

Regional variation in timing, duration, and production of flowers by allergenic ragweed

K. A. Stinson  · J. A. Wheeler · S. Record · J. L. Jennings

Received: 15 March 2018 / Accepted: 12 July 2018
© Springer Nature B.V. 2018

Abstract Plant reproduction has broad implications for ecology and society (e.g., production of allergenic pollen), and a number of processes that affect flowering are affected by climate. In this study, we tested for variation in traits related to pollen production by the allergenic plant, common ragweed, across a climate gradient in Massachusetts. We tested whether traits that are easily-measured in the field could be used to predict differences in spike length, a known proxy for allergenic pollen production, and the timing

and duration of flowering. We also tested whether flowering time and allometric estimates of spike length varied across the climate gradient, to better understand climate effects on future pollen production. We found that height predicts inflorescence length, but the slope of the relationship between the two traits is steeper in cooler climates, suggesting ragweed growing in cool climates produces more pollen per unit of vegetative height than ragweed in warm climates. Cool climates were also associated with larger and higher numbers of flowers and earlier and longer periods of flowering. Thus, we provide improved estimates of local pollen exposure by establishing variation in timing and output of flowering across a regional climate gradient, and at a spatial scale that may be useful for developing management strategies for allergenic plants.

Communicated by Devan Allen McGranahan.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11258-018-0860-0>) contains supplementary material, which is available to authorized users.

K. A. Stinson (✉)
Department of Environmental Conservation, University of
Massachusetts, Amherst, MA 01003, USA
e-mail: kstinson@eco.umass.edu

J. A. Wheeler
Agriculture and Agri-Food Canada, St. John's Research
and Development Centre, 308 Brookfield Rd, St. John's,
NL A1E 0B2, Canada

S. Record
Department of Biology, Bryn Mawr College, Bryn Mawr,
PA 19010, USA

J. L. Jennings
Department of Health Sciences, University of Wisconsin,
Madison, WI 53705, USA

Keywords Ragweed · *Ambrosia artemisiifolia* ·
Climate · Flowering · Pollen

Introduction

Wild populations of plant species are known to express different traits across environmental gradients at many different spatial scales, underscoring the importance of documenting intraspecific variation in response to ongoing climate change (e.g., Clausen

et al. 1940; Lau et al. 2010; Valladares et al. 2014). Climate-driven variation in flowering patterns can affect mating success, dispersal, gene flow, and species interactions (Fitter and Fitter 2002; Bolmgren and Cowan 2008; Stinson et al. 2011)—and thus can influence current and future geographic patterns of plant distribution and reproductive output (Wang et al. 2015). Further, growth-related traits such as height and number of branches tend to vary with climate, which can affect total plant reproduction (Reekie and Bazzaz 1987; Ackerly and Jasieński 1990; Nakahara et al. 2018). While flowering times are known to vary over strong climate gradients such as latitude or altitude, detailed measurements of natural flowering variation across more subtle climate gradients are less readily available, even for problematic species such as weedy and allergenic plants (e.g., Katz and Carey 2014; Zhang et al. 2015a; Stinson et al. 2016). Data that capture regional variation in reproductive timing, duration, and flower production are thus needed to improve predictive models of pollen from allergenic plants (Fumanal et al. 2007; Beggs 2014; Katz and Carey 2014).

Common ragweed (*Ambrosia artemisiifolia* L., Asteraceae), or “ragweed,” is native to North America and is a widely distributed, monoecious annual weed in the daisy family. Its pollen is the leading cause of hay fever and a major cause of childhood and adult seasonal asthma (Beggs 2014; El Kelish et al. 2014). A dominant early successional species, ragweed forms dense stands in a variety of habitats, especially roadsides and other disturbed places (Bassett and Crompton 1975). Among the factors that determine allergic reactions in humans to ragweed pollen are the size, total number of male inflorescences produced, and duration and timing of flowering (Frenz et al. 1995), which affect the onset, peak, and duration of pollen allergies (Rogers et al. 2006; Ziska et al. 2011; Zhang et al. 2015b). Total pollen production is correlated with male inflorescence size (spike length; e.g., Rogers et al. 2006), but field measurements of flowering phenology and spike length are difficult to obtain, and involve exposure to the allergen itself. Although phenotypic traits related to plant size are easier to measure and are known to scale with male inflorescence biomass (Nakahara et al. 2018; Ackerly and Jasieński 1990), simple allometric estimators for spike length and the effects of climate on these relationships have not been published. Thus,

establishing any linkage between morphology and male reproductive biomass across environmental gradients can simplify and improve predictions of pollen production, and help establish how plants prioritize growth and reproduction along this climate gradient.

Several studies suggest that tradeoffs between growth and reproduction have led to divergent strategies for reproductive success in cool versus warm climates (e.g., Turesson 1922; Grime 1977). Species in cooler climates tend to flower quickly to ensure reproduction within a short growing season at the cost of reaching small size at reproduction and/or exhibiting low seed production (e.g., Clausen et al. 1940; Grime 1977; Stanton et al. 1997). In warmer climates, plants tend to mature more slowly to enhance growth, competitiveness, and fecundity, but they may also invest less in reproduction (Molau 1993; Stanton et al. 1997). Such variation has been observed not only across taxa along broad geographic gradients but also within species (Stanton et al. 1997; Leiblein-Wild et al. 2014; Li et al. 2015; Stinson et al. 2016). Ragweed flowering phenology is tightly linked to temperature, and growing season length across latitudes determines the duration of flowering (Deen et al. 2001). Although ragweed pollen disperses long distances (Liu et al. 2016), there is evidence for divergence among populations in distinct climate zones. Floral initiation in cooler climates occurs more quickly, and at smaller size, to accommodate a shorter growing season, whereas plants in warmer climates flower earlier in the calendar year but at a larger overall size (Dickerson and Sweet 1971; Stinson et al. 2016, 2017). The broad climatic niche of this species raises the possibility that some ragweed populations are more likely to affect human exposure to pollen than others through variation in onset, peak, and duration of flowering times as well as overall flower production, as has been shown for birch (Ahlhom et al. 1998) and for ragweed at a broader geographic scale (Rogers et al. 2006; Stinson et al. 2016). Determining the degree to which populations in regional climate zones vary in their current and future flowering patterns is critical for predicting whether ongoing environmental changes will affect pollen loads and seasonal allergy timing in different geographic locations.

