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SPECIAL TOPIC – HIGHLIGHTED STUDENT RESEARCH

Interactions between rising CO_2 and temperature drive accelerated flowering in model plants under changing conditions of the last century

S. Michael Walker II[1] · **Joy K. Ward**[1]

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Abstract

Past studies have shown that flowering times have accelerated over the last century. These responses are often attributed to rising temperature, although short-term field experiments with warming treatments have under-estimated accelerations in flowering time that have been observed in long-term field surveys. Thus, there appears to be a missing factor(s) for explaining accelerated flowering over the last century. Rising atmospheric CO_2 concentration ($[CO_2]$) is a possible candidate, and its contributions to affecting flowering time over historic periods are not well understood. This is likely because rising $[CO_2]$ is confounded with temperature in the field and preindustrial $[CO_2]$ studies are relatively rare. To address this, we tested the individual and interactive effects of rising $[CO_2]$ and temperature between preindustrial and modern periods on flowering time in the model system, *Arabidopsis thaliana*. We used a variety of genotypes originating from diverse locations, allowing us to test intraspecific responses to last-century climate change. We found that accelerated flowering time between the full-preindustrial and full-modern treatments was mainly driven by an interaction between rising $[CO_2]$ and temperature, rather than through the individual effects of either factor in isolation. Furthermore, accelerated flowering time was driven by enhanced plant growth rates and not through changes in plant size at flowering. Thus, the interaction between rising $[CO_2]$ and temperature may be key for explaining large accelerations in flowering times that have been observed over the last century and that could not be explained by rising temperature alone.

Keywords Climate change · CO_2 by temperature interaction · Flowering time · Global change · Low CO_2 · Past conditions · Phenology · Plant development · Preindustrial

Introduction

Flowering time influences, and is influenced by, ecological and evolutionary processes and produces major developmental changes within the life cycle of plants (Franks 2015; Huang et al. 2017; Menzel 2002). At the plant level, flowering time affects carbon accumulation and overall fitness (Elzinga et al. 2007; Franks 2015). At the ecosystem scale, primary productivity and water use are influenced by flowering time, as well as interspecific competition and interactions with pollinators and herbivores (Cleland et al. 2007; Craufurd and Wheeler 2009; Felton and Smith 2017; Primack and Miller-Rushing 2011; Rafferty and Ives 2012; Schmalenbach et al. 2014), also see (Bartomeus et al. 2011). Plants sense a variety of endogenous and exogenous cues for appropriately timing the vegetative to floral transition. Such cues include (but are not limited to) carbohydrate status, photoperiod, light quality, vernalization, and ambient

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Using model plants, S. Michael Walker (Ph.D. student) demonstrated that the interaction between rising CO_2 and temperature better explains accelerated flowering time over changing conditions of the last century compared with the individual effects of either factor.

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✉ Joy K. Ward
joyward@ku.edu

[1] Department of Ecology and Evolutionary Biology, University of Kansas, 1200 Sunnyside Avenue, Lawrence, KS 66044, USA

temperature (Burghardt et al. 2015; Simpson and Dean 2002; Wahl et al. 2013). Importantly, climate change factors influence a number of these cues, along with altering plant growth trajectories, all of which can have potential impacts on flowering time (Anderson et al. 2012; Cleland et al. 2007; Franks 2015; Richardson et al. 2016; Wadgymar et al. 2018).

As a result of fossil fuel combustion, atmospheric [CO_2] has increased from 270 to 402 ppm since the onset of the Industrial Revolution (≈ 120 years ago), and average global temperatures have increased by 0.85 °C (IPCC 2013). Along with this, field surveys have documented accelerations in flowering time over the last century in a variety of plant species (Dunnell and Travers 2011; Ellwood et al. 2013; Fitter and Fitter 2002; Jagadish et al. 2016; Menzel et al. 2006; Wolkovich et al. 2012). The most comprehensive surveys and meta-analyses to date show that flowering time advances are statistically significant in 5–30% of species (depending on the study), with average accelerations of 5–15 days occurring over the past several decades (Dunnell and Travers 2011; Fitter and Fitter 2002; Menzel et al. 2006; Wolkovich et al. 2012).

In the vast majority of field surveys, accelerated flowering has been attributed to the effects of rising temperature, although in many instances, this assumption has not been directly tested (Dunnell and Travers 2011; Fitter and Fitter 2002; Menzel et al. 2006; Wolkovich et al. 2012). Importantly, Wolkovich et al. (2012) found that in a large meta-analysis with over 1500 taxa, warming treatments in field experiments greatly under-estimated accelerations in flowering time that were observed in long-term field surveys (Wolkovich et al. 2012). In fact, on a per degree basis, experimental warming treatments did not produce a change in flowering time on average and even produced delays in some cases, whereas flowering was accelerated by an average of 4.6 days/°C in long-term field surveys in similar environments (Wolkovich et al. 2012). Thus, there appears to be a major missing factor(s) for explaining accelerations in flowering time that have occurred in natural field settings over the last century. Given that flowering time is a major proxy for detecting the impacts of climate change and that there is a need to better understand historical shifts in flowering time to make inferences about future changes (Allen et al. 2014; Ellwood et al. 2013; Wang et al. 2016), it is urgent that we identify this missing factor(s).

To date, the majority of flowering time studies have focused on the impacts of increasing temperature, while rarely considering the effects of rising atmospheric [CO_2]. This may prove problematic since in a literature review, 57% of wild species and 62% of crop species exhibited altered flowering times when grown at elevated (700 ppm) versus modern (350–400 ppm) [CO_2] (with no change in temperature). The extreme responses at elevated [CO_2] ranged from accelerations of 60 days to delays of 16 days depending on the species, with high levels of intraspecific variation also being reported (Jagadish et al. 2016; Springer and Ward 2007). In addition, effects of rising [CO_2] on absolute flowering time responses were as large or larger as corresponding effects of temperature increase across similar time ranges. Taken together, this suggests that differences in flowering time responses observed in long-term field studies relative to warming-only field experiments (Wolkovich et al. 2012) may potentially require the effects of rising [CO_2] to explain this discrepancy.

Rising temperature and [CO_2] each have the potential to alter flowering time (Jagadish et al. 2016; Lutz et al. 2015), although less is known about their interactive effects. The main focus of recent studies has been on responses to *future* conditions with less known about responses to past conditions (likely due to the need for controlled removal of CO_2 from ambient air to simulate past conditions). Some studies report that rising temperature and [CO_2] above current levels can interact to affect flowering time (Craufurd and Wheeler 2009; Rogers et al. 2006), and when interactions do occur, the results are often mixed (Rawson 1992). For example, future [CO_2] levels serve to enhance the accelerating effect of warming on flowering time in *Lotus corniculatus* (Carter et al. 1997), whereas in other species, including a variety of naturalized European annual grass species, they interact to eliminate the effect of warming (Cleland et al. 2006). Unfortunately, it is unknown how the interaction of rising temperature and [CO_2] impacted flowering time across *preindustrial* through *modern* periods, mainly since increasing [CO_2] and temperature are confounded in the field, and historical CO_2 studies are relatively rare. This knowledge gap hampers our ability to predict future flowering time trends since it is unclear how even recent climate change factors have contributed to affect flowering times to date (Springer and Ward 2007).

Here, we tested the effects of increasing [CO_2] and temperature on flowering time between preindustrial and modern conditions in a controlled and replicated study. We used diverse, field-collected genotypes of the well-characterized model system, *Arabidopsis thaliana*, to quantify intraspecific variation to rising [CO_2] and temperature. The advantages of using this model system are that (1) it is an annual for which growth rate trajectories on whole plants can be measured, (2) the plant size is amenable to full-factorial experiments in growth chambers, whereby CO_2 can be scrubbed to achieve historical conditions, (3) replication of genotypes is straight forward since this is a selfing species, and (4) the genetics of flowering are well characterized, positioning future studies to resolve mechanisms of evolutionary and developmental responses to both past and future global change. This was also the first species that was found to exhibit altered expression of flowering genes in response to elevated [CO_2] (Springer et al. 2008). We performed this

study under controlled conditions to isolate the individual and interactive effects of [CO_2] and temperature to better understand the developmental drivers of accelerated flowering time over the last century.

Materials and methods

Seed sources

We used eight genotypes of *Arabidopsis thaliana* that were originally field collected from diverse locations, and seed stocks were obtained from the *Arabidopsis* Biological Resource Center (The Ohio State University). This allowed us to quantify intraspecific variation in flowering time responses within this model system. Seeds were homozygous lines that were derived from single-seed descent. Genotypes with accession numbers are as follows: Cape Verde, CS902; Tajikistan, CS916; Ukraine, CS927; Belgium, CS948; Austria, CS6752; British Columbia, Canada, CS6842; Sweden, CS22548; Portugal, CS22645. All genotypes are annuals that display rapid-cycling life histories (Kim et al. 2005). Six of the genotypes were collected from the Eurasian temperate zone, one from the North American temperate zone in British Columbia, and one from the tropics at Cape Verde. The Eurasian genotypes are native to their collection sites, whereas the Cape Verde and British Columbia genotypes were introduced and have since naturalized in their respective locations (Koornneef et al. 2004). We included Cape Verde to examine potentially interesting ramifications of adaptation to warm temperatures. The genotypes originated from 38°N to 56°N latitude, with the exception of Cape Verde which originated from 15°N. They experienced average historic growing season temperatures ranging from 18–24 °C for temperate genotypes and 23–27 °C for the tropical, Cape Verde genotype in their original field locations.

Experimental treatments and controlled environments

CO_2 and temperature treatments were controlled at 270 ± 10 ppm CO_2 and at $20.0/13.0 \pm 0.2$ °C day/night for the preindustrial period and 380 ± 10 ppm CO_2 and $21.3/14.3 \pm 0.2$ °C for the modern period. We selected 20 °C for the initial preindustrial temperature because it falls within the historical and seasonal range for the origins of the majority of genotypes (with the exception of Cape Verde). The 1.3 °C increase represents average warming in the temperate zone over the last century where the majority of genotypes originated (Harris and Chapman 2001; IPCC 2007). Integrated sensors within the growth space of the chambers (Conviron BDR16, Winnipeg, Manitoba) continuously monitored ambient [CO_2], temperature, light intensity, and humidity. Accuracy of these sensors was independently verified using a Li-6400 Portable Photosynthesis System (LI-COR, Lincoln, NE), an AccuPAR LP-80 light meter (Decagon Devices, INC., Pullman, WA), and HOBO temperature data loggers (Onset, MacArthur, MA). As needed, CO_2 gas was automatically injected from gas cylinders into chambers and excess CO_2 was removed by pulling chamber air through JorVet soda lime (Jorgensen Labs, Inc, Loveland, CO) in a customized and automated scrubbing system. Growth chambers utilized an external and customized glycol-based heat exchanger that allowed for tight control of temperature. Light lamps were housed in separate banks outside of the growth space to avoid interference with temperature control.

Plant growth conditions

Seeds were individually grown in 750 ml pots filled with a 1:1:1 (v/v) mixture of vermiculite, gravel, and Turface (Profile Products, Buffalo Grove, IL). Imbibed seeds were maintained at 4 °C for 4 days prior to being placed in controlled chambers to promote uniform germination. Because we utilized open, field-adapted genotypes, light levels were maintained at ≈ 1000 µmol m^{-2} s^{-1} with a 14-h photoperiod, which allowed for inductive long days for flowering that would have occurred under field conditions during summer months (when the life cycles of these genotypes are usually completed). Relative humidity was approximately 65% during the light period and 90% during the dark period with automated control. All plants were watered to saturation twice daily, with one dose of half-strength Hoagland's solution each morning and de-ionized water each afternoon.

Experimental measurements and design

We used a fully factorial design and every treatment was repeated twice. Each experimental treatment was randomly assigned to two different growth chambers to control for chamber effects. Placement of plants within each chamber was randomized across genotype. We measured flowering time as the number of days for the inflorescence (bolt) to reach 1 cm in length, whereby the beginning of the growth phase was controlled as the emergence of seedlings above the soil. Our total sample size for flowering time (across both rounds) was 774 plants in total, where *n* ranged from 15 to 28 plants per genotype per treatment. These sample sizes allowed us to maximize chamber size such that plants were not in competition for space or light. On a random subset of plants, total plant mass was measured (after drying at 70 °C for at least 48 h) at 2 days after emergence and at the time of flowering (as defined above), which included both above- and belowground components. Relative growth rate was calculated as the difference between ln of total plant dry

mass at flowering and seedling mass shortly after emergence (2 days) divided by the number of days between harvests. This represents growth rate during the full pre-reproductive growth phase leading up to flowering. For growth measurements, total sample size was 347, where *n* ranged from 6 to 15 plants per genotype per treatment.

Statistical analysis

Flowering time, relative growth rate, and total biomass at the time of flowering were assessed via three-way ANOVA with main effects of genotype, [CO_2] and temperature, along with their interactions. Biomass at flowering was ln transformed to meet the assumptions of ANOVA. Bonferroni tests were used to conduct targeted comparisons between genotypic responses to [CO_2] and/or temperature when significant effects were detected in the overall ANOVA. All analyses were performed in JMP (SAS, Cary, NC).

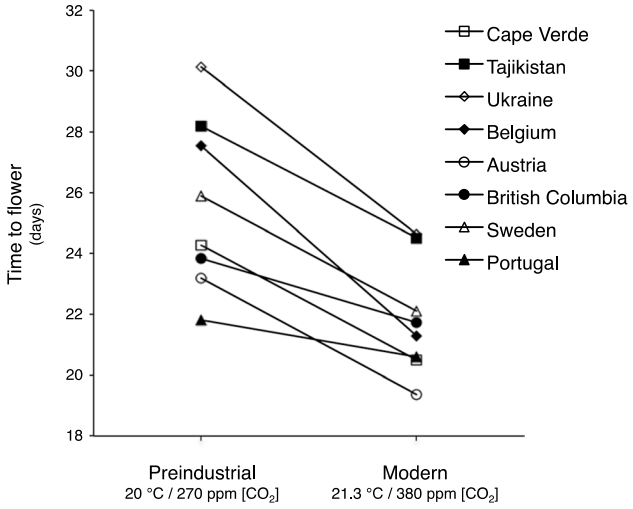

Fig. 1 Mean time to flower between full-preindustrial and full-modern treatments (increases in both [CO_2] and temperature) for eight genotypes of *Arabidopsis thaliana* from diverse locations (see text for more information)

Results

We examined how the combined and individual effects of an increase in [CO_2] by 110 ppm and temperature by 1.3 °C between preindustrial and modern conditions affected individual genotypes of *Arabidopsis thaliana* for flowering time, growth rate, and total biomass at flowering. With respect to flowering time, we found a significant three-way interaction between [CO_2], temperature, and genotype in the overall ANOVA (Table S1; $p = 0.0008$), indicating that genotypes exhibited varying response patterns to the interacting effects of [CO_2] and temperature. Across all genotypes, there was an average 3.8 day mean reduction in flowering time between the full-preindustrial and full-modern treatment (Figs. 1, 2a). We found that five of the eight genotypes exhibited significant accelerations in flowering time between these treatments (Cape Verde, $p = 0.002$; Ukraine, $p = 0.002$; Belgium, $p < 0.0001$; Austria, $p < 0.0001$; Sweden, $p < 0.0001$ from Bonferroni tests), with other genotypes showing a similar trend, although less pronounced, in this same direction (Figs. 1, 2a).

To determine how individual increases in [CO_2] and temperature contributed to flowering time responses at the full-modern treatment, we examined the effects of increasing either [CO_2] or temperature in isolation between preindustrial and modern conditions (Fig. 2a–c). Increasing [CO_2] alone did not have a significant effect on flowering time across the eight genotypes (Fig. 2b; $p > 0.05$ from Bonferroni tests for all genotypes). When only temperature was increased, genotypes exhibited an average 1.3 day acceleration in time to flower between preindustrial and modern conditions (Fig. 2c), with responses ranging from a 2.4 day delay (Tajikistan) to accelerations up to 4.5 days (Ukraine). Furthermore, accelerations were significant for three of the eight genotypes (Ukraine; $p = 0.0044$, Belgium; $p = 0.0022$, and Austria; $p = 0.0008$ from Bonferroni tests). Interestingly, across all treatments, the Cape Verde genotype that was pre-adapted to warmer conditions exhibited similar response patterns as the other temperate genotypes with respect to flowering time. Taken together, we found that the primary driver for accelerated flowering times between the full-preindustrial and full-modern treatment (Fig. 2a) was the interaction between rising [CO_2] and temperature, and not the individual effect of either factor (Fig. 2a–c).

To better understand how whole-plant responses affected flowering time, we assessed how growth rate (above- and belowground) was affected between preindustrial and modern conditions (Fig. 2d–f). We found a significant two-way interaction in the overall ANOVA between [CO_2] and temperature (Table S1; $p < 0.0001$), although there was not a significant three-way interaction with genotype. Growth rates were significantly increased by 14% on average among genotypes when both [CO_2] and temperature were increased between the full-preindustrial and full-modern treatment ($p < 0.0001$ for all genotypes from Bonferroni tests; Fig. 2d). However, when analyzed in isolation, neither [CO_2] (Fig. 2e) or temperature (Fig. 2f) had significant effects on growth rates among the genotypes (from Bonferroni tests among genotypes). Thus, the increase in growth rates at the full-modern condition was due to an emerging interaction between increasing [CO_2] and temperature (Fig. 2d–f).

Lastly, we assessed the effects of temperature and [CO_2] rise between preindustrial and modern conditions on total plant biomass at the time of flowering (total plant size). In the overall ANOVA, we did not find a significant three-way

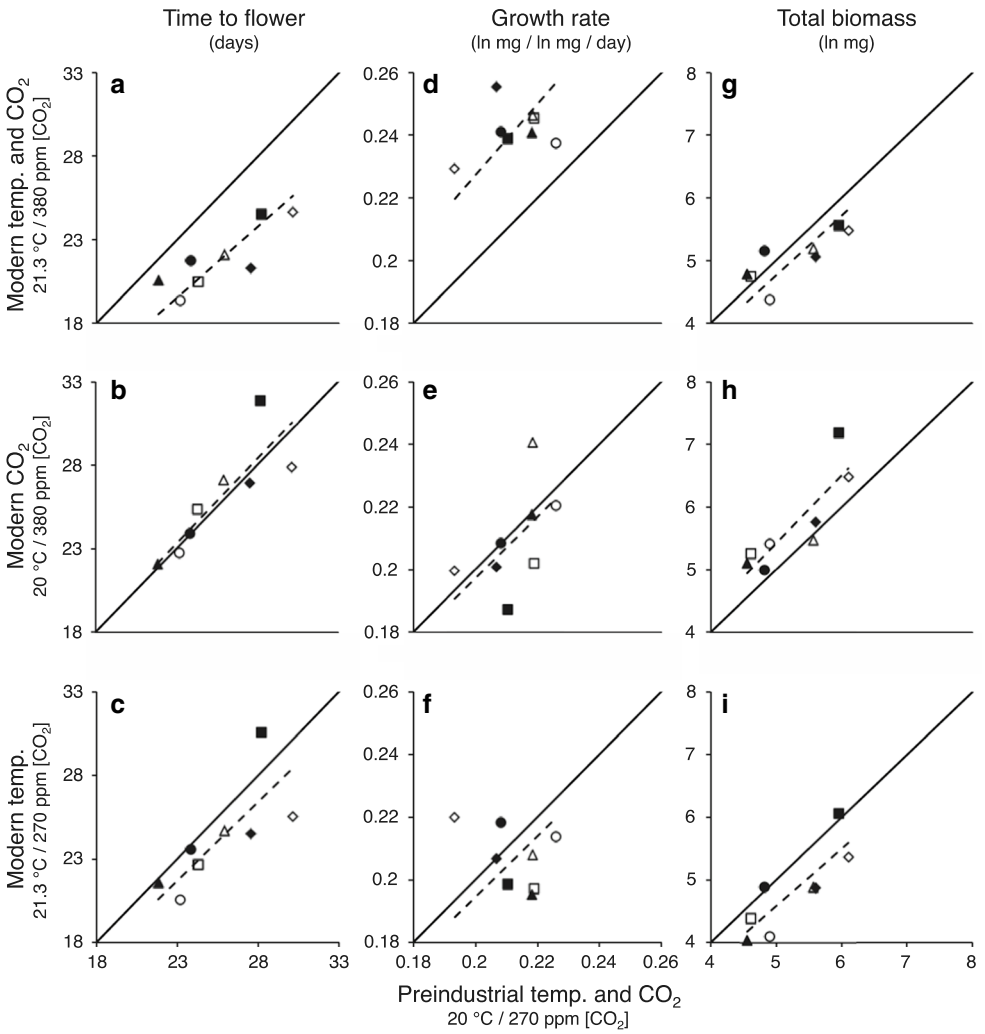

Fig. 2 Mean time to flower (**a**–**c**), growth rate (**d**–**f**), and total biomass at flowering (**g**–**i**; total of above and belowground components) for eight genotypes of *Arabidopsis thaliana*. Symbols are as follows: open squares, Cape Verde; closed squares, Tajikistan; open diamonds, Ukraine; closed diamonds, Belgium; open circles, Austria; closed circles, British Columbia; open triangles, Sweden; closed triangles, Portugal. Responses between full-preindustrial and full-modern conditions (increases in both [CO_2] and temperature) are shown in panels **a**, **d**, and **g**. Responses to increases in only [CO_2] are shown in panels **b**, **e**, and **h**, and responses to increases in only temperature are shown in panels **c**, **f**, and **i**. A solid theoretical one-to-one line is shown, whereby points falling on this line would indicate no change in response between the treatment listed on the *y*-axis relative to the full-preindustrial treatment (*x*-axis). Best-fit lines of the data are shown with dashed lines

interaction between [CO_2], temperature, and genotype (Table S1). There was, however, a significant interaction between temperature and genotype for total biomass at flowering (Table S1; $p = 0.03$), indicating that genotypes varied in their relative response patterns to increasing temperature. In addition, although [CO_2] was significant as a main effect in the overall ANOVA, the genotypes did not vary in their relative response patterns to increasing [CO_2]), as indicated by a non-significant genotype by [CO_2] interaction. Across genotypes, we found that increasing [CO_2] alone led to moderate, yet significant increases in total biomass at flowering (Fig. 2h; $p = 0.005$ from Bonferroni test). However, in isolation, temperature increase between preindustrial and modern conditions reduced average biomass at flowering by over 8% among genotypes (Fig. 2i) and these reductions were significant for three genotypes (Belgium, $p = 0.0006$; Austria, $p < 0.0001$; Portugal, $p = 0.0002$ from Bonferroni tests). When temperature and [CO_2] rise were combined in the full-modern treatment, gains in biomass at flowering as a result of increasing [CO_2] (Fig. 2h) were offset by reductions in biomass at flowering brought on by warming (Fig. 2i), resulting in no significant change in biomass at flowering between the full-preindustrial and full-modern treatments (Fig. 2g; non-significant Bonferroni tests).

Discussion

When looking broadly across the tested *Arabidopsis thaliana* genotypes, we found that accelerated flowering time between the full-preindustrial and full-modern treatments was mainly driven by an interaction between rising [CO_2] and temperature, rather than through the individual effects of either factor. We also found that whole-plant growth rates were enhanced between preindustrial and modern conditions and this was also driven by an interaction between rising [CO_2] and temperature. In addition, there was not a change in plant size at flowering due to the opposing effects of increasing [CO_2] and temperature, and therefore, accelerated flowering

was primarily driven by changing growth rates and not alterations in plant size at flowering.

For short-lived annuals such as *Arabidopsis*, the average 3.8-day acceleration in flowering time between the full-preindustrial and full-modern treatments represents an approximate 10% reduction in pre-reproductive growth time for rapid-cycling genotypes. Such large relative shifts in developmental timing would be expected to have large implications for competitive interactions and overall productivity under field scenarios. For example, it has been shown that earlier flowering in *Arabidopsis* correlates with higher productivity and survival under high intensity stress that occurs at the end of the growing season, whereas earlier flowering tends to decrease plant productivity under long term and consistent levels of mild stress in the field (Schmalenbach et al. 2014). Thus, the implications of flowering time responses to [CO_2] and temperature may manifest in different ways depending on the context of seasonality and levels of abiotic stress under field scenarios. This may have also been more pronounced in ancient ecosystems during the last glacial period since Ward et al. (1999) demonstrated that biomass accumulation at the end of the life cycle was much greater in *Arabidopsis* genotypes grown at 350 ppm CO_2 relative to lower glacial levels of 200 ppm CO_2, with some genotypes showing a doubling or more of total biomass across these treatments. These responses were also accompanied by accelerated flowering times between 200 and 350 ppm CO_2 by 5–14 days, depending on the genotype.

Even though we only investigated a narrow, historically relevant temperature gradient, we can convert our results to per degree celsius effects on flowering time for comparative purposes with other studies. In doing so, we observed an average 3 day/°C acceleration in flowering time among genotypes with the addition of rising [CO_2] (110 ppm increase) between preindustrial and modern conditions, and a 1 day/°C acceleration in response to warming alone. Recall that Wolkovich et al. (2012) found little to no changes in flowering time per degree celsius in warming-only experiments, but 4.6 day accelerations/°C in long-term field surveys that would have incorporated both temperature and [CO_2] rise (see the "Introduction"). By testing the effects of both rising [CO_2] and temperature with model plants, we demonstrated the potential to explain some of this discrepancy, whereby flowering accelerations occurred mainly through an interaction between rising [CO_2] and temperature, rather than through an increase in temperature alone.

It is also important to note that Wolkovich et al. (2012) may have seen larger responses in long-term field studies per degree celsius since these studies incorporate both changes from earlier initiation of growth (e.g., germination or leaf-out) as a result of shifting seasonality and also altered time to flowering via changes in growth/development rates. In our case, we only studied the latter effects of growth and development by controlling for the timing of the onset of growth, which was defined as seed emergence above the soil. It is also worth noting that individual and/or interactive effects of [CO_2] and temperature may affect germination, and although not assessed in this study, these responses should be measured across historical gradients in future work, since the main focus in this area has been on future climate scenarios (Footitt et al. 2018). Nonetheless, if the discrepancy we observed between warming alone versus the interaction between rising [CO_2] and temperature is relevant outside of *Arabidopsis* and under field scenarios, it suggests that increasing [CO_2] may have played a major role, in conjunction with temperature, for driving accelerated flowering times over the last century as a result of shifts in growth and development rates. Furthermore, if [CO_2] and temperature continue to interact to alter flowering time, or if the nature of this interaction changes in the future, this may complicate our ability to predict future shifts in flowering time. In addition, unexpected changes may also occur such as the large delays in flowering time (at a larger plant size and higher leaf number) that occurred in an elevated CO_2-selected genotype of *Arabidopsis* when grown at elevated CO_2 due to altered expression of the floral repressor, *FLOWERING LOCUS C*, that occurred even under highly accelerated growth rates at elevated [CO_2] (Springer et al. 2008).

While it is perhaps surprising that warming alone did not lead to greater accelerations in flowering, it should be noted that while the temperature gradient used here (1.3 °C) is of historical relevance, it is lower than most temperature gradients used in previous studies with *Arabidopsis* (Balasubramanian et al. 2006; Lutz et al. 2015). For example, Lutz observed that shifts in temperature between 15 and 21 °C produced large accelerations in flowering in the Columbia line of *Arabidopsis* through modulation of the ambient temperature floral regulator gene *Flowering Locus M*, where plants flowered after nearly 60 days under 15 °C and at approximately 25 days under 21 °C (Lutz et al. 2015). It is likely that the historical temperature increase used here was not substantial enough to trigger strong accelerations in flowering via the ambient temperature regulatory mechanisms identified by Balasubramanian et al. (2006) and Lutz et al. (2015), and that temperature gradients at lower ranges may include higher responses per degree Celsius in some cases.

It is clear that increasing [CO_2] and temperature increased growth rates that had a subsequent effect on flower times, and these were likely manifested through effects on net photosynthesis rates. Bunce (2008) found that the Columbia ecotype of *Arabidopsis* showed increasing rates of net photosynthesis when measured across 10–30 °C (with stabilization between 30 and 35 °C), and this included the 21.0–21.3 °C range used in our study. Interestingly, he also showed that long-term growth temperature conditions

(15, 21 or 27 °C) did not alter photosynthetic responses to changing temperature, and therefore, it was concluded that *Arabidopsis* may have limited potential for photosynthetic acclimation to temperature (Bunce 2008). Furthermore, photosynthetic response curves with *Arabidopsis* showed very steep increases in net photosynthetic rates across c_i (intercellular [CO_2]) values ranging from 200 to 400 ppm CO_2, which would encompass the c_i ranges occurring in our study (Easlon et al. 2015). Furthermore, increasing [CO_2] from preindustrial to modern values has been shown to increase photosynthetic rates and biomass accumulation across a broad range of C_3 species (Gerhart and Ward 2010). In the present study, it is likely that the combination of increasing temperature and [CO_2] from preindustrial to modern levels were both needed to enhanced photosynthetic rates and subsequently growth rates, since the gradients were relatively small for both factors (to accommodate historical changes), and because increasing [CO_2] has the capacity to enhance the effects of warming on photosynthesis across non-stressful ranges (Norby and Luo 2004).

It has been hypothesized that elevated [CO_2] may drive accelerated flowering via stomatal closure that may increase leaf temperature through loss of evaporative cooling, resulting in accelerated flowering through indirect effects of warming. From a meta-analysis of free-air carbon enrichment (FACE) studies, average stomatal conductance was found to decrease by 22% among C_3 species grown at elevated [CO_2] conditions (Ainsworth and Rogers 2007). Stomatal conductance was also found to decrease in *Arabidopsis* plants grown at elevated [CO_2] (800 ppm) by 32% compared with current conditions (Wang et al. 2015). Across a wide array of species, however, reduced stomatal conductance at elevated [CO_2] produces only an average 0.7 °C increase in leaf temperature (Kimball 2016), and this would not have had a large effect on flowering times based on the direct temperature responses observed in the current study. Additionally, it has been observed that stomatal conductance tends to be relatively unresponsive to rising [CO_2] between preindustrial and modern treatments (Farquhar and Sharkey 1982; Ward et al. 1999). Therefore, it is unlikely that a secondary effect of warming from potential stomatal closure between preindustrial and modern conditions had a large effect on flowering time in our case.

The effects of microRNAs may provide a potential molecular mechanism through which rising [CO_2] and temperature could interact to accelerate flowering. Interestingly, all microRNAs in *Arabidopsis* that exhibited altered expression levels at elevated [CO_2] also showed inverse responses at higher temperatures (3–6 °C increases). Furthermore, both of these factors were shown to influence miR156/157 and miR172 expression, which may influence development rates, including flowering times (May et al. 2013). It is not clear if these miRNAs respond to the magnitude of temperature and [CO_2] change that occurred over the last century, and therefore, this issue warrants further study for both past and future climate change.

In closing, our study with the *Arabidopsis* model system suggests that the interactive effects of rising [CO_2] and temperature over the last century may explain why there has been observed accelerations in flowering times in long-term field surveys that cannot be replicated in warming-only studies (Wolkovich et al. 2012). Future work with additional species will be critical for weighing the generality of this finding across non-model systems. Furthermore, it is possible that the interactions between [CO_2] and temperature on flowering time may be complicated by other factors in the field, such as alterations in the length of the growing season, competition, nutrient availability, and light regimes. Although these factors need consideration, we conclude that rising [CO_2] may have had the potential to interact with warming to drive advances in flowering times that have been observed over the last century. Such interactive effects should be considered when investigating both past and future responses of flowering time to climate change drivers.

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Author contribution statement SMW and JKW conceived and designed the experiments, performed the experiments, analyzed the data, and wrote the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflicts of interest.

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