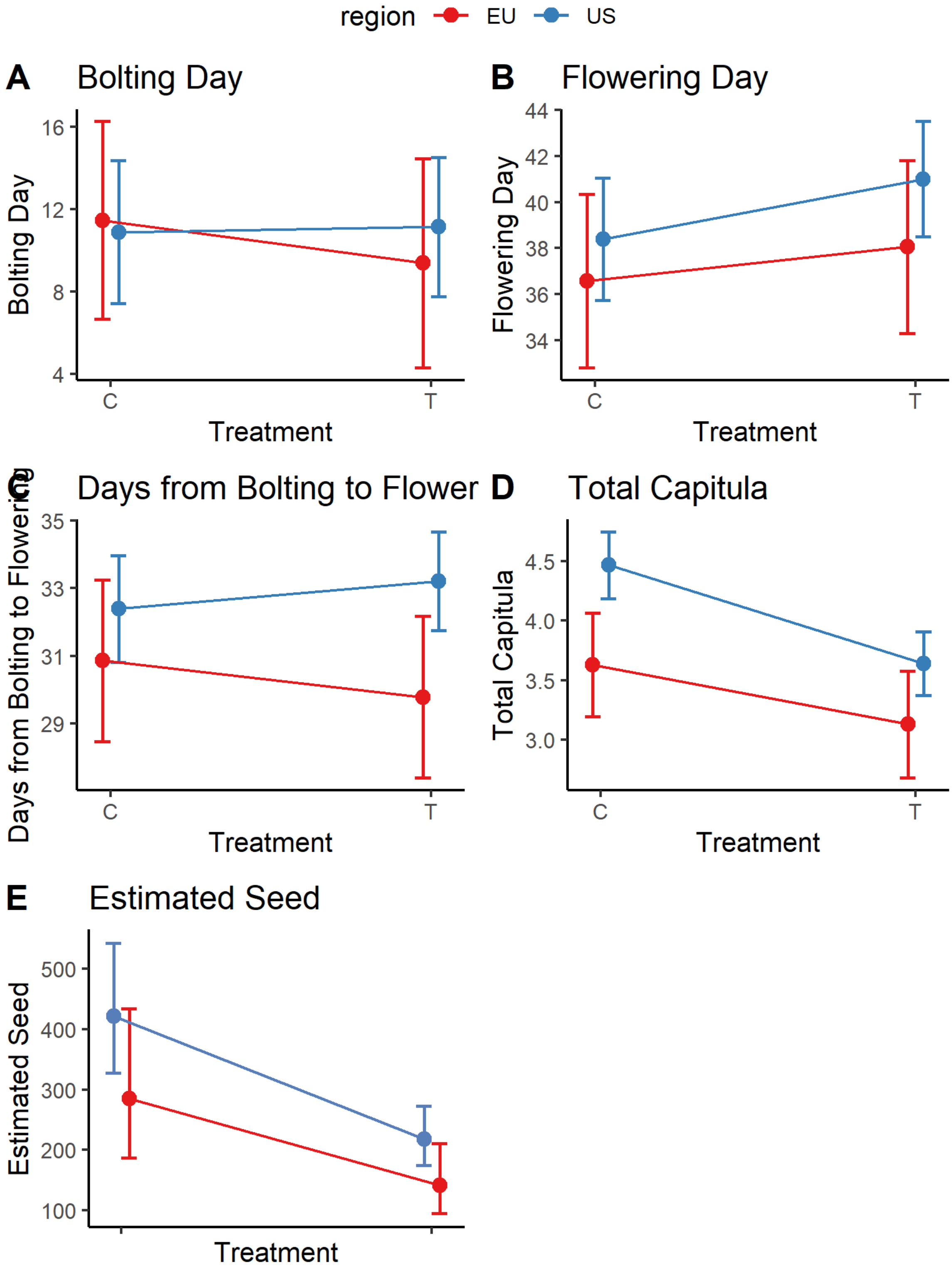

Figure 9 Reproductive traits LMM or GLMM (Estimated Seed) predicted values across region in each treatment.

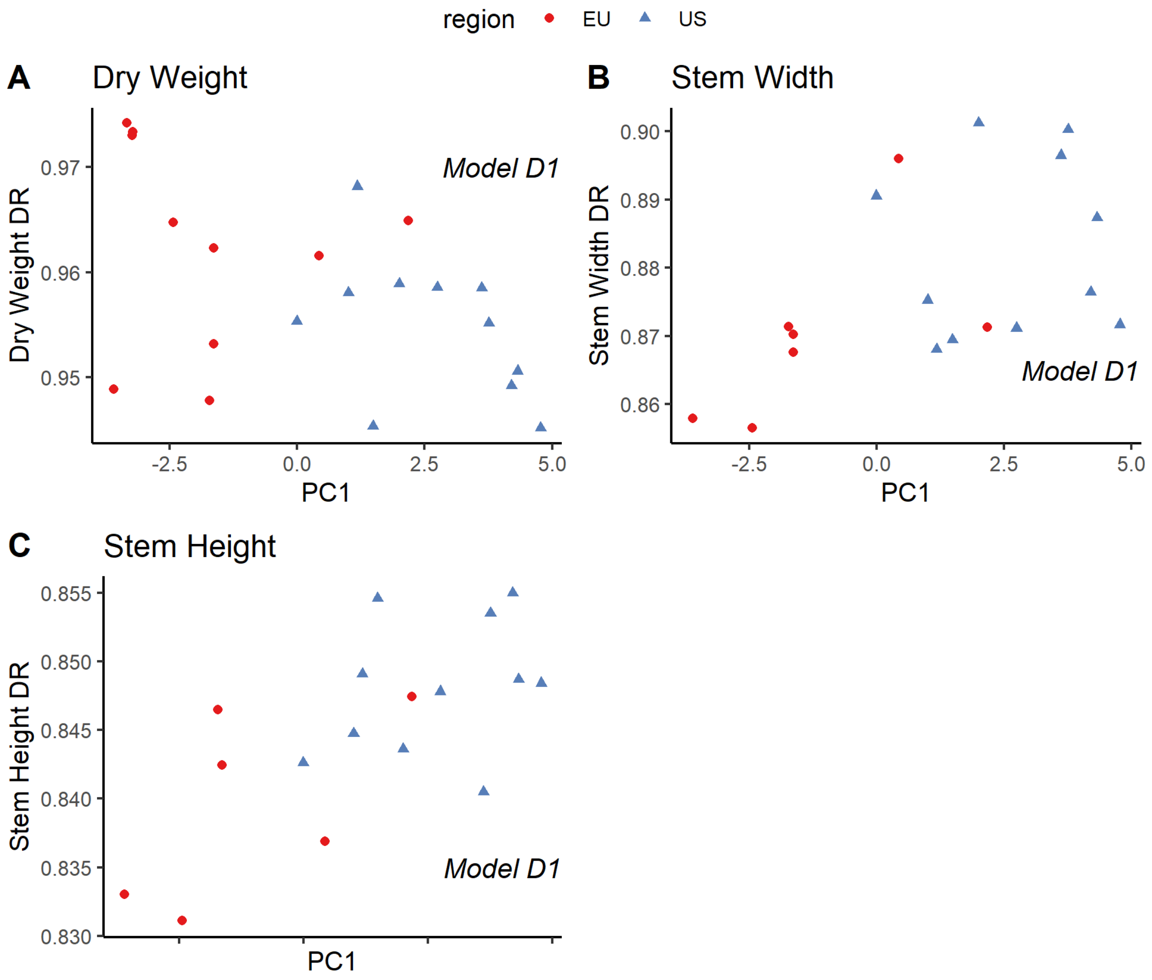

Figure 10 Drought Ratio (DR) for growth traits of EU and US populations over PC1 score. Best fitting linear model based on AIC score included on graph. A = preadaptation, B = generalist, C = post-introduction adaptation without a cline, D1/D2 = post-introduction adaptation with a cline.

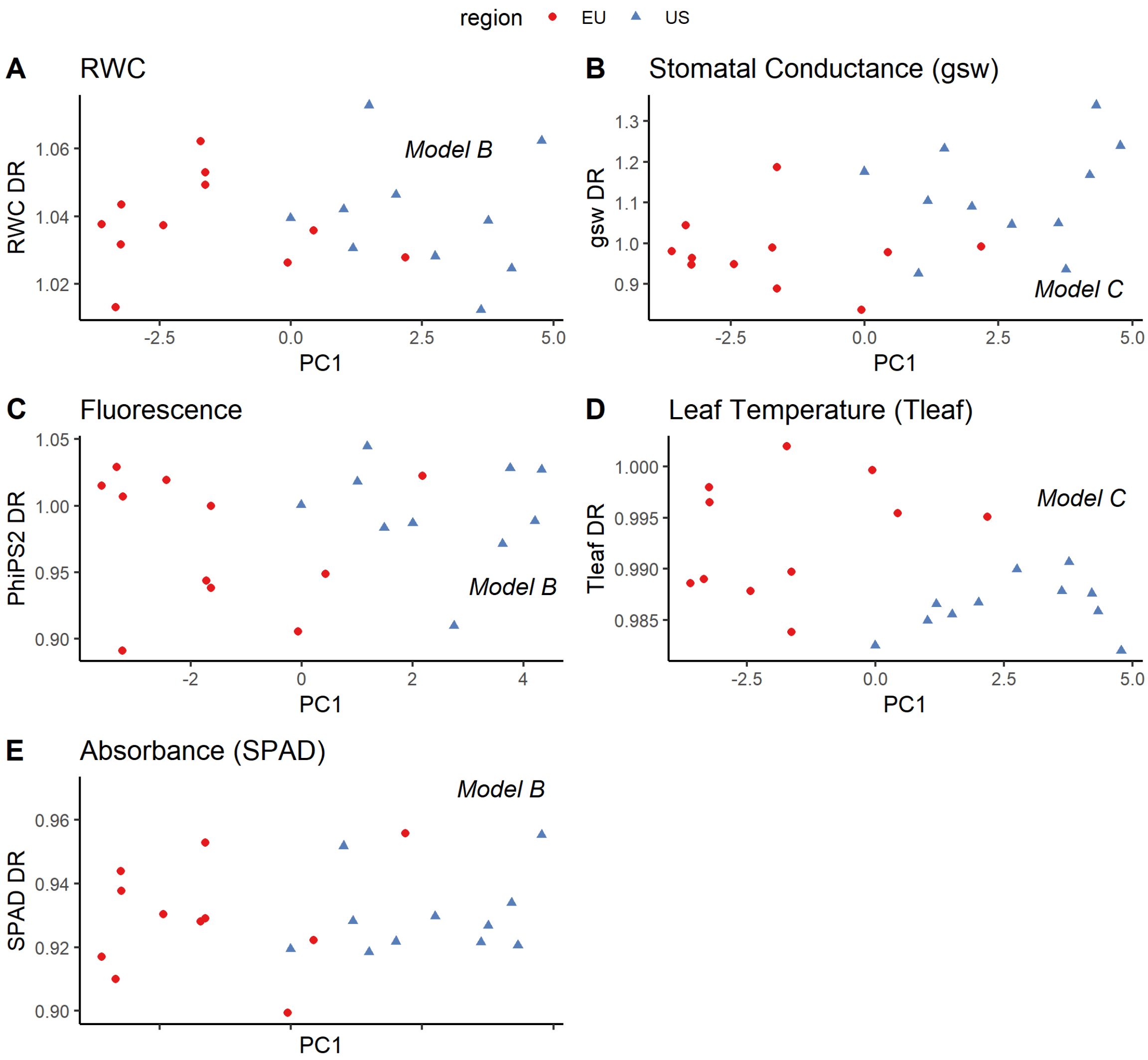

Figure 11 Drought Ratio (DR) for physiological traits of EU and US populations over PC1 score, including chlorophyll absorbance (SPAD). Best fitting linear model based on AIC score included on graph. A = preadaptation, B = generalist, C = post-introduction adaptation without a cline, D1/D2 = post-introduction adaptation with a cline.

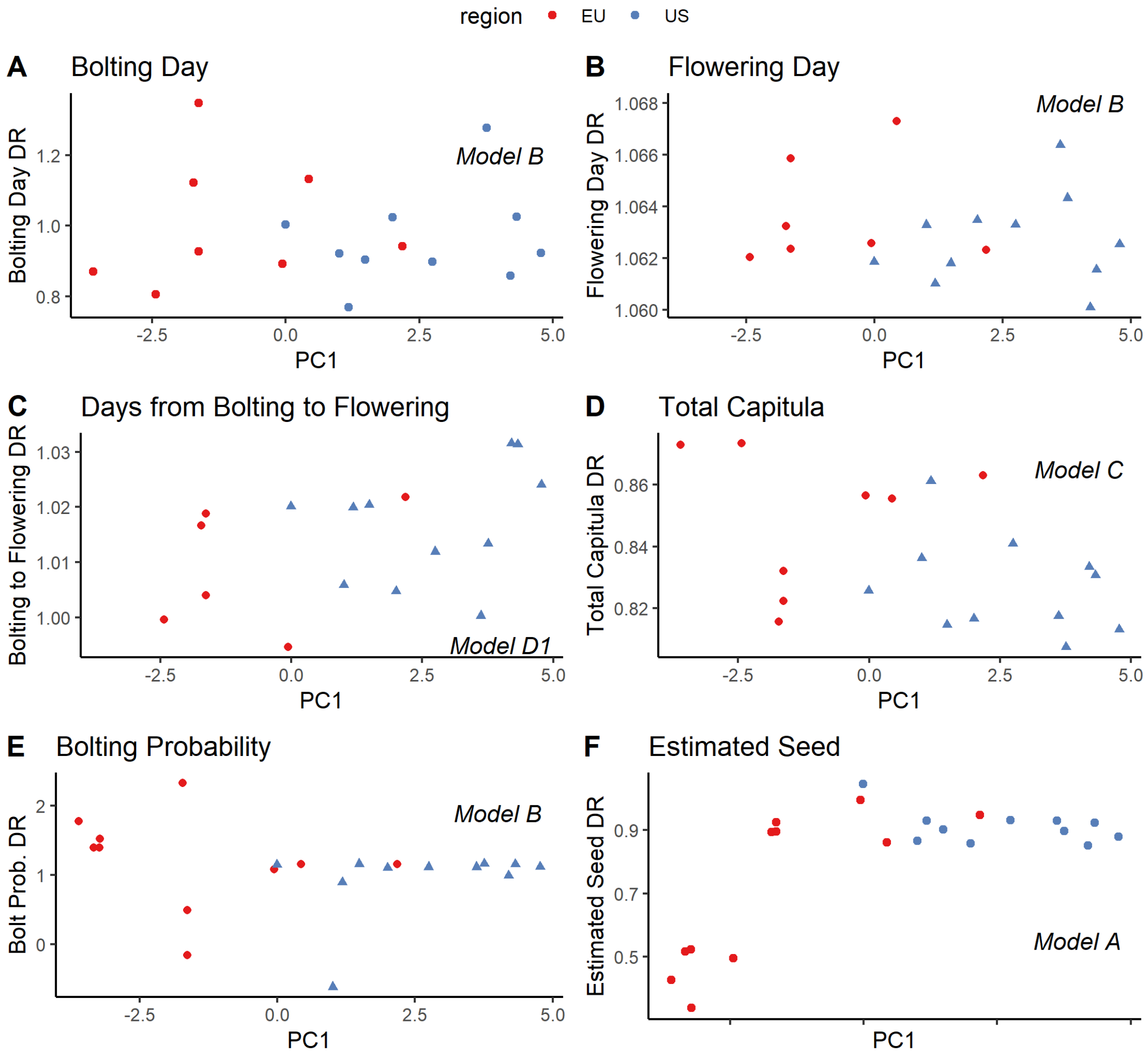

Figure 12 Drought Ratio (DR) for growth traits of EU and US populations over PC1 score, including bolting probability and estimated seed. Best fitting linear model based on AIC score included on graphs. A = preadaptation, B = generalist, C = post-introduction adaptation without a cline, D1/D2 = post-introduction adaptation with a cline.

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