In this study, we documented inter-population flowering variation in ragweed across a climate

gradient in Massachusetts. The longitudinal temperature gradient is influenced by the mountains and rural regions of Western MA grading into the warmer urban and coastal regions of the eastern part of the State, and the range of current and projected climate variability across greater New England (Meehl and Tebaldi 2004). We tested whether flowering variation observed in this widespread species at the continental extent (Allard 1945; Li et al. 2015; Stinson et al. 2016) would also occur across this regional gradient—at spatial extents and temporal extents and resolutions that are relevant to human exposure to allergenic pollen and management of allergenic plants. We hypothesized that populations in cooler climates in Massachusetts would demonstrate accelerated development and flowering relative to those in warmer climates. We also tested whether populations in warmer climates have lower total male reproductive output, as a function of size, due to prioritization of growth traits (Reekie and Bazzaz 1987; Grime 1977). Specifically, we looked for differences among climate types in their correlations between plant size and a known proxy for pollen production, male inflorescence length (Rogers et al. 2006; Fumanal et al. 2007). Regarding total production of male spikes, we tested two alternative hypotheses: (a) cool climate types prioritize reproduction over growth, and thus they produce larger and more male spikes than warm climate plants; or (b) warm climate types reach a larger overall size, and thus they produce more male spikes than cool climate plants.

Methods

Study plots

We used satellite imagery in Google maps and GIS to identify ~ 90 random search points within probable ragweed habitat across an average summer temperature gradient of ~ 4 °C in Massachusetts (Hijmans et al. 2005). We visited each point to determine the presence (or absence) and abundance of ragweed. Of these, we chose eight sites with ragweed populations in each of three average summer temperature groups: cool, 6–7 °C; medium, 7–9 °C; and warm, 9–12 °C. Growing degree-days are strongly correlated with temperature in this region; average annual precipitation is less variable, ranging from 100 to 152 cm (Daly

et al. 2002; PRISM Group 2010). Sites were deemed suitable for study if they contained at least 20 ragweed plants per square meter and were accessible to us for research (e.g., through private landowner or agency permission). We established a transect through each study population (~ 10–30 m), and randomly selected positions along the transect for three 1 × 1 m study plots. To separate out the effects of climate from land-use in this study, sites were selected to have similar land cover (i.e., early successional, open habitat). As a further check to ensure that climate did not co-vary with land-use, we extracted information on land-use from the 2011 National Land Cover Database (i.e., the percent impervious surface and percent forest) for each site to ensure that land-use did not correlate with longitude (Homer et al. 2015). To explore co-variation between elevation and longitude, we extracted elevation for each site from the National Aeronautic and Space Administration's Shuttle Radar Topography 30 m resolution data product (Farr et al. 2007).

Plant measurements

At each plot, we randomly selected 15 target individuals in each population for biweekly measurements of survival, plant crown height (cm), number of branches, number of inflorescences, and length (cm) of five randomly chosen inflorescences per plant to determine underlying phenotypic and developmental variation that might contribute to variation in flowering. Measurements were taken from ~ June 15–September 15. In mid-September, we harvested the above-ground biomass of the 15 target plants to assess biomass of all male inflorescences and seeds. We measured the dry weight of the inflorescence spikes and seeds to a precision of 0.001 g. At each plot we also counted the total number of stems and total number of flowering individuals on a biweekly basis during the flowering season, from mid-July through plant senescence in early October.

Statistical analyses

We constructed linear and generalized linear mixed models (LMM and GLMM, respectively) with R 3.5.0 (R Core Team 2018) using categories based upon mean temperature of the warmest quarter (bioclimatic variable 10) of the 'current' (i.e., interpolated weather

station data averaged across 1950–2000) Worldclim data (Hijmans et al. 2005) (hereafter, cool, medium and warm ‘climate’) as the main fixed explanatory variable. Site was included as a random factor to account for site to site variation. The response variables were onset, peak, and duration of flowering, maximum inflorescence size, number of flowering spikes, male inflorescence dry mass, seed dry mass, number of branches, and crown height. We also analyzed the effects of climate on plot-level density of ragweed and the proportion of individuals flowering. Cook’s distance tests were used to identify and remove statistical outliers prior to analyses. For LMMs, we used residual diagnostic plots to confirm good model fit and normal residual distribution. For count data we applied a GLMM with Poisson error structure, and for proportional data we used a binomial error structure using the *glmer* function from the ‘lme4’ package (Bates et al. 2015). Differences in phenology, reproduction, and growth traits were compared post-hoc between climate types using the *ghlt* function from the ‘multcomp’ package in R (Hothorn et al. 2008).

To assess variation in developmental rates and flowering phenology, we used repeated measures with climate as the main fixed effect and site as a random effect as noted above. For developmental rates, day of year was specified as the measure of the rate of increase in a trait over time; the climate $\times$ date interaction term was included for response variables of height, spike length, and number of spikes as a measure of variation in developmental rates among different climate types.

To test for proportional relationships between inflorescence length and crown height or inflorescence mass, we constructed GLMMs in JMP Pro 13.2 (SAS Institute Inc., Cary, NC 2017). We specified height and inflorescence mass as continuous predictors (covariates), of inflorescence length within each climate type. To test whether the slopes of these relationships varied by climate type, we then constructed a global model and examined the climate $\times$ covariate interaction term. We included site as a random effect in all cases. The continuous variables for this analysis were log transformed to account for the multiplicative nature of growth and reproductive data and to provide an assessment of proportional change in resource allocation independent of the units or scale of measurement (Kerkhoff and Enquist 2009). We interpreted the fit and slope of the

trait:inflorescence relationship as indicators of whether and how height or inflorescence mass could directly predict inflorescence length. The climate $\times$ height and climate $\times$ inflorescence mass interaction terms were assessed as measures of variation in the slopes of the relationships between each trait and inflorescence length.

Results

Landscape variation

Correlations between remotely sensed land-use and longitude indicated that climate at the study sites were not confounded with land-use (longitude vs. percent impervious surface Pearson correlation coefficient $r = -0.17$, longitude vs. percent forest Pearson correlation coefficient $r = -0.07$). Elevation ranged from 37.5 to 344.75 m at our sites and longitude was inversely correlated with elevation (longitude vs. elevation Pearson correlation coefficient $r = -0.7629$, $p = 0.001$).

