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CHANGE IN CARBON DIOXIDE EFFECT ON REPRODUCTIVE MORPHOLOGY AND PHENOLOGY OF *ARABIDOPSIS* ECOTYPES

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ABSTRACT: An increase in anthropogenic generation of carbon dioxide (CO_2) is one of the characteristic features of global climate change. Elevated CO_2 affects plant reproduction and development, both of which influence plant fitness. The aim of this project was to quantify the effects of increasing CO_2 on reproductive morphological features and phenology of ecotypes of *Arabidopsis thaliana*, a primary model plant species, originating from different altitudes and geographical origins. Ecotypes of *A.thaliana* were grown in controlled environment chambers to assess genetic constraints on phenotypic plasticity across a wide range of atmospheric CO_2 concentrations. Elevated CO_2 had a significant positive impact on plant fitness in terms of seed mass and silique size. Sub-ambient CO_2 had the opposite effect of reducing plant fitness; all measured variables except fruit size were significantly reduced by sub-ambient CO_2. Flowering and fruiting time was hastened under elevated CO_2 and delayed under sub- ambient CO_2 conditions. This study therefore show that elevated CO_2 improves plant fitness and low CO_2 is stressful to *Arabidopsis thaliana*. All plant fitness variables failed to show any significant relationship with altitude of origin under different CO_2 concentrations.

Key words: *Arabidopsis thaliana;* CO_2 enrichment; Sub-ambient CO_2; Plant fitness; Altitude; Reproductive phenology

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INTRODUCTION

The reported increase in atmospheric carbon dioxide is a primary driver of global climate change with overwhelming consensus on the future increase of this environmental factor. Plants constantly monitor their surroundings and responds by changing their life forms. It is important to understand how plants respond to atmospheric changes in order to enhance our ability to predict plant responses to future CO_2 changes [1]. In addition low CO_2 studies provide a baseline for defining plant responses to minimum CO_2 of the geologic past such that changes in plant functioning in response to rising CO_2 can be assessed within a broader context [2].

Growth at elevated CO_2 affects plant reproduction and development, both of which influence plant fitness, [3]. Seeds are strong carbon sinks [4] and therefore likely to increase in their mass in high CO_2 therefore yield may increase with CO_2 elevation. Examples include rice (*Oriza sativa*) yield increase by 57% [5], seeds of *Triticum aestivum* and *Glycine max* increased mass significantly at a CO_2 concentration of 690 ppm [6].

The magnitude of seed number response in this study was species-specific for example rice (*Oryza sativa*) had an increase of 42%, soybean (*Glycine max*) (of 20%, wheat (*Triticum aestivum*) of 15% and maize (*Zea mays*) of 5%. However Way *et al.* [7] realized an insignificant difference in the mass of *Pinus taeda* mature seeds grown under ambient CO_2 and elevated CO_2. This implies that carbon allocation to seeds may increase seed numbers but not mass.

Alongside photosynthesis and yield increases at elevated CO_2, total biomass of plants also increases at elevated CO_2. For example Saxe *et al.* [8] reported a 130% and 49% increase of biomass in conifers and deciduous plants. Kimbal *et al.* [9] found the same results of highly stimulated biomass by CO_2 enrichment in terms of fruit biomass, trunks, stumps, branches and large roots of sour orange trees. In their meta- analysis study involving 159 CO_2 enrichment papers in which 79 species were studied, Jablonski *et al.* [10] found that elevated CO_2 of between 500 and 800 ppm increased flower numbers up to 19%, fruit numbers up to 18% and total seed mass increased by 25%. Phenology in terms of the flowering time of species is also affected. Springer and Ward [11] reported variable responses to CO_2 elevation. Among economically cultivated crops *Sorghum bicolor* exhibited delayed flowering, *Oryza sativa* and barley (*Hordeum vulgare*) exhibited early flowering. These authors reported that the commonly studied ecotype of *Arabidopsis thaliana* Col-0 exhibited delayed flowering with elevated CO_2 (1000 ppm). The above results suggest that effects of CO_2 on flowering time are species- specific. Flower senescence is also affected by variation in CO_2, for example that of *Tropaeolum majus* was delayed under elevated CO_2 [12].

Although there is a vast amount of information that has addressed the gross effects of increasing CO_2 on plants many of these investigations focus on inter-specific or community responses. Little work has investigated within species effects, yet investigating differences within species reduces problems associated with genetic background and constraints of comparing different species [13].

The aim of this study was to quantify the effects of changing CO_2 concentration on reproductive characteristics of plants of ecotypes of *A.thaliana* with different geographical and altitude origin (Table 1). This broad distribution encompasses different growing conditions hence phenotypic variation among ecotypes is expected to reflect the genetic variation that enables them to adapt to specific growing conditions [14]. Altitudinal differences are important to plants because the partial pressure of CO_2 and temperature decline with increasing altitude whereas solar irradiation increases.

It is reported that plants originating from different altitudes remain different when grown at the same altitudes [15]. There is little reported literature on investigations that involve ecotypes from different altitudes, however a similar study to the present investigations performed on *Arabidopsis's* genotypes was that of Ward and Strain [16] in which they measured biomass and reproduction. None of the ecotypes used in this study were used in their study. An ecotype in this context is a distinct race of a species genetically adapted to a particular habitat (wild homozygous lines) [14].

Here we report on reproductive morphological traits including reproductive phenology. We test the following hypotheses:

1. There would be no significant variations in the responses of different ecotypes to elevated, ambient, and sub-ambient CO_2.
2. Altitudinal origin would have no significant effect on responses to the different CO_2 concentrations.

A. thaliana was chosen for this project because it provides a rapid experimental throughput having a life from germination to seed set approximately nine weeks. It also has a rich source of natural genetic diversity. *A. thaliana* is distributed widely in the moderate temperate zones of the world [16] and provides an excellent research model for determining plant responses to environmental change [17].

MATERIALS AND METHODS

Plant material and description

Nine ecotypes from different geographical and altitudinal origins of *Arabidopsis thaliana* (Nottingham Arabidopsis stock centre, UK, http://www.nasc.nott.ac.uk/home.html) were used in these investigations (Table 1). The seeds of these ecotypes were a maximum of two generations old and therefore the risk of genetic drift was minimal [18].

Growing conditions

Seedlings were grown as in Lake and Woodward [19]. In addition, every week the growing conditions and the plants were transferred between chambers and rotated within each chamber set at 400 (ambient CO_2 or ACO_2) and either 800 (elevated CO_2 or ECO_2) ppm or 280 (sub-ambient CO_2 or subACO_2) ppm to minimize chamber effects. Pots were watered regularly from the base to maintain moist compost conditions at all times. The treatments were maintained from germination to maturity as when the siliques turned brown.

Table 1: Ecotypes of A. thaliana and their place of origin, altitude and description [18]

Ecotype & Stork No	Country	Location	Altitude (m)	Brief description of ecotype
Col-0 N1092	US	Columbia	1-100	Hairy leaves with serrate margins, H: 15-24cm
Ksk-1 N1634	UK	Keswick, UK	1-100	(no information)
Su-0 N1540	UK	Southport	1-100	Early leaves rounded, late leaves elongated, H: 24-32cm
Lan-0 N1304	UK	Lanack	100 -200	Height: 33-44cm, Large leaves, weakly hairy
Mt-0 N1384	Libya	Martuba/Acyre naika	100 -200	Height: 16-26cm
Rsch-4 N1494	USSR	Rschew/Starize	100 -200	H: 34-42cm. Large rosette, single flowering stem, slightly serrated leaves
Ba-1 N952	UK	Blackmount	500 -600	H: 15-25cm. *Sinuate* leaves
Mc-0 N1362	UK	Mickles Fell	790	Bright green thin leaves with serrate margins. H: 28-38cm.
Can-0 N1064	Spain	Canary Islands	1260	H: 15-22cm. Rosette turns pink on maturing. Possibly late flowering

Floral stem height, floral branch number and inflorescence number:

These measurements were collected after plants had reached maturity. The height of each floral stem was measured with a ruler from where the reproductive stalk emerged from the compost to the tip of the main reproductive branch (floral stem). Primary branches (co florescences) were counted from five plants *per* ecotype. The total number of inflorescences (was counted from the same number of plants for each ecotype).

Silique lengths and number of siliques:

From each plant, the length of 5 siliques was measured after the siliques had ripened. The measurement included that of the silique and its stalk. The number of siliques per plant was found by counting all the brown siliques from each plant for a period of one month after the plants had reached maturity. After each count the ripe siliques were removed from the plant to avoid recounts. The counting was conducted every two days.

Mass of dry seeds:

To obtain the mass of the total number of seeds, ten intact ripe siliques were harvested *per* plant dried in an oven at 50 °C for five days. With the use of forceps the seeds were removed manually and put in labelled ependorfs. The seeds were then weighed to give the mass of seeds per silique. This then multiplied by the total number of siliques per plant. The total number of plants harvested per ecotype per treatment was 5.

Reproductive phenology:

The number of flowering days and ripening (maturing of siliques) days were recorded.

Statistical analysis

Results were analysed using ANOVA general linear model procedures in Minitab, statistical program version 15 and 16 (Minitab Ltd, USA). Two-way ANOVA was used to assess the effects of CO_2 and ecotypes on the different variables and significant interactions between ecotypes and treatments (main effects). Correlation analysis was performed to determine the relationship between altitude and the different plant traits. Coefficients of linear regressions were calculated using Minitab.

RESULTS AND DISCUSSION

Floral stem height, branch number and inflorescence number

The increase in CO_2 concentration had no significant effect on floral stem height for most ecotypes except for Ksk-1 ($p \leq 0.01$) and Su-0 ($p \leq 0.05$) which had significantly shorter floral stems. Though plants growing under sub-ambient CO_2 were shorter in size, the decrease was significant for only one ecotype, Can-0 ($p \leq 0.01$) (Figure1). There was no significant correlation between altitude of origin and floral heights. Variation in CO_2 concentration did not significantly affect the number of branches (Figure 2). Despite this insignificant result, the CO_2 enriched plants looked bushier due to many leaves and branches than those grown under ACO_2 and sub-ambient CO_2 ones.

There was a highly significant and positive correlation between altitude of origin and number of branches R2= 0.67, p-value = 0.007. High altitude ecotypes had the largest number of branches when grown under ECO_2 enrichment (Figure 3). This relationship was positive but not significant under Sub-ACO_2.

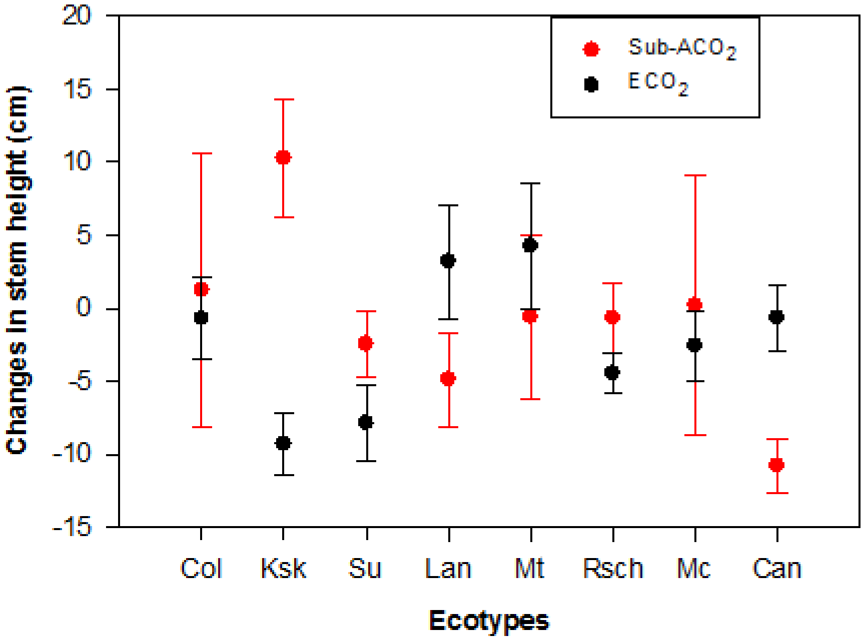

Figure 1: Variations in the floral stem height of ecotypes of *A.thaliana* grown in different CO$_2$ concentrations. Values = mean differences between ECO$_2$/ACO$_2$ and between sub-ACO$_2$/ACO$_2$ and mean differences. Error bars = standard error of mean (SE mean)

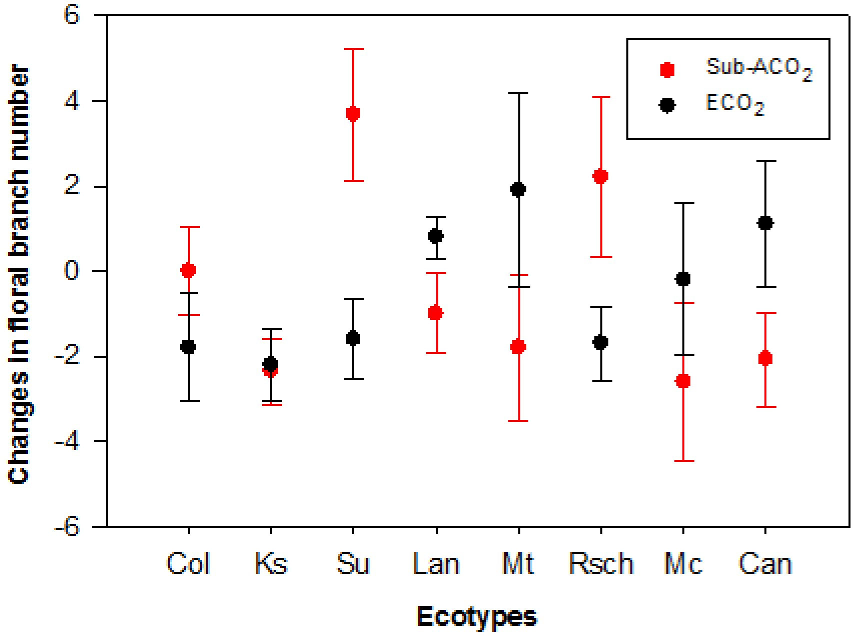

Figure 2: Variations in the floral branch of ecotypes of *A.thaliana* grown in different CO$_2$ concentrations. Values = mean differences between ECO$_2$/ACO$_2$ and between Sub-ACO$_2$/ACO$_2$ and mean differences.

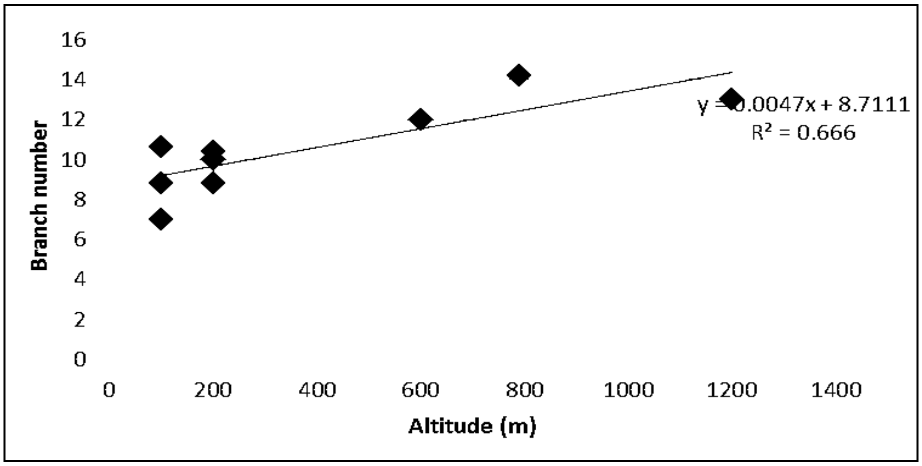

Figure 3: Relationship between altitude of origin and the number of branches in ECO_2. Each point represent the mean of each ecotype. Values = means. (n=5)

CO_2 enrichment significantly reduced the number of inflorescences. The inflorescence numbers of Ksk-1 and Can-0 were significantly reduced ($p \leq 0.05$) (Figure 4). Ecotypes from above 100 m altitude produced a higher number of inflorescences under ECO_2 while the 100 m ecotypes produced a smaller number of inflorescence on average. Under sub-ACO_2 the trend was the same as for that of the number of branches, there was no significant effect of sub-ACO_2 on inflorescence number. There was no correlation between this variable and altitude of origin in both CO_2 concentrations.

Plant fitness

Ecotypes varied in their silique length with most having longer siliques under ECO_2, and shorter under sub-ACO_2. The increase was highly significant for Mt-0 under ECO_2 ($p \leq 0.001$) and only significant for Lan-0 under sub-ACO_2 ($p \leq 0.01$) (Figure 5). Silique number was counted only for sub-ACO_2 experiment and all ecotypes had a reduced number of siliques under sub-ACO_2, but only Mc-0, and Can-0 had significantly reduced silique numbers ($p \leq 0.05$) (Figure 6).
Seed mass was strongly dependent on the CO_2 concentration; most ecotypes having increased seed mass under CO_2 enrichment. The increase under ECO_2 was statistically significant for Mt-0 ($p \leq 0.01$), Rsch-4 ($p \leq 0.01$) and Can-0 ($p \leq 0.05$). Ksk-1 was the only ecotype with significantly reduced seed mass under CO_2 enrichment ($p \leq 0.05$) (Figure 7). Ecotypes were significantly different in absolute mass of seeds and the increase expressed as a percentage varied from 7 to 300% from ACO_2 to ECO_2. Sub-ACO_2 had no significant effect on the mass of seeds. Under both sub-ACO_2 and ECO_2 the correlation between altitude of origin and all plant fitness variables was insignificant.

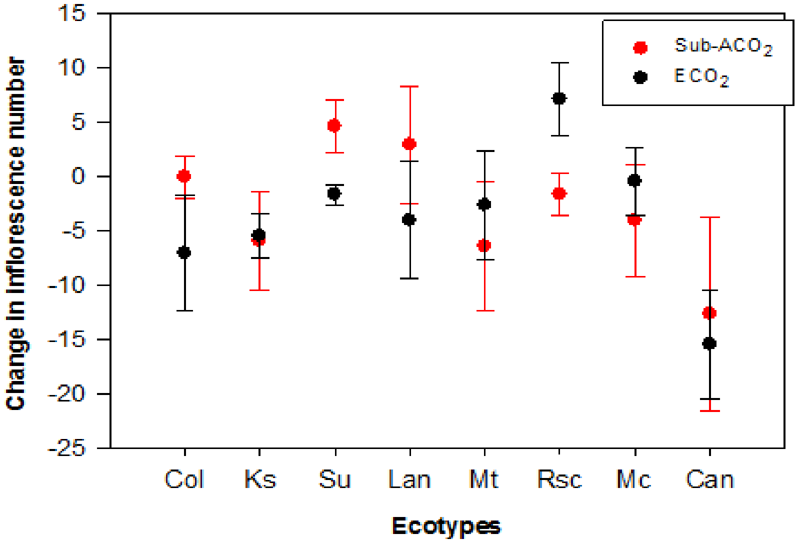

Figure 4: Variations in the number of inflorescence of ecotypes of *A.thaliana* grown in different CO_2 concentrations. Values = mean differences between ECO_2/ACO_2 and between sub-ACO_2/ACO_2 and mean differences.

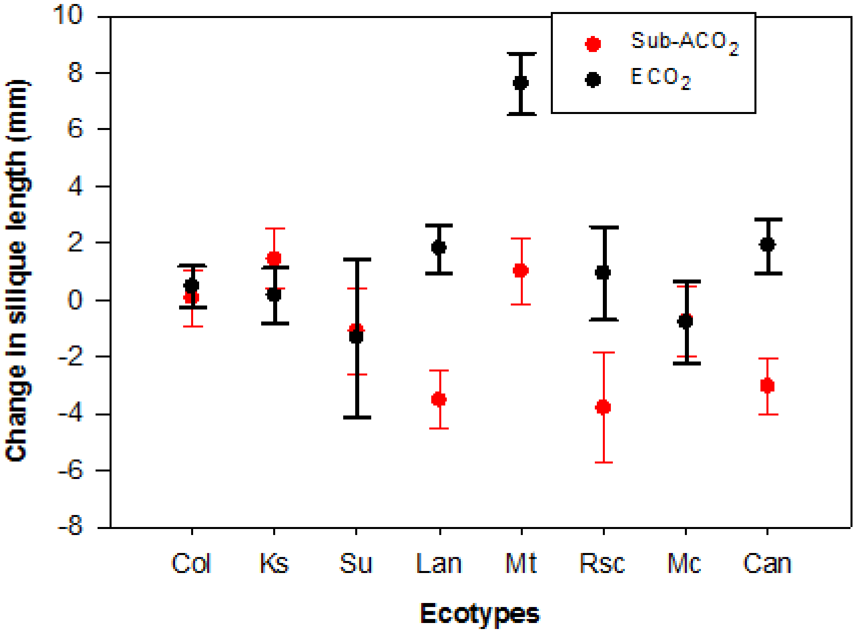

Figure 5: Variations in the silique length of ecotypes of *A.thaliana* grown in different CO_2 concentrations. Values = mean differences between ECO_2/ACO_2 and between sub-ACO_2/ACO_2 and mean differences.

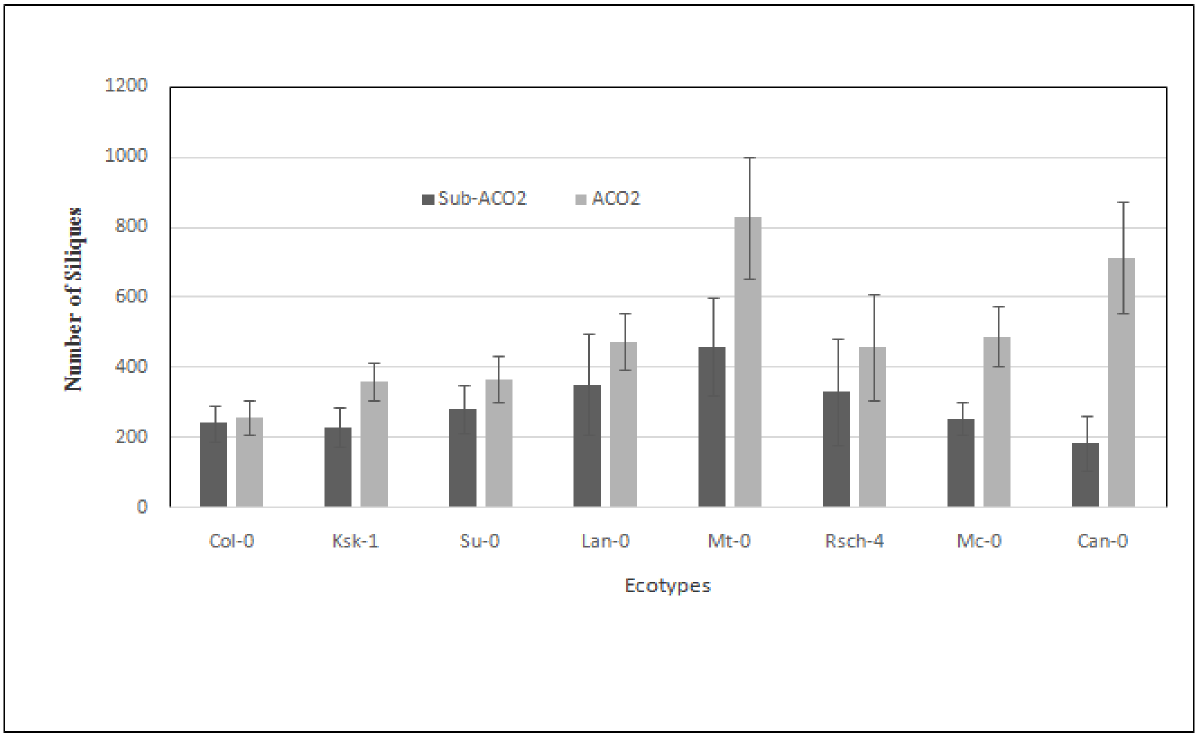

Figure 6: Number of siliques of ecotypes of *A. thaliana* grown under Sub-ACO_2 and ACO_2. Values = Means and SE

Reproductive phenology: Flowering and maturing time:
Flowering occurred earlier under CO_2 enrichment and was delayed under sub-ACO_2 in all ecotypes (Table 2). Ecotypes took 43 to 60 days to flower under ECO_2 and 64 to 138 days under sub-ACO_2. The ecotypes also matured earlier under ECO_2 compared to under sub-ACO_2 (Table 2). In ECO_2 the time of maturing was between 63 and 79 days and under sub-ACO_2 was between 85to 158 days. But there was no correlation between the numbers of days to flower/mature and altitude of origin for these ecotypes. For both flowering and maturing time Su-0 took longer days than other ecotypes.

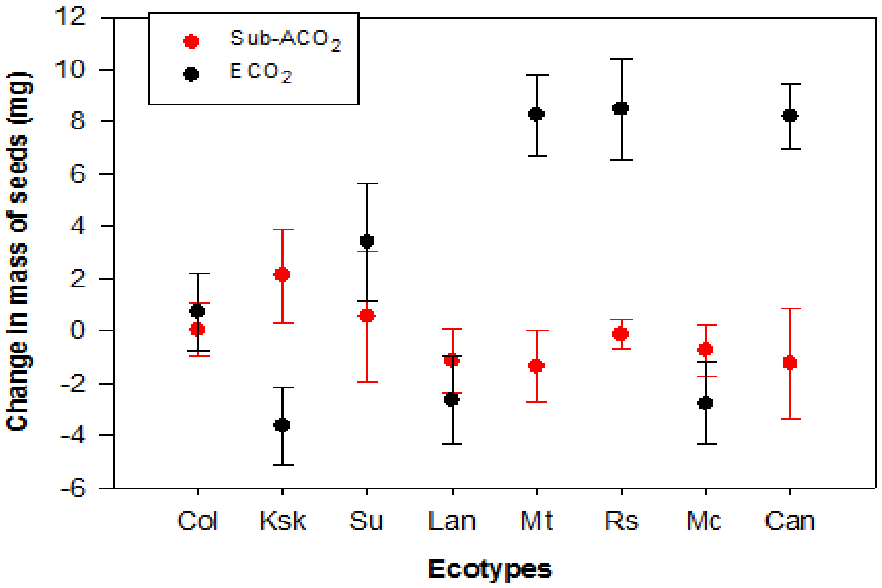

Figure 7: Variations in the mass of seeds of ecotypes of *A.thaliana* grown in different CO_2 concentrations. