

Elevated CO₂ boosts reproduction and alters selection in northern but not southern ecotypes of allergenic ragweed¹

Kristina A. Stinson^{2–4,6}, Jennifer M. Albertine^{2,3}, Tristram G. Seidler⁴, and Christine A. Rogers⁵

PREMISE OF THE STUDY: Many plants increase reproduction in response to rising levels of atmospheric CO₂. However, environmental and genetic variation across heterogeneous landscapes can lead to intraspecific differences in the partitioning of CO₂-induced carbon gains to reproductive tissue relative to growth.

METHODS: We measured the effects of rising atmospheric CO₂ on biomass allocation in the allergenic plant *Ambrosia artemisiifolia* (common ragweed) across a geographic climate gradient. We grew plants from three latitudes at 400, 600, and 800 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ and analyzed biomass allocation and natural selection on flowering phenology and growth.

KEY RESULTS: Both the latitude of origin and CO₂ treatment had significant effects on allocation and on estimates of selection. Northern plants were under stronger selection than southern plants to flower quickly, and they produced larger seeds and more reproductive mass per unit of growth. Northern plants were under stronger selection than southern plants to flower quickly, and they produced larger seeds and more reproductive mass per unit of growth. While all plants grew larger and produced heavier seeds at higher CO₂, only northern plants increased male flower production. Both size and time to flowering were under selection, with a relaxation of the size–fitness function in northern ecotypes at high CO₂.

CONCLUSIONS: Northern ecotypes allocate more CO₂-induced carbon gains to reproduction than do southern plants, pointing to a geographic gradient in future pollen and seed production by this species arising from local adaptation. Relaxed selection on size at elevated CO₂ could amplify reproductive enhancements to northern ecotypes, although more growth and seed provisioning can be expected overall. Our results demonstrate potential for ecotypic divergence in ragweed responses to climate change.

KEY WORDS *Ambrosia artemisiifolia* (Asteraceae); allergen; carbon dioxide; climate gradient; ecotype; flowering; latitude; natural selection; ragweed; reproductive allocation

Reproduction in higher plants is fundamentally linked to the photosynthetic assimilation of CO₂ from the atmosphere into biomass of flowers and fruits, which must be balanced against structural growth and resource acquisition (Reekie and Bazzaz, 1987). Although many plants increase growth and reproduction as a result of rising atmospheric CO₂ levels (Ainsworth et al., 2002; Ward and Kelly,

2004), the relative partitioning of CO₂-induced carbon gains to reproduction vs. other plant functions varies considerably within and among species (Curtis et al., 1994; Bazzaz et al., 1995; Stinson et al., 2011; van Loon et al., 2016). Increasing evidence suggests that population divergence in life history traits and phenotypic plasticity may give rise to intraspecific variation in responses to CO₂, but whether and how intraspecific trait variation affects reproductive success at the landscape scale is not well understood (e.g., Anderson et al., 2012; Leakey and Lau, 2012; Valladares et al., 2014; Moran et al., 2016).

Because reproductive allocation affects fitness, intraspecific differences in a species' response to CO₂ can also affect long-term evolutionary processes. In some cases, carbon gains from elevated CO₂ reduce trade-offs between growth and reproduction, thereby mitigating natural selection on size (Stinson et al., 2011). Estimates of

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² Department of Environmental Conservation, University of Massachusetts, Amherst, MA 01003;

³ Harvard University, Harvard Forest, Petersham, Massachusetts 01366;

⁴ Biology Department, University of Massachusetts, Amherst, Massachusetts 01003; and

⁵ Environmental Health Sciences, University of Massachusetts, Amherst, Massachusetts 01003

⁶ Author for correspondence (e-mail: kstinson@eco.umass.edu); ORCID id 0000-0003-4571-048X

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natural selection under predicted levels of CO₂ suggest that the evolutionary effects of CO₂ alone are small in comparison to ecological effects, i.e., plants are highly plastic in their responses (Lau et al., 2007; Leakey and Lau, 2012). However, effects of CO₂ on selection estimates may be more pronounced in some populations than in others for a number of reasons, including prior selection and differentiation of life history traits in contrasting environments (Lau et al., 2010; Leakey and Lau, 2012). To better predict broader patterns of plant reproduction in a changing world, we need to further quantify how plasticity, genetic variation, and natural selection differ among populations regarding the effects of elevated CO₂ on plant reproduction.

We tested for intraspecific variation across a regional climate gradient in biomass allocation, natural selection, and partitioning of CO₂-induced carbon gains to reproduction. We focused on the widespread, allergenic plant, common ragweed in the northeastern United States. As outlined further below, we hypothesized that preadaptations to flowering over a short time would disproportionately enhance allocation to reproduction in northern populations exposed to elevated CO₂ relative to southern populations that are preadapted to delay onset of reproduction in favor of structural growth. We also predicted that CO₂ enrichment would alleviate size–reproduction trade-offs and thus reduce natural selection on size overall across the gradient.

The annual monoecious plant *Ambrosia artemisiifolia* L. (Asteraceae), also known as common ragweed, produces wind-dispersed, allergenic pollen that triggers autumn hay fever reactions (pollenosis) and is linked to seasonal asthma in humans (Rogers, 2001; White and Bernstein, 2003; Ziska et al., 2003, 2008). Experimentally elevated CO₂ enhances ragweed photosynthesis, growth, and reproduction (Stinson et al., 2006; Stinson and Bazzaz, 2006) and stimulates pollen production by up to 60% (Ziska and Caulfield, 2000; Wayne et al., 2002; Rogers et al., 2006). A widespread agricultural weed and early-successional dominant, common ragweed occupies a broad range of habitats in its native range of North America as well as Europe, where it is invasive (Tamarcaz et al., 2005). Distinct ecotypes have been described at the continental scale in both the native and invaded range, with northern plants flowering in less time and at small size, and southern plants growing for a longer period and reproducing at larger size (Dickerson and Sweet, 1971; Hodgins and Rieseberg, 2011; Stinson et al., 2016). Ragweed responses to elevated CO₂ have been largely studied within single populations or otherwise limited geographic areas (Ziska and Caulfield, 2000; Wayne et al., 2002; Rogers et al., 2006; Stinson et al., 2006; Stinson and Bazzaz, 2006). However, Ziska et al. (2003, 2007) demonstrated increased growth and pollen production by plants near urban centers compared to more rural populations due to temperature and CO₂ gradients. Intraspecific variation in this species' reproductive responses to CO₂ enrichment remains a critical knowledge gap for understanding future ecological roles and societal impacts.

Our study area encompassed a 40–44° latitudinal gradient in the northeastern United States, where ragweed is a ubiquitous weed and has the potential to affect many people in diverse settings, but for which CO₂ effects have not been extensively documented. We conducted a common garden experiment with plants from the northern, middle, and southern latitudes across the northeastern USA to determine whether and how CO₂ and latitude affect (1) phenotypic variation and plasticity patterns in growth and reproductive traits, (2) allometric relationships between biomasses of

growth structures (roots and shoots) and reproductive components (male inflorescences and seeds), and (3) natural selection on size and flowering time.

Northern ecotypes in this region are known to flower in less time and experience earlier frost events and to disproportionately accelerate flowering in response to experimentally elevated CO₂ compared to larger, southern ecotypes with longer time to flowering (Stinson et al., 2016). We thus predicted stronger selection on time to flowering and greater reproductive allocation with increasing latitude as is commonly reported for ecotypes adapted to cooler, shorter growing seasons (Dickerson and Sweet, 1971; Grime, 1977; Reekie and Bazzaz, 1987; Bolmgren and Cowan, 2008). We also predicted that under common conditions, northern plants would flower in less time and thus allocate disproportionately more CO₂-induced biomass gains to reproduction, while southern plants would delay reproduction and thus respond to high CO₂ with increased overall growth. Alternatively, strong population differentiation could occur with no or low plasticity. We tested among these scenarios by comparing reaction norms and modeling the effects of CO₂, latitude, and their interactions on the traits in question.

The effects of CO₂ on sex allocation are less-well documented, but it is generally accepted that female structures are more costly and time-consuming to produce and that higher carbon availability is likely to favor male function over seed provisioning if nutrient conditions are similar (Obeso, 2002; Paquin and Aarssen, 2004; Darbah et al., 2008). We predicted that elevated CO₂ treatments on ragweed plants grown in ideal soil conditions would shift sex ratios in favor of male function, as demonstrated in other wind-pollinated species (Darbah et al., 2008).

MATERIALS AND METHODS

Species information and seed collection—*Ambrosia artemisiifolia* L. (Asteraceae) is a wind-pollinated, monoecious, annual weed native to North America (Allard, 1943; Bassett and Crompton, 1975), that typically forms dense stands in disturbed habitats, roadsides, forest edges, croplands, and old fields (Allard, 1943; Bazzaz, 1970, 1974). Male inflorescences develop from the apical meristem as branching ensues, begin to bud in the summer, and produce pollen-bearing male flowers throughout the reproductive stage of the life cycle. Female flowers typically develop in axillary positions along the stem later in the season, and fruits ripen toward the end of the season. We collected wild *A. artemisiifolia* seeds from three latitudes in the northeastern United States. This regional gradient corresponds to mean temperature declines by ~4°C and a shortening of the frost-free period by up to ~60 d with increasing latitude and similar precipitation across the region (Ollinger et al., 1995; Daly, 2002; PRISM Climate Group, 2014). We used satellite images and land cover data layers (MassGIS) to identify potential ragweed habitat based on our understanding of its presence in nonforested habitats with recent soil disturbance (Allard, 1943; Bazzaz, 1970). We selected eight distinct source populations located within a ~40-km radius of each of three urban centers along the gradient (New York, New York [40.71°N, –74.00°W]; Boston, Massachusetts [42.35°N, –71.06°W]; and Burlington, Vermont [44.48°N, –73.21°W]). Each of the 24 source populations contained at least 50 plants and were a minimum of 2 km from one another. Approximate locations of seed collection points are shown in Appendix S1 (see the Supplemental Data with this article).

We bulk-collected ripe seeds from 15 to 20 randomly selected mature plants in each of the 24 source populations. Seeds from each source population were placed into large, separately labeled paper bags indicating the location point and the latitude of origin. The seeds were taken back to the laboratory, cleaned of all debris, and stored over winter at 4°C for 4 months in a laboratory refrigerator at University of Massachusetts, Amherst, Massachusetts. We stratified the seeds from each population in separate trays of quartz sand for 16 weeks with 14-h day length, 6°C days and 4°C nights, according to previously established methods (Pickett and Baskin, 1973).

CO₂ treatments—We constructed 12 outdoor growth chambers from lumber framing and 6-inch diameter PVC hoops covered with 6 mil polyethylene film (AT Plastics, Milwaukee, Wisconsin). Each chamber was 3.5 m wide × 6 m long. The chambers were spaced 10 m apart in all directions within a large, open field at the experimental garden enclosure at the Harvard Forest, Petersham, Massachusetts. Four chambers each were randomly selected to be maintained at one of three experimental CO₂ levels: 400 μL·L⁻¹ (ambient), 600 μL·L⁻¹ (intermediate), and 800 μL·L⁻¹ (high). CO₂ was supplied via injection from a liquid tank located on site, for the duration of the experiment. Set points were maintained within each chamber using a computer-controlled infrared gas analyzer and solenoid valve system (LI-800, LiCor, Lincoln, Nebraska, USA), which sampled air concentrations at a frequency of 5 min and supplied CO₂ levels to within ±50 μL·L⁻¹ of the set point. Air temperature was maintained within 1.5°C of ambient using thermocouple-controlled electric fans located at the roof gables of each chamber.

Experimental design and measurements—In early June, we sowed 10 seeds from each population into each of 288 replicate 4-L plastic pots, containing Sunshine Mix #1 (Sun Gro Horticulture, Bellevue, Washington, USA). We randomized the pots within each of 12 CO₂ fumigation chambers, in the following experimental design: 3 CO₂ levels × 3 latitudes × 8 populations × 4 replicates per population × 4 chambers per CO₂ level = 1152 plants, or 96 pots per chamber (32 pots per latitude per chamber).

The pots were monitored to determine germination rates. After 10 d, plants were thinned to a single, experimental seedling (the individual closest to the center of the pot), and an average germination date was obtained for each pot. The soil mixture was amended in late June with slow-release NPK 14:14:14 (Osmocote; Scott's, Marysville, Ohio, USA) to standardize nutrient conditions to an ideal growth range for this species. Pots were watered daily to saturation with an automated drip irrigation system to maintain even moisture levels across all treatments. The date of flowering onset (flower date) and number of days in active flowering (flower duration) was recorded each week for each plant. Plants were destructively harvested in early September when plants were still actively shedding pollen but fruits were mature and basal leaves had begun to show signs of senescence. Biomass was separated into roots, shoots, seeds, and male inflorescences, with this last highly correlated with pollen production (Ziska and Caulfield, 2000). All tissue from every organ from each plant was dried (70°C for 48 h), weighed on a laboratory balance, and recorded to the nearest 0.10 g. We also determined maternal plant allocation to individual seeds on a subset of the experiment ($N = 288$) by weighing three sets of 10 seeds per plant, to obtain average mass per seed. We assigned random numbers to each pot within each treatment and latitude, and selected the 32 individuals with the highest randomly assigned numbers for analysis.

Statistical analyses—We constructed a restricted maximum likelihood (REML) linear mixed model to test the main effects of CO₂ level (400, 600, 800), latitude (NY, MA, or VT), and the CO₂ × latitude interaction on plant traits (organ biomasses) in JMP 12.0 (SAS Institute, Cary, North Carolina, USA). Population was included as a random factor, and the trait values for each population were pooled by chamber (Gotelli and Ellison, 2004). We considered CO₂ effects as “E”, or evidence for plasticity in response to CO₂, and latitude effects as evidence for conserved trait expression by populations from northern, middle, and southern parts of the study region (i.e., ecotypic variation, or “G” in the broadest sense). We plotted norms of reaction for biomass traits from each latitude as a function of CO₂ level, and interpreted model effects of latitude × CO₂ as evidence for genetic variation in plasticity patterns among ecotypes in the response to experimental CO₂ levels (Clausen et al., 1948; Lande and Arnold, 1983). We performed linear regressions to evaluate relationships between plant organs (i.e., seed, male flower, shoot, and root biomasses). We performed a multiple regression on the number of days from germination to flowering (“flower date”) and the number of days of flowering (“flower duration”) on organ biomasses to determine whether variation in timing and duration of active flowering affected allocation to growth and reproduction.

We analyzed the fit and tested for heterogeneity of slopes in the resulting linear relationships using a mixed model approach in which CO₂ and latitude were specified as fixed main effects. In this model, the continuous values for reproductive traits were specified as the responses, and traits significantly correlated with reproductive traits (shoot biomass and flower date) were specified as the covariates.

To measure the strength and direction of natural selection on plant size and flowering time, we performed phenotypic selection analyses (Lande and Arnold, 1983) by regressing relative fitness against standardized phenotypic values of traits that were correlated with reproduction. Relative fitness was calculated as the individual's total reproductive biomass divided by the population mean reproductive biomass for all combinations of latitude and CO₂. Mean values of each predictor trait were subtracted from the mean trait value and divided by the standard deviation for the trait. We calculated the univariate selection differential, S , as the slope of the fitness function derived from each standardized trait, and the multivariate selection gradients, β , as the partial regression coefficients from multiple regression of all predictor traits against relative fitness. To test whether selection regimes varied across latitude and CO₂ level, we used a REML mixed model specifying latitude and CO₂ treatment as fixed effects and population as a random factor. Standardized traits were considered as covariates, and relative fitness was specified as the response variable.

RESULTS

Norms of reaction to elevated CO₂—The reaction norms for biomass demonstrated clear differences in phenotypes among latitudes, as well as plasticity in the production of shoots, roots, and male flowers with elevated CO₂ (Fig. 1A–D, Table 1). While all plants grew larger and produced more seed mass at higher CO₂, only northern plants produced more male flowers under enriched conditions. There were strong latitudinal effects on several plant traits, as well as plastic responses to CO₂ (Table 1). There was a clear CO₂ × latitude interaction effect on male inflorescence mass, with

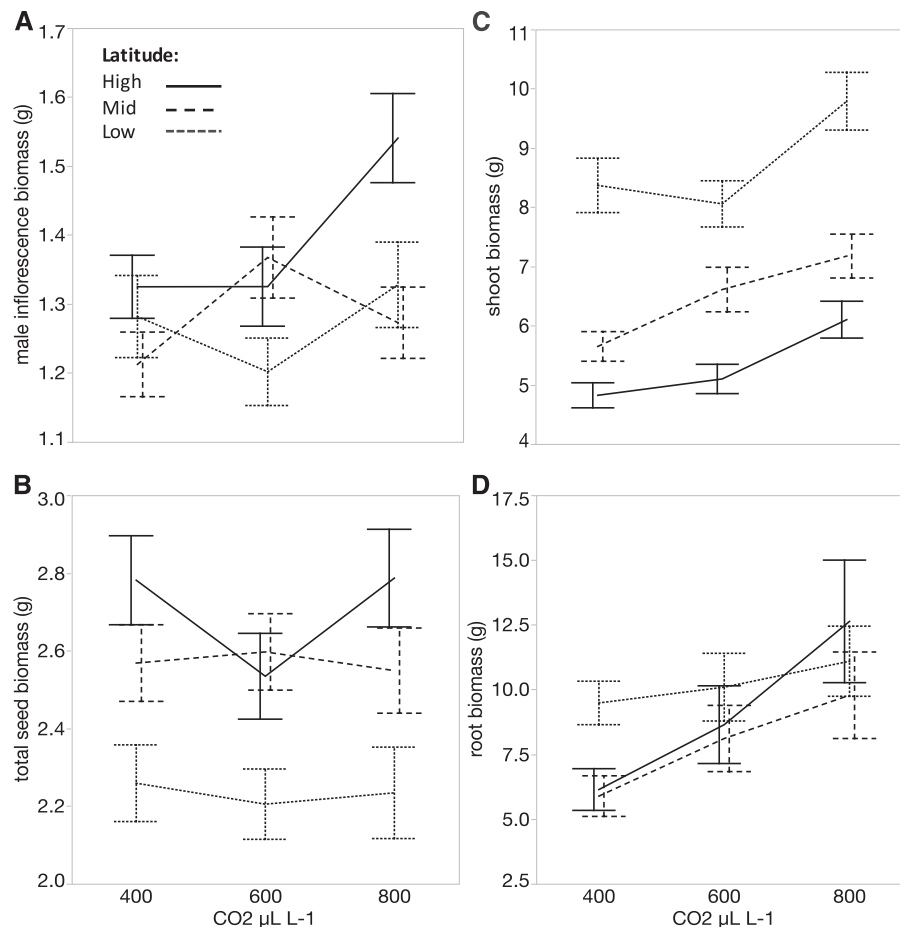


FIGURE 1 Reaction norms for mean dry biomass (g) of (A) male flowers, (B) seeds, (C) shoots, and (D) roots. Line styles represent trait means for plants from low, intermediate and high latitude of origin, as expressed when grown at 400, 600, and 800 ppm CO_2 . Bars represent ± 1 standard error of the mean.

northern plants demonstrating disproportionately higher male flower production at high CO_2 than plants from the other latitudes (Fig. 1A). Plants from northern latitudes also produced the most seed mass (the mass of all seeds combined per plant; Fig. 1B), whereas shoot mass was up to 5 g greater in plants from the southern vs. intermediate and northern latitudes (Fig. 1C). Root mass was stimulated by elevated CO_2 consistently in all plants and did not differ by latitude of origin (Fig. 1D). Plants from the most southern latitude produced significantly lighter seeds (~ 0.004 g/seed) than those from the mid and northern latitudes (~ 0.005 g/seed), and seed mass increased slightly in the 600 and 800 $\mu\text{L L}^{-1}$ CO_2 treatments compared to ambient (Table 1). Mean number of days to flowering (“flower date”) and number of days in active flower (“flower duration”) were reported by Stinson et al. (2016): as noted therein, northern plants had more rapid onset of flowering than southern plants and accelerated onset of reproduction at elevated CO_2 more than southern ecotypes when grown in common experimental environments.

Effects of CO_2 and latitude on allometry and sex allocation—Plants from higher latitudes allocated more biomass to reproduction per unit of above ground growth than those from lower latitudes. While shoot mass was positively correlated with root, male flower, and seed

mass in all cases (Table 2A), plants sourced from higher latitudes had steeper shoot to male flower mass and shoot to seed mass ratios (Fig. 2, Table 2A: latitude $\times$ shoot interactions, $P < 0.0001$). A significant dampening effect of CO_2 on these relationships was also detected (Table 2A: $\text{CO}_2 \times$ shoot interactions). The relationship between root mass and shoot mass was positive, but did not differ by latitude or treatment. There was a positive relationship between female and male mass production, with sex ratios more female-biased in the northern and middle latitude populations and becoming slightly more male-biased with increasing seed size in plants from southern populations as CO_2 levels increased (Fig. 3, Table 2B).

Phenotypic selection on size and timing of reproduction

Two of the standardized predictor traits, shoot mass and flower date were significantly correlated with relative fitness. We thus calculated univariate selection differentials (S) for each predictor trait separately, as a measure of total selection, and multivariate selection gradients (β) with a model including both of these traits as a measure of direct selection controlling for fitness effects of the other trait (Table 3A, B). Natural selection favored larger and more rapidly flowering plants overall, demonstrating trade-offs between size and timing of reproduction (Tables 3, 4, Fig. 4). Univariate selection differentials for shoot mass were significantly positive across all latitudes especially at ambient CO_2 in northern plants, with significant effects of latitude and CO_2 on the fitness

functions (Table 3A, latitude $\times$ shoot and $\text{CO}_2 \times$ shoot interactions, Table 4A). Univariate selection differentials for onset of flowering were significantly negative across the experiment (Fig. 4). Examination of individual selection differentials revealed the pattern was strongest in high latitude plants, but the relationship deteriorated at increasing CO_2 levels and was not significant in the southernmost populations (Table 3A). However, there were no effects of latitude or CO_2 on selection differentials for onset of flowering in the univariate model (Table 4A). Multivariate selection gradients, β , for shoot mass were also positive, indicating strong direct selection on plant size in all cases. For flowering time, β values from all latitudes were negative at ambient CO_2 , but with elevated CO_2 levels, β for shoot size was only negative in high latitude plants (Tables 3B, 4B: shoot $\times$ latitude interaction).

DISCUSSION

A growing literature now recognizes that plant responses to global change factors are context-dependent, due in part to divergent selective pressures across a range of habitats that can affect fixed phenotypic traits as well as plasticity patterns (Poorter, 1993; Long et al., 2004; Anderson et al., 2012; Wang et al., 2015; Moran et al., 2016).

TABLE 1. Results of restricted maximum likelihood linear mixed model (REML) testing for effects of explanatory variables CO₂ level (400, 600, 800), latitude (NY, MA, or VT), and the CO₂ × latitude interaction on the response variables, plant organ masses (g), with *P* value threshold of 0.05. Significant effects are in bold text.

Effect	df	Male flowers		Seeds		Flowers + seeds		Shoots		Roots		Individual seed	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
CO ₂	2	4.08	0.02	0.52	0.6	0.68	0.51	18.2	<0.01	6.05	<0.01	18.20	<0.01
Latitude	2	1.25	0.31	4.24	0.03	3.09	0.07	7.37	<0.01	1.69	0.21	31.71	<0.01
CO ₂ × latitude	4	3.85	<0.01	0.52	0.72	1.37	0.26	1.56	0.2	0.98	0.43	0.86	0.49

Whether and how this translates to predicted patterns of plant reproduction across the landscape has not been fully explored. Further, the evolutionary consequences of population-level differences in reproductive allocation at elevated CO₂ are not well understood and may vary across species and environmental conditions (Ward and Kelly, 2004; Fridley and Grime, 2010; Clark et al., 2011; Anderson et al., 2012; Bocedi et al., 2013; Valladares et al., 2014; Moran et al., 2016). Such data are important for predicting future patterns of plant reproduction across heterogeneous landscapes in the wild. Here we explored how local adaptations across a regional climate gradient contribute to geographic variation in the reproductive output of an allergenic annual plant, common ragweed.

Intraspecific variation in CO₂ response and evidence for ecotypes—

We found several lines of evidence for intraspecific variation in ragweed's reproductive allocation and responses to elevated CO₂. First, latitude was a clear predictor of phenotype for many of the

measured traits, consistent with classical interpretations of ecotypic variation in this and other species (e.g., Clausen et al., 1948; Dickerson and Sweet, 1971). As is typical of plants adapted to shorter/cooler growing seasons, in our study the northern plants with a shorter time to flowering were smaller overall but allocated more biomass to reproduction relative to growth. In contrast, southern plants delayed reproduction and maximized growth, as is typical in longer growing seasons and exposure to more biotic stress (Grime, 1977; Reekie and Bazzaz, 1987; Stinson, 2004; Bolmgren and Cowan, 2008). In addition, while the plasticity patterns in plants from all latitudes showed similar increases in shoot and overall reproductive biomass with increasing CO₂ levels, the divergent reaction norms for male reproduction and the CO₂ × latitude interactive effect on male flower mass demonstrate ecotypic variation in CO₂ responsiveness of male reproduction. Disproportionate increases in male function at elevated CO₂ among plants is also consistent with the prediction that the rapid-flowering northern populations will allocate more CO₂-driven photosynthetic gains to reproduction, and with prior work showing that northern ragweed ecotypes from the study area flower earlier and longer and rank consistently higher in male flower production at elevated CO₂ (Stinson et al., 2016).

Mechanisms for variable responses to elevated CO₂ include differences in the allocation of photosynthetic gains to reproduction (Bocedi et al., 2013; Wang et al., 2015) and cell and molecular level stimulation (Long et al., 2004; Ainsworth and Rogers, 2007; Li et al., 2008; Leakey et al., 2009) from the CO₂ enrichment. Because male flowers are produced at the onset of branching in ragweed (Ackerly and Jasienski, 1990), it is possible that flowering in a short time established an ontogenetic trajectory that favored greater allocation to reproduction in our northern ecotypes (Diggle, 1995; Obeso, 2002; Diggle and Miller, 2013), i.e., northern plants flowered sooner within the life cycle and thus allocated relatively more CO₂-induced biomass gains to male reproduction, compared to the southern plants, which delayed reproduction in favor of growth. Consistent with this idea, northern plants from these populations have also been shown to rank consistently higher in male function and branching over time at elevated CO₂ (Ackerly and Jasienski, 1990; Stinson et al., 2016). In other species, population-level divergence in phenotypic responses to elevated CO₂ include distinct growth responses to CO₂-enrichment in *Acer rubrum* from different provenances (Mohan et al., 2004), and ecotypic differences in the provisioning of carbon gains from CO₂ to different plant functions in *Arabidopsis thaliana* (Ward and Kelly, 2004; Li et al., 2008), *Impatiens cylindrica* (Runion et al., 2016), and *Raphanus raphanistrum* (Curtis et al., 1994). Further, Stinson et al. (2011) found that elevated CO₂ reversed the dominance hierarchies of small and large genotypes of ragweed by increasing relative reproduction and relaxing selection on size in smaller, shaded individuals. The current study is the first, to our knowledge, to document ecotypic variation

TABLE 2A. Results of restricted maximum likelihood linear mixed model (REML) testing for effects of explanatory variables CO₂ level (400, 600, 800 ppm), latitude (high, intermediate, and low), shoot mass (g), and all interaction terms on the mass (g) of male flowers, seeds, and roots. Shoot mass is specified as a continuous covariate. Threshold for *P* value was set to <0.05; significant values are in bold.

Effect	df	Male flower mass		Seed mass		Root mass	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
CO ₂	2	1.24	0.29	6.17	<0.001	0.73	0.48
Latitude	2	14.83	<0.001	35.52	<0.001	2.98	0.066
Shoot biomass	1	979.56	<0.001	380.91	<0.001	46.40	<0.001
CO ₂ × latitude	4	2.57	0.04	1.60	0.18	0.43	0.79
CO ₂ × shoot	2	3.03	0.05	3.45	0.03	0.34	0.71
Latitude × shoot	4	19.87	<0.001	17.12	<0.001	0.12	0.89
CO ₂ × latitude × shoot	2	0.60	0.67	0.30	0.88	0.26	0.90

TABLE 2B. Results of restricted maximum likelihood linear mixed model (REML) testing for effects of explanatory variables CO₂ level (400, 600, 800 ppm), latitude (high, intermediate, and low), and female function (seed mass [g]) on male flower mass. CO₂ × latitude × seed interaction terms tests for differences in the slope of linear relationship of male mass per unit of seed mass. Threshold for *P* value was set to <0.05; significant values are in bold.

Effect	df	<i>F</i>	<i>P</i>
CO ₂	2	3.23	0.04
Latitude	2	1.25	0.32
Seed biomass	1	240.21	<0.0001
CO ₂ × latitude	4	3.30	0.01
CO ₂ × seed	2	3.78	0.02
Latitude × seed	4	7.21	<0.001
CO ₂ × latitude × seed	2	1.66	0.16

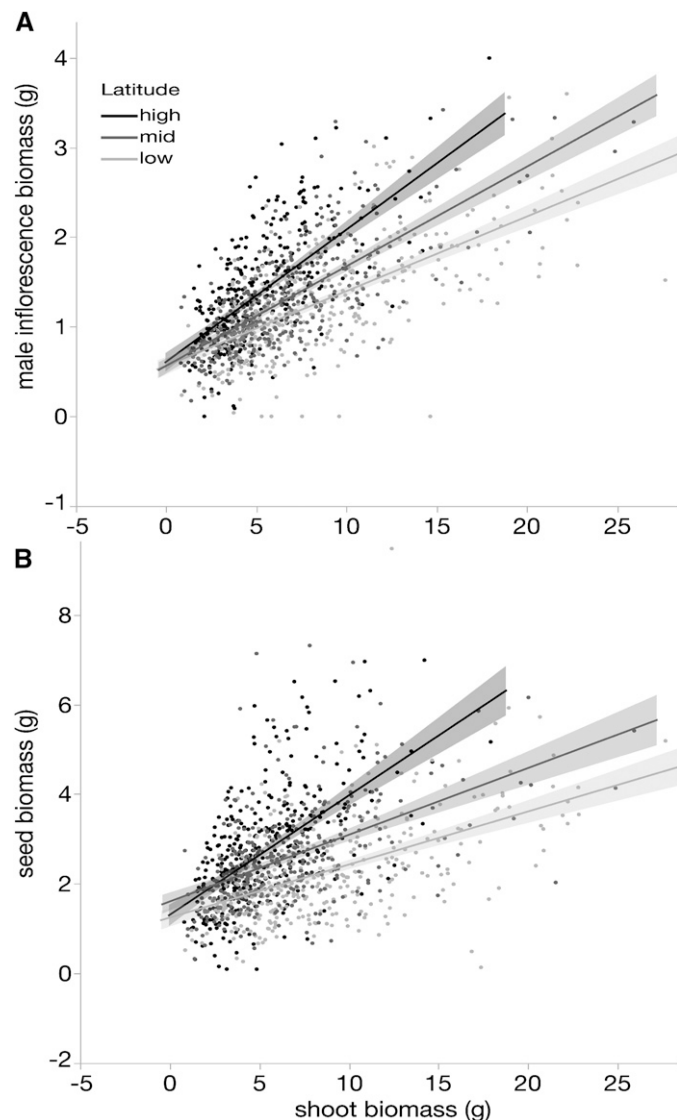


FIGURE 2 Allometric relationships between shoot biomass and reproductive biomass in plants from different latitudes. (A) Male flower biomass as a function of shoot mass. (B) Seed biomass as a function of shoot mass. Shaded area represents 99% confidence interval for regression line.

in the reproductive output of ragweed from different latitudes at elevated CO_2 .

Effects of CO_2 and latitude on seed provisioning and sex allocation—The effect of CO_2 on sex ratios varies widely across species (Ainsworth et al., 2002; Darbah et al., 2008; Wang et al., 2015) and because maternal provisioning has implications for both evolutionary trajectories and demographic processes (e.g., Donohue, 2009), additional data on the general effects of CO_2 on sex ratios are needed. Although we did not see an effect of CO_2 on total seed mass per plant, several lines of evidence suggest that both CO_2 and latitude are as important for seed provisioning as they are for pollen-producing structures in common ragweed. First, increased individual seed mass (i.e., mass per individual seed) with elevated CO_2 is consistent with earlier observations of plasticity in seed provisioning in this species, and the possibility for higher germination

rates in the future (Ziska and Caulfield, 2000; Wayne et al., 2002; Rogers et al., 2006; Stinson and Bazzaz, 2006; Stinson et al., 2006). Higher maternal provisioning to seed in the northern populations further reflects the general trend to greater reproductive allocation at higher latitudes, possibly in response to selection for more rapid germination in a shorter growing season (Donohue, 2009). Second, significant CO_2 and $\text{CO}_2 \times \text{latitude}$ effects on the allometric relationships between size and seed output suggest that the structural reproductive cost of seed production is mitigated by enhanced shoot mass, again to a greater extent in northern populations. The bias toward seed production with increasing latitude also supports the “size advantage” and “time limitation” hypotheses for sex allocation in northern plants, i.e., that seeds are more costly to produce than pollen-bearing male flowers and thus are a disproportionate component of resource budgets with a shorter growing season (Paquin and Aarssen, 2004). We also note that soil nutrient status could affect our findings and that we did not measure C to N ratios of plant tissues in this experiment. While our study held soil nitrogen levels at a constant, it would be informative to further test the interactive effects of nitrogen and CO_2 on seed provisioning, carbon balance of tissues, and biomass allocation in general. Since seed provisioning also affects germination success, further research on how CO_2 and other environmental factors affect sex ratios in this and other species would provide insights into future scenarios regarding plant demography and population structure.

Divergent patterns of natural selection—Our selection estimates provide further evolutionary context for the observed divergence in plasticity patterns and allocation in ragweed and are among the first available comparative estimates of evolutionary responses to CO_2 in plants from different latitudes. In general, we show that positive selection on size is counterbalanced by negative selection on flowering time, in keeping with our prediction that maximizing size at reproduction involves a fitness trade-off to flowering earlier overall. These findings are consistent with other studies in which selection on size and flowering time remain strong under experimental CO_2 conditions (Lau et al., 2007). Although we did not find evidence for a latitude effect on flowering time–fitness functions, the fact that selection estimates themselves were negative only in northern plants is notable. At the same time, stronger selection on size in northern latitudes does indicate a higher fitness cost to smaller plants, which in light of significantly smaller shoot size may outweigh the importance of flowering quickly for northern plants.

The differential effects of CO_2 on selection for size, though small, further raise the possibility for geographic variation in future evolutionary trajectories. Specifically, the latitude and CO_2 effects on the size–fitness function appear to be driven by opposing effects on directional selection, becoming relaxed by CO_2 in northern plants, but changing little or becoming slightly exacerbated with decreasing latitude and/or increasing levels of CO_2 . While CO_2 -induced photosynthetic gains appear to offset the cost of smaller size in higher latitudes, increased size–fitness functions may reflect overall increases in biomass in the southern populations as well. It is not surprising that the patterns were subtle, as CO_2 effects on selection are typically small (Leakey and Lau, 2012). Of the few studies testing for changes in phenotypic selection under high CO_2 , Steinger et al. (2007) and Bazzaz et al. (1995) reported CO_2 -induced changes to patterns of natural selection on size, whereas Lau et al. (2007) found no variation in selection or heritability despite large changes in phenotype. As suggested in a recent review, it

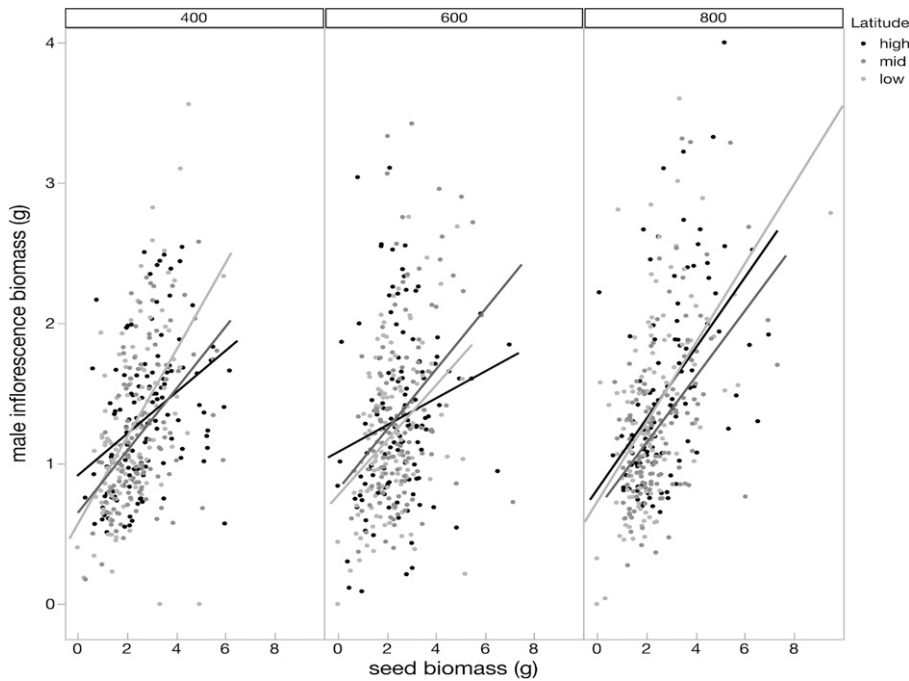


FIGURE 3 Male flower biomass as a function of seed mass (sex ratio) for plants from low (light gray line), intermediate (dark gray line), and high (black line) latitude, shown separately for each experimental concentration of CO₂. Shaded area represents 99% confidence interval for regression line.

is possible that CO₂ effects on fitness functions are exacerbated under stress (Leakey and Lau, 2012), such as increased levels of herbivory (Lau et al., 2010). The current study indicates a need for manipulative experiments that test the interactive effects of CO₂ with other abiotic and biotic factors, e.g., cooler temperature at higher latitudes, competition, and herbivory at lower latitudes.

Whether the latitudinal differences in CO₂ responses we observed are due to underlying adaptive genetic differentiation is not clear, as epigenetic and maternal effects may also play a role in both trait variation and selection estimates. For instance, as is the case in most latitudinal studies, maternal ragweed plants from the southern part of the current study may have been exposed to slightly higher ambient CO₂ conditions due to their proximity to a larger city, as well as higher levels of overall competition, drought stress, and/or herbivory than those from the northern part of the study, all of which could affect seed provisioning and offspring phenotype. At the highest latitudes, plant growth including that of ragweed is

typically much lower (Li et al., 2015), but is increasing even more rapidly as a result of climate change (Myneni et al., 1997). Characterizing the molecular diversity within and among latitudes could also be highly informative, to differentiate cytoplasmic from nuclear genetic effects, and to measure the effects of maternal environment (Roach and Wulff, 1987; Donohue, 2009). Further analyses are needed, including experiments in which maternal effects are homogenized by a refreshing generation to more fully assess potential for adaptation to current and future climate conditions in this and other species (Curtis et al., 1994; Volk and Körner, 2001; Ward and Kelly, 2004; Valladares et al., 2014).

Implications for future ragweed presence and pollen production

Humans are breathing increasing numbers of irritants every year, and cases of asthma and allergic rhinitis are on the rise (Ziska et al., 2008). A major culprit is airborne allergens from pollen produced by widespread plants such as common ragweed (Taramarcas et al., 2005; Ziska et al., 2008). Our study highlights landscape-level variation in the growth and reproduction of ragweed in New England with rising

CO₂ conditions, a finding not previously examined at a regional scale nor understood at a mechanistic level. Our data suggest that plants from northern latitudes are likely to represent ecotypes pre-adapted to allocate resource gains from elevated CO₂ to male flower production, while southern plants are under less selection pressure to achieve reproductive size quickly. As discussed above, this idea is supported both in the current study and elsewhere where ragweed demonstrates ecotypic variation in flowering time (e.g., Dickerson and Sweet, 1971; Stinson et al., 2016). While these findings must be interpreted in context with other environmental factors, our data point to possible disproportionate shifts toward reproduction, especially pollen-bearing male flowers, in northern ragweed ecotypes. Since male flower mass is highly correlated with pollen production (Ziska and Caulfield, 2000), exposure to allergenic pollen, at least in the short term, may increase in the northern part of the region, while ragweed vigor and increased seed dispersal could be hypothesized to increase in the middle and southern parts of the region.

TABLE 3. (A) Univariate selection differentials (*S*) and (B) multivariate partial regression gradients (*β*) for plants originating at high, intermediate, and low latitudes grown at experimental 400, 600, and 800 ppm CO₂ levels. Univariate or multivariate regression coefficients are reported; positive or negative values indicate direction of the relationship between the trait and relative fitness. NS indicates no relationship.

CO ₂ treatment	High latitude		Mid latitude		Low latitude		All latitudes	
	Flower date	Shoot mass	Flower date	Shoot mass	Flower date	Shoot mass	Flower date	Shoot mass
A) Univariate selection differentials								
400	−0.44	0.75	−0.55	0.55	NS	0.6	−0.5	0.63
600	−0.2	0.44	NS	0.68	NS	0.56	−0.2	0.56
800	−0.22	0.59	NS	0.69	NS	0.64	−0.22	0.64
B) Multivariate partial regression gradients								
400	−0.5	0.77	−0.45	0.45	−0.35	0.65	−0.43	0.62
600	−0.5	0.56	NS	0.67	NS	0.57	−0.5	0.61
800	−0.3	0.61	NS	0.69	NS	0.68	−0.3	0.66

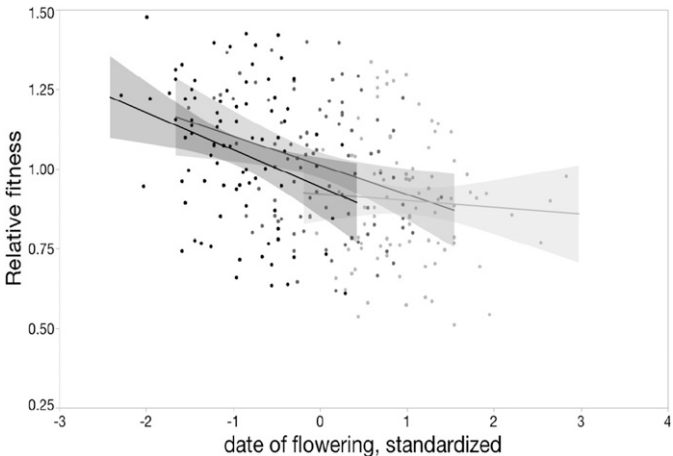


FIGURE 4 Univariate fitness functions for relative fitness as predicted by standardized timing of flowering within each latitude (no effects of CO₂ on fitness functions for flowering time). Shaded area represents 99% confidence interval for regression line.

Many of the predicted environmental changes for the northeastern United States are associated with increased performance of ragweed, such as historical warming in the pollen record (Faison et al., 2006), heat island and CO₂ effects near cities (Ziska et al., 2003), and ongoing soil disturbance and landscape fragmentation

TABLE 4. Effects of treatment and latitude on (A) univariate selection differentials (*S*) and (B) multivariate partial regression gradients (*β*). Results of restricted maximum likelihood linear mixed model (REML) for the univariate relationship between explanatory variables number of days to flowering (flower date) or shoot mass on relative fitness, as determined by CO₂ level (400, 600, 800 ppm) and latitude of origin. Threshold is set to *P* < 0.01; significant values are in bold.

Effect	df	F	P
A) Univariate selection differentials			
CO ₂	2	0.05	0.95
Latitude	2	0.84	0.44
CO ₂ × latitude	4	0.26	0.90
Flower date	1	11.39	<0.001
Flower date × CO ₂	2	0.70	0.50
Flower date × latitude	2	0.98	0.38
Flower date × CO ₂ × latitude	4	0.73	0.57
CO ₂	2	4.17	0.05
Latitude	2	25.81	<0.0001
CO ₂ × latitude	4	2.75	0.03
Shoot	1	160.41	<0.0001
Shoot × CO ₂	2	3.88	0.02
Shoot × latitude	2	3.28	0.04
Shoot × CO ₂ × latitude	4	1.13	0.34
B) Multivariate partial regression gradients			
CO ₂	2	3.44	0.04
Latitude	2	5.82	0.01
CO ₂ × latitude	4	1.53	0.20
Flower date	1	11.50	<0.0001
Flower date × CO ₂	2	1.78	0.17
Flower date × latitude	2	1.62	0.20
Flower date × CO ₂ × latitude	4	0.68	0.60
Shoot	1	149.95	<0.0001
Shoot × CO ₂	2	1.78	0.17
Shoot × latitude	2	3.07	0.05
Shoot × CO ₂ × latitude	4	1.03	0.39

(Bazzaz, 1974; Ziska et al., 2007). Variation in temperature, soil types, water, and nutrients can affect plant biomass allocation, production of pollen and seeds, and trajectories of natural selection (Körner, 2006; Ainsworth and Rogers, 2007; Reich et al., 2014). As such, environmental factors other than CO₂ might offset, or synergistically amplify, geographic variation in response to the CO₂ treatments in this study. This work thus advances our understanding of future reproductive potential in an annual, widespread species, and we emphasize the need for similar research in other plant species and functional types. Our study exemplifies the need for documenting landscape-level variation in both plant species and the environment when predicting impacts of environmental change on ecological processes and, in some cases, on society.

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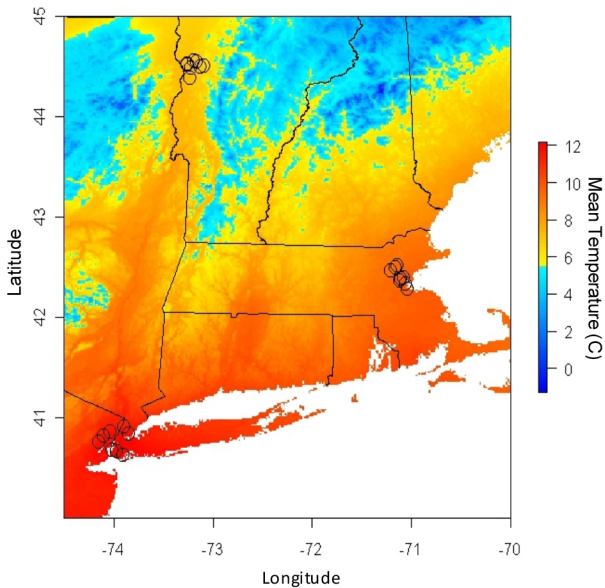
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Appendix S1. Approximate locations of the twenty-four *Ambrosia artemisiifolia* study populations used in the study. Populations were identified during a landscape survey across the climate gradient in Northeastern USA. Each open circle represents a study population. There were eight populations in each of the three climate zones centered around the cities of New York, NY, Boston, MA, and Burlington, VT.