Climate variation in individual traits: size and flower production

There was a significant effect of climate on spike length (Table 1; Fig. 1), with the length of longest inflorescence averaging mean = 9.28 mm (SE = 0.48) in the cool zone, mean = 8.19 mm (SE = 0.46) in the intermediate zone, and mean = 7.21 mm (SE = 0.40) in the cool zone. Male inflorescence dry mass (cool mean = 0.431 g, SE = 0.057; intermediate mean = 0.25 g, SE = 0.066; warm mean = 0.14 g, SE = 0.068) also differed by climate type (Table 1). Post-hoc tests revealed that cool climate types produced longer spikes with more male inflorescence mass than the warm climate types (Table S1).

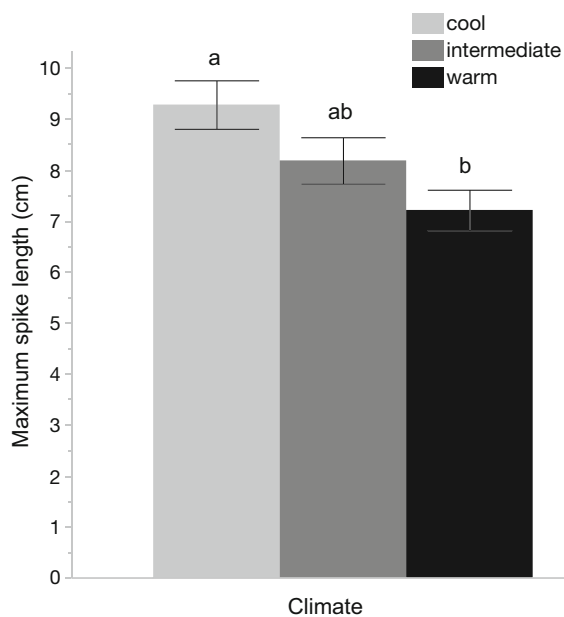
There was no significant effect of climate on number of flowering spikes, crown height, or seed mass (Table 1). The number of flowering spikes produced by individual plants averaged 11.95 inflorescences per plant (SE = 1.93) compared to 6.32 (SE = 0.87) and 7.78 (SE = 0.76) in the intermediate and warm climates, respectively (Table 1; Fig. 1). Vegetative size was similar across climate types (cool mean = 39.13, SE = 1.75; intermediate mean = 41.24, SE = 1.76; warm mean = 40.02, SE = 2.02).

Table 1 Effects of climate on the maximum values for individual plant traits: crown height, number (N) of flowering spikes, length of longest inflorescence, inflorescence dry mass,

and seed mass in common ragweed across a natural climatic gradient in Massachusetts during the 2011 growing season

Response	Model distribution	Climate (DF, 2)
Crown height (cm)	Gaussian	$F = 0.328$, $p = 0.720$
N flowering spikes	Poisson	$\chi^2 = 2.242$, $p = 0.326$
Length of longest inflorescence (mm)	Gaussian	$F = 5.874$, $p = 0.003$
Inflorescence dry mass (g)	Gaussian	$F = 6.605$, $p = 0.002$
Seed mass	Gaussian	$F = 1.794$, $p = 0.168$

The data are from linear and generalized linear mixed models, with site included as a random factor and F - or Chi square test statistics and p values reported for the main effect of climate

**Fig. 1** Maximum number of flowering spikes in common ragweed growing across a climatic gradient in Massachusetts. Error bars denote standard error (SE). Lowercase letters indicate significant differences between climate types using post-hoc Tukey test pairwise comparisons; climate types with non-matched letter labels are significantly different at the $p = 0.05$ level

More than 90% of measured individuals produced no secondary branches, and all multi-branched plants were statistical outliers using the Cook's distance test. Thus, branching was extremely uncommon and single-stem plants were the dominant growth form in all three climate types, and no tests were applied.

Allometric predictors of spike length

Both crown height and inflorescence biomass were strong predictors of inflorescence length within each climate type (Table 2; Fig. 2a, b; $p < 0.0001$). The trait $\times$ climate interaction in the global model comparing the regression lines among climate types was only significant for height, such that taller plants from the cool climate zone produced longer inflorescences per unit of height compared to those from the intermediate and warm climate zones (Fig. 2a). The slopes within each climate type were similarly positive but varied in magnitude, and thus the trait $\times$ climate interaction was not attributable to crossover interaction (VanderWeele and Knol 2014). Thus, smaller plants with lighter inflorescences produced shorter spikes overall regardless of climate type. However, the slope of the relationship between height and spike length differed among climate types and was steepest in the cool climate zone.

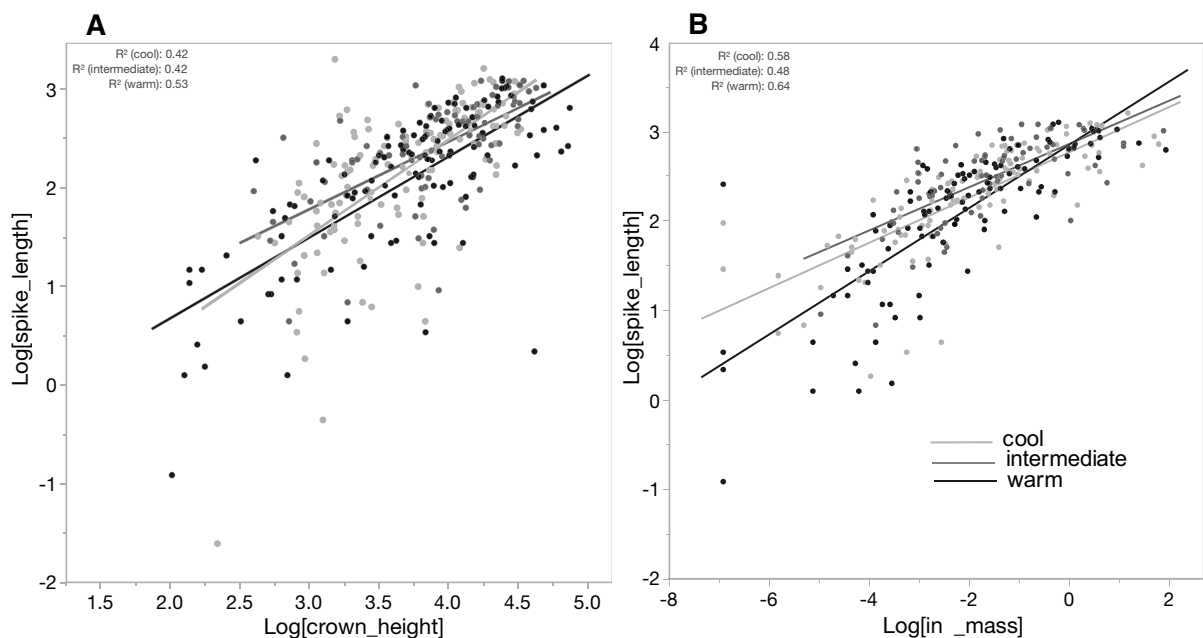
Developmental rates and flowering phenology

There was significant variation among climate zones in terms of plant developmental rates and flowering phenology (Table 3; Fig. 3a, b). The climate $\times$ date interaction terms were significant for height, number of spikes, and spike length, with plants in the warm zone generally developing at a slower rate than those in the intermediate and cool zones for all traits. Plant height varied somewhat over the season, with plants in the cool climate type beginning smaller but converging with the other climate types in size by the end of the season. Intermediate plants overtopped warm types initially and at week seven, but they were

Table 2 Generalized linear mixed model effects of climate on the relationships between inflorescence length and the covariate estimators (a) inflorescence mass or (b) crown height with site specified as a random effect

Covariate	DF	Model effects on response variable inflorescence length			
		(a) Inflorescence mass		(b) Height	
Effect	DF	F	p	F	p
Cool	1	131.896	< 0.001	135.532	< 0.0001
Intermediate	1	41.131	< 0.0001	31.801	< 0.0001
Warm	1	126.09	< 0.0001	79.470	< 0.0001
Covariate × Climate	2	2.503	0.084	4.482	0.01

Results of individual models are shown for each climate zone; the covariate × climate interactions from the global model measures differences among climate types in the slopes of the allometric relationships

**Fig. 2** Allometric predictions of inflorescence length for each climate type from the plant traits **a** crown height and **b** total inflorescence biomass. The X-axis is the log of the predictor

variable. The Y-axis is the log of the length of the inflorescence, a proxy for total pollen production

otherwise similar in height to warm types. Individual plants from the cool climate flowered earlier, and throughout the flowering season they had more flowers than those from the other two climate types (Fig. 3a). In the warm climate, plant inflorescences remained smaller than in the other two climate types throughout the season (Fig. 3b) and had a shorter flowering phase, averaging 21 days flowering compared to ~ 31 days in the cool and intermediate climate types (Fig. 4; Table 1).

Plot density and proportion flowering

Mean plot densities of ragweed were 107.1, 83.7, and 74.7 plants m² in the cool, intermediate, and warm climate types respectively, but density was not affected by climate (DF = 2; $\chi^2 = 1.078$; $p = 0.5833$). The proportion of plants flowering within each plot was uniformly high across climate types, with 92% (SE = 2.8) of plants flowering on cool sites, 90% (SE = 3.8) in intermediate types, and 87% (SE = 4.7) in warm types (DF = 2, $\chi^2 = 3.890$,

Table 3 Results of repeated measures model testing effects of climate on ragweed height, number (*N*) of inflorescences, and length of inflorescence through time

Effect	Response variables						
	Height			No. of inflorescences		Inflorescence length	
	DF	F	<i>p</i>	χ^2	<i>p</i>	F	<i>p</i>
Climate	2	0.835	0.444	91.332	< 0.0001	4.331	0.014
Time $\times$ Climate	2	4.643	< 0.01	494.656	< 0.0001	7.444	< 0.01

The time $\times$ climate interaction is included as a test of differences between climate types in developmental rates. Site is included as a random effect in the model

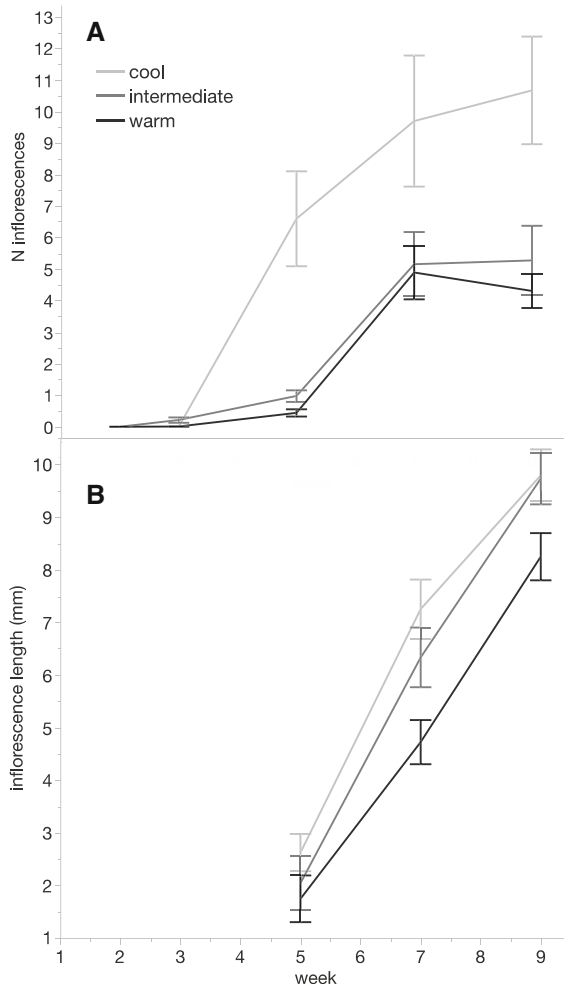


Fig. 3 **a** Mean spike length (mm), and **b** mean number of flowering spikes per plant. Data are derived from weekly observations of individual plants, beginning June 15. Error bars are derived as one standard error of the mean. Tukey post-hoc tests indicate differences among climate zones at the $p = 0.05$ level wherever error bars do not overlap (not lettered in figure)

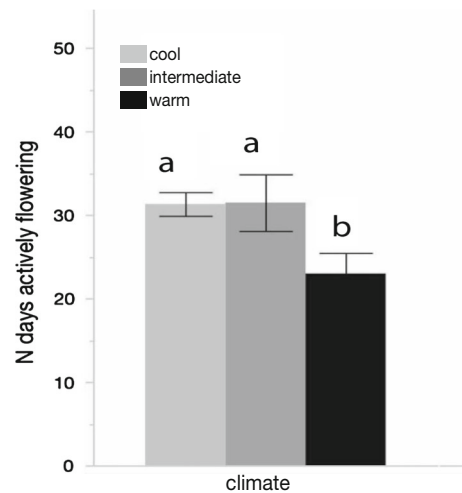


Fig. 4 Average duration of flowering of individual plants across a climatic gradient in Massachusetts. Error bars are derived as one standard error of the mean. Tukey post-hoc tests indicate differences among climate zones at the $p = 0.05$ level

$p = 0.143$). The phenophase for proportion flowering in each climate type is shown in Fig. 5.

Discussion

The purpose of this study was to document natural variation in ragweed populations growing across a regional climate gradient, particularly regarding traits related to allergenic pollen exposure to humans. Specifically, we looked for variation in male spike length, a known proxy for total pollen production (Rogers et al. 2006), and its relationship to expected phenotypic variation in aspects of plant growth. We tested whether traits that are easily-measured in the field could be used to predict differences in spike

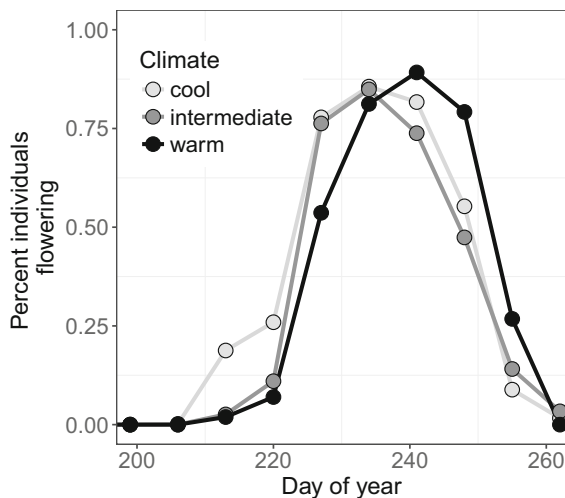


Fig. 5 Day of onset and duration of flowering in ragweed growing across a climatic gradient in Massachusetts as measured by plot-level proportion of plants flowering. White points/light gray lines indicate means for plants in cool climate types, medium gray points/lines for intermediate climate types, and dark gray points/lines for warm climate types

length, and thus provide better estimates of local pollen loads at a regional spatial extent that may be experienced by human populations.

We also tested whether flowering time and allometric estimates of spike length, varied across the longitudinal climate gradient in Massachusetts, to better understand climate effects on future pollen production. Due to time constraints on growth and reproduction in cooler climates, we expected to see earlier onset and peaks in flowering, and higher overall investment into male spikes with decreasing temperature. We further tested whether overall male flower production increased or decreased along the temperature gradient.

We found that inflorescence length can be predicted by plant height, but the slope of the relationship is steeper in cooler climates, suggesting ragweed growing in cool climates produce more pollen per unit of height than ragweed in warm climates. Likewise, plants in cool climates flowered earlier and for a longer duration, and produced longer and heavier spikes than those in warmer climates. Thus, we demonstrated variation in timing and output of flowering across the regional climate gradient, and discuss the findings in terms of potential strategies for managing ragweed in current and potential future conditions.

Climate effects on phenotypes—size, flower production, and spike length

Male inflorescences in ragweed consist of individual spikes that develop from axillary buds along the main stem. The spikes elongate as new flowers are produced, so longer inflorescences generally contain more individual flowers per spike (Deen et al. 2001). Here we found that plants in the cooler climate type produce longer and heavier spikes but a similar number of inflorescences to those in warmer climate types. Since pollen is borne on the male spikes, and longer spikes produce more pollen grains (Rogers et al. 2006), our data suggest that more pollen overall is produced in cooler climate types than in warmer climates across the study region. It is also possible that a longer flowering spike can improve pollen dispersal distance (e.g., Ackerly and Jasiński 1990; Nakahara et al. 2018), though this requires further study.

Both height and inflorescence mass were strong predictors of spike length, indicating that larger plants within each climate type tend to produce longer spikes and therefore more male flowers. The fact that height can predict spike length is important for two reasons. First, it provides a simple metric that can be collected in the field to predict pollen exposure in different populations, with reasonable confidence that taller plants are producing more pollen. Elsewhere, it has been noted that even very short plants will flower and release pollen (Stinson et al. 2006; Simard and Benoit 2011) particularly in suburban/urban areas subjected to repeated mowing. Here we show that the relative contribution of shorter plants to overall pollen loads are likely to be lower, however, than that of populations that support taller plants. From a management perspective, this suggests that mowing ragweed can indeed reduce local pollen burdens, with the caveat that shorter plants in cooler climates are also producing more and longer pollen-bearing spikes. Second, the height $\times$ climate interaction shown in Fig. 2 demonstrates a difference among climate types in the slopes of the height:spike length relationship, such that plants in the cooler climate type produce longer spikes per unit of height than those in the warmer climates. Thus, plants of similar height may contribute disproportionately more to pollen loads in the cooler than in the warmer parts of the region, making it even more important to remove tall plants in areas with a cooler climate.

Our findings are consistent with the prediction that cooler climate types prioritize reproduction over growth, reflecting a general proclivity toward rapid reproduction in response to shorter growing seasons (Reekie and Bazzaz 1987; Bolmgren and Cowan 2008). Several plant taxa produce larger and more abundant flowers relative to their size in more extreme habitats such as high latitudes and altitudes, as reproduction is thought to be prioritized over competitive growth in more extreme environments (e.g., Turesson 1922; Stanton et al. 1997; Bolmgren and Cowan 2008). However, we did not measure the density of other species in this study, and thus cannot determine whether variability across the longitudinal climate gradient in this study also represents variation in the competitive environment. We also note that we selected sites that would not be mowed during the growing season, so our data may not be representative of the height and spike length relationships at mowed sites.

Onset, timing, and duration of flowering

Accelerated phenology may help explain how individual plants in the cooler climates produced longer and heavier spikes in this study as well as broader geographic trends towards enhanced pollen production in the north. First, spike length remained consistently lower in the warmest climate as measured over time, indicating a distinctly short-spiked phenotype in warm climate types compared to that of the intermediate and cool phenotypes. However, despite similar height and number of spikes overall, individual plants in the cool climate type began producing male inflorescences earlier in the season than those from the warmer two climates (Fig. 3), and flowering persisted longer in the intermediate and cool climates than in the warm climate (Fig. 4). This pattern is consistent with observations in ragweed at continental and broader regional extents (Rogers et al. 2006), and in many other plant taxa that accelerate reproduction in cooler climates due to time constraints on reproduction within a short growing season (Bolmgren and Cowan 2008). Thus, in addition to producing longer spikes, individual plants in the cooler climates flower earlier than elsewhere; potentially creating pollen in these areas at least 1 week sooner than in the warmer two climates. In terms of flowering duration, pollen loads may persist up to 10 days longer in intermediate

and cooler types compared to that of the warmer climate type, at least in some years (Figs. 4, 5).

Role of landscape heterogeneity other than climate

We found no evidence to suggest that particular land-use variables (e.g., land-use as measured by percent impervious surface or percent forest) other than climate varied significantly across the gradient, and thus attribute variation among climate zones to the documented variability in temperature across the gradient. However, we did find evidence that elevation covaries with climate, as cooler sites were also those at higher elevation. It is possible that plants at higher elevations also disperse pollen further due to effects of wind speed and exposure (Blando et al. 2012). Other unmeasured factors such as competition, soil fertility, or weed management practices across sites and at the microsite scale may co-vary and/or interact with climate to affect male flower production and pollen dispersal (Grime 1977; Ackerly and Jasienski 1990; Fox and Morin 2001; Bartkova-Scevkova 2003; Ziska et al. 2007; Bolmgren and Cowan 2008; Leskovsek et al. 2012). We also note that our results differ from that of a prior study, where ragweed from a common seed source flowered earlier and longer in urban than rural locations in Maryland (Ziska et al. 2002), rather than in situ populations in our study, possibly due to intraspecific variation in climatic response across climate types (Li et al. 2015; Stinson et al. 2016; Ziska et al. 2011). Also, we did not measure variation in atmospheric carbon dioxide levels, which are known to co-vary with temperature along urban–rural gradients such as that in Massachusetts (Ziska et al. 2007) and to affect ragweed reproduction differentially across climate gradients and size classes (Ziska et al. 2002, 2007; Stinson et al. 2011). However, after accounting for site to site variation in our models, we can conclude that the variation we observed in spike length, spike biomass, and flowering patterns do scale with regional variability in temperature, and thus can be estimated from current or predicted climate data. We also show that height can be used as a straightforward field metric to predict spike length and thus estimate pollen output among populations in this and possibly other regions. Further work is needed to determine the role of site-specific and interannual temporal environmental variation in the patterns we observed here.

Conclusions

With globally fluctuating temperatures, plants and humans alike are being exposed to changes in ecological processes. Past climate records indicate strong correlations between ragweed pollen abundance and hot, dry growing conditions and past land-use by humans (Faison et al. 2006), suggesting the potential for an uptick in ragweed reproduction under future warming scenarios throughout its range (Kiss and Beres 2006; Ziska and Beggs 2012; Zhang et al. 2015a). Here we improve our understanding of regional climate effects on ragweed by showing significant climate-driven variation in the timing, duration, and output of male flower spikes and thus the potential for landscape-level differences in human exposure to pollen. It is therefore likely that changes in climate patterns could differentially affect ragweed output across the region. The present study indicates that plants in cooler climates contribute more to pollen loads by flowering earlier and longer than those in the warmer climate zone. An underlying proclivity towards rapid onset of reproduction amongst cool climate type plants may result in disproportionately higher allocation to reproduction if spring begins earlier with warming trends. This same mechanism may help explain observations of more enhanced pollen output in response to recent warming trends in cooler climates near ragweed's northern range limit (Ziska et al. 2011; Stinson et al. 2017). Finally, we show that allometric relationships with height not only provide a simple field metric for pollen output, but also a mechanistic understanding of complex interactions between climate and ragweed flowering. While taller plants produce larger male flower spikes, those in cooler regions produce longer spikes per unit of height and may thus respond differently to warming than those in warm climates. From an ecological standpoint, these findings suggest fine-scaled climate variation explains timing of and investment into male inflorescences. From a public health perspective, our data place the future of ragweed allergies into the practical realm of human demographic scales that may be useful in the analysis of epidemiological data (Beggs and Bambrick 2005; Blando et al. 2012). Overall, we suggest that basic field measurements of plant height, and the integration of regional patterns of climatic variation, can successfully contribute to ragweed pollen models and management strategies.

Acknowledgements This research was funded by the US Environmental Protection Agency (EPA) Science to Achieve Results (STAR) Program Grant # SU834359010. We gratefully acknowledge: David Foster for collegial support, and the Harvard Forest Lab Group for thoughtful comments during project development. We thank Christine Rogers for comments on sampling design and Israel Marquez, Sam Safran, and Tristram Seidler for assistance with data collection and data curation.

References

- Ackerly DD, Jasieński M (1990) Size-dependent variation of gender in high-density stands of the monoecious annual, *Ambrosia artemisiifolia* (Asteraceae). *Oecologia* 82:474–477. <https://doi.org/10.1007/BF00319788>
- Ahlhom JU, Helander ML, Savolainen J (1998) Genetic and environmental factors affecting the allergenicity of birch (*Betula pubescens* ssp. *Czerepanovii* [Orl.] Hamet-Ahti) pollen. *Clin Exp Allergy* 28:1384–1388
- Allard HA (1945) Flowering behavior and natural distribution of the eastern ragweeds (*Ambrosia*) as affected by length of day. *Ecology* 26:387–394. <https://doi.org/10.2307/1931660>
- Bartkova-Scevkova J (2003) The influence of temperature, relative humidity and rainfall on the occurrence of pollen allergens (*Betula*, *Poaceae*, *Ambrosia artemisiifolia*) in the atmosphere of Bratislava (Slovakia). *Int J Biometeorol* 48:1–5. <https://doi.org/10.1007/s00484-003-0166-2>
- Bassett IJ, Crompton CW (1975) Biology of Canadian Weeds 11. *Ambrosia artemisiifolia* L. and *A. psilostachya* Dc. *Can J Plant Sci* 55:463–476. <https://doi.org/10.4141/cjps75-072>
- Bates D, Maechler M, Bolker B, Walkder S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- Beggs PJ (2014) Impacts of climate change on allergens and allergic diseases: knowledge and highlights from two decades of research. *Clim Chang Glob Health*. <https://doi.org/10.1079/9781780642659.0105>
- Beggs PJ, Bambrick HJ (2005) Is the global rise of asthma an early impact of anthropogenic climate change? *Environ Health Perspect* 113:915–919. <https://doi.org/10.1289/ehp.7724>
- Blando J, Bielory L, Nguyen V, Diaz R, Jeng HA (2012) Anthropogenic climate change and allergic diseases. *Atmosphere* 3:200–212. <https://doi.org/10.3390/atmos3010200>
- Bolmgren K, Cowan PD (2008) Time-size tradeoffs: a phylogenetic comparative study of flowering time, plant height and seed mass in a north-temperate flora. *Oikos* 117:424–429. <https://doi.org/10.1111/j.2007.0030-1299.16142.x>
- Clausen J, Keck DD, Hiesey WM (1940) Experimental studies on the nature of species: volume I: experimental effects of varied environments on Western North American plants. Publication 540. Carnegie Institution of Washington, Washington, DC
- Daly C, Gibson WP, Taylor GH, Johnson GL, Pasteris P (2002) A knowledge-based approach to the statistical mapping of climate. *Clim Res* 22:99–113

- Deen W, Swanton CJ, Hunt LA (2001) A mechanistic growth and development model of common ragweed. *Weed Sci* 49:723–731
- Dickerson CT, Sweet RD (1971) Common ragweed ecotypes. *Weed Sci* 19:64–66
- Frenz DA, Palmer MA, Hokanson JM, Scamehorn RT (1995) Seasonal characteristics of ragweed pollen dispersal in the United States. *Ann Allergy Asthma Immunol* 75:417–422
- El Kelish A, Zhao F, Heller W, Durner J, Winkler JB, Behrendt H, Traidl-Hoffmann C, Horres R, Pfeifer M, Frank U, Ernst D (2014) Ragweed (*Ambrosia artemisiifolia*) pollen allergenicity: superSAGE transcriptomic analysis upon elevated CO₂ and drought stress. *BMC Plant Biol* 14:176. <https://doi.org/10.1186/1471-2229-14-176>
- Faison EK, Foster DR, Oswald WW, Hansen BCS, Doughty E (2006) Early holocene openlands in southern New England. *Ecology* 87:2537–2547
- Farr TG, Rosen PA, Caro E, Crippen R, Duren R, Hensley S, Kobrick M, Paller M, Rodriguez E, Roth L, Seal D (2007) The shuttle radar topography mission. *Rev Geophys* 45:1–33. <https://doi.org/10.1029/2005RG000183>
- Fitter AH, Fitter R (2002) Rapid changes in flowering time in British plants. *Science* 296:1689–1691. <https://doi.org/10.1126/science.1071617>
- Fox JW, Morin PJ (2001) Effects of intra- and interspecific interactions on species responses to environmental change. *J Anim Ecol* 70:80–90
- Fumanal B, Chauvel B, Bretagnolle F (2007) Estimation of pollen and seed production of common ragweed in France. *Ann Agric Environ Med* 14:233–236
- Grime JP (1977) Evidence for existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am Nat* 111:1169–1194. <https://doi.org/10.1086/283244>
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–1978
- Homer C, Dewitz J, Yang L, Jin S, Danielson P, Xian G, Coulston J, Herold N, Wickham J, Megown K (2015) Completion of the 2011 National land cover database for the conterminous United States—representing a decade of land cover change information. *Photogramm Eng Remote Sensing* 81:345–354. <https://doi.org/10.14358/PERS.81.5.345>
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* 50:346–363
- Katz DSW, Carey TS (2014) Heterogeneity in ragweed pollen exposure is determined by plant composition at small spatial scales. *Sci Total Environ* 485:435–440. <https://doi.org/10.1016/j.scitotenv.2014.03.099>
- Kerckhoff AJ, Enquist BJ (2009) Multiplicative by nature: why logarithmic transformation is necessary in allometry. *J Theor Biol* 257(3):519–521. <https://doi.org/10.1016/j.jtbi.2008.12.026>
- Kiss L, Beres I (2006) Anthropogenic factors behind the recent population expansion of common ragweed (*Ambrosia artemisiifolia* L.) in Eastern Europe: is there a correlation with political transitions? *J Biogeogr* 33:2156–2157. <https://doi.org/10.1111/j.1365-2699.2006.01633.x>
- Lau JA, Shaw RG, Reich PB, Tiffin P (2010) Species interactions in a changing environment: elevated CO₂ alters the ecological and potential evolutionary consequences of competition. *Evol Ecol Res* 12:435–455
- Leiblein-Wild MC, Kaviani R, Tackenberg O (2014) Germination and seedling frost tolerance differ between the native and invasive range in common ragweed. *Oecologia* 174:739–750. <https://doi.org/10.1007/s00442-013-2813-6>
- Leskovsek R, Eler K, Batic F, Simoncic A (2012) The influence of nitrogen, water and competition on the vegetative and reproductive growth of common ragweed (*Ambrosia artemisiifolia* L.). *Plant Ecol* 213:769–781. <https://doi.org/10.1007/s11258-012-0040-6>
- Li X, She D, Zhang D, Liao W (2015) Life history trait differentiation and local adaptation in invasive populations of *Ambrosia artemisiifolia* in China. *Oecologia* 177:669–677. <https://doi.org/10.1007/s00442-014-3127-z>
- Liu L, Solmon F, Vautard R, Hamaoui-Laguel L, Torma CZ, Giorgi F (2016) Ragweed pollen production and dispersion modelling within a regional climate system, calibration and application over Europe. *Biogeosciences* 13:2769–2786. <https://doi.org/10.5194/bg-13-2769-2016>
- Meehl GA, Tebaldi C (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* 305:994–997. <https://doi.org/10.1126/science.1098704>
- Molau U (1993) Relationships between flowering phenology and life history strategies in tundra plants. *Arct Alp Res* 25(4):391
- Nakahara T, Fukano Y, Hirota SK, Yahara T (2018) Size advantage for male function and size-dependent sex allocation in *Ambrosia artemisiifolia*, a wind-pollinated plant. *Ecol Evol* 8:1159–1170. <https://doi.org/10.1002/ece3.3722>
- PRISM Climate Group, Oregon State University, <http://prism.oregonstate.edu>, created 10 Aug 2010
- R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Reekie EG, Bazzaz FA (1987) Reproductive effort in plants 3. Effect of reproduction on vegetative activity. *Am Nat* 129:907–919. <https://doi.org/10.1086/284683>
- Rogers CA, Wayne PM, Macklin EA, Muilenberg ML, Wagner CJ, Epstein PR, Bazzaz FA (2006) Interaction of the onset of spring and elevated atmospheric CO₂ on ragweed (*Ambrosia artemisiifolia* L.) pollen production. *Environ Health Perspect* 114:865–869. <https://doi.org/10.1289/ehp.8549>
- Simard M, Benoit DL (2011) Effect of repetitive mowing on common ragweed (*Ambrosia artemisiifolia* L.) pollen and seed production. *Ann Agric Environ Med* 18:55–62
- Stanton ML, Galen C, Shore J (1997) Population structure along a steep environmental gradient: consequences of flowering time and habitat variation in the snow buttercup, *Ranunculus adoneus*. *Evolution* 51:79–94. <https://doi.org/10.1111/j.1558-5646.1997.tb02390.x>
- Stinson KA, Tranz JH, Petzold JL, Bazzaz FA (2006) Architectural and physiological mechanisms of reduced size inequality in CO₂-enriched stands of common ragweed (*Ambrosia artemisiifolia*). *Glob Chang Biol* 12:1680–1689. <https://doi.org/10.1111/j.1365-2486.2006.01229.x>
- Stinson KA, Brophy C, Connolly J (2011) Catching up on global change: new ragweed genotypes emerge in elevated CO₂

- conditions. *Ecosphere*. <https://doi.org/10.1890/es10-00168.1>
- Stinson KA, Albertine JM, Hancock LMS, Seidler TG, Rogers CA (2016) Northern ragweed ecotypes flower earlier and longer in response to elevated CO₂: what are you sneezing at? *Oecologia* 182:587–594. <https://doi.org/10.1007/s00442-016-3670-x>
- Stinson KA, Albertine JM, Seidler TG, Rogers CA (2017) Elevated CO₂ boosts reproduction and alters selection in northern but not southern ecotypes of allergenic ragweed. *Am J Bot* 104:1313–1322. <https://doi.org/10.3732/ajb.1700222>
- Turesson G (1922) The genotypical response of the plant species to the habitat. *Hereditas* 3:211–350. <https://doi.org/10.1111/j.1601-5223.1922.tb02734.x>
- Valladares F, Matesanz S, Guilhaumon F, Araujo MB, Balaguer L, Benito-Garzon M, Cornwell W, Gianoli E, van Kleunen M, Naya DE, Nicotra AB, Poorter H, Zavala MA (2014) The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol Lett* 17:1351–1364. <https://doi.org/10.1111/ele.12348>
- VanderWeele TJ, Knol MJ (2014) A tutorial on interaction. *Epidemiol Methods* 3:33–72
- Wang X, Taub DR, Jablonski LM (2015) Reproductive allocation in plants as affected by elevated carbon dioxide and other environmental changes: a synthesis using meta-analysis and graphical vector analysis. *Oecologia* 177:1075–1087. <https://doi.org/10.1007/s00442-014-3191-4>
- Zhang Y, Bielory L, Cai T, Mi Z, Georgopoulos P (2015a) Predicting onset and duration of airborne allergenic pollen season in the United States. *Atmos Environ* 103:297–306. <https://doi.org/10.1016/j.atmosenv.2014.12.019>
- Zhang Y, Bielory L, Mi Z, Cai T, Robock A, Georgopoulos P (2015b) Allergenic pollen season variations in the past two decades under changing climate in the United States. *Glob Chang Biol* 21:1581–1589. <https://doi.org/10.1111/gcb.12755>
- Ziska LH, Beggs PJ (2012) Anthropogenic climate change and allergen exposure: the role of plant biology. *J Allergy Clin Immunol* 129:27–32. <https://doi.org/10.1016/j.jaci.2011.10.032>
- Ziska LH, Gebhard DE, Frenz DA, Faulkner S, Singer BD, Straka JG (2002) Ragweed pollen production in response to urban-induced CO₂/temperature gradients. *Ecol Soc Am Annu Meet Abstr* 87:456
- Ziska LH, George K, Frenz DA (2007) Establishment and persistence of common ragweed (*Ambrosia artemisiifolia* L.) in disturbed soil as a function of an urban-rural macro-environment. *Glob Chang Biol* 13:266–274. <https://doi.org/10.1111/j.1365-2486.2006.01264.x>
- Ziska L, Knowlton K, Rogers C, Dalan D, Tierney N, Elder MA, Filley W, Shropshire J, Ford LB, Hedberg C, Fleetwood P, Hovanky KT, Kavanaugh T, Fulford G, Vrtis RF, Patz JA, Portnoy J, Coates F, Bielory L, Frenz D (2011) Recent warming by latitude associated with increased length of ragweed pollen season in central North America. *Proc Natl Acad Sci USA* 108:4248–4251. <https://doi.org/10.1073/pnas.1014107108>

Regional variation in timing, duration, and production of flowers by allergenic ragweed

Stinson, K.A., Wheeler, J.A., Record, S., and Jennings, J.L.

Supplementary Material

Table S1 Posthoc output for traits significantly affected by climate in Table 1 of the main Manuscript.

Climate	<u>spike length</u> <u>(cm)</u>		<u>infl mass (g)</u>	
	t	p	t	p
warm - cool	-3.437	0.002	3.393	0.002
warm - interm	-1.342	0.373	-1.365	0.360
interm - cool	-1.806	0.169	-1.804	0.170

Figure S1 Map of annual mean summer temperature in Massachusetts overlaid with the 24 study sites (black circles). Climate maps were generated from Worldclim bioclimatic variable 10, which are interpolated weather station data averaged across 1950-2000 for the warmest quarter (Hijmans et al. 2005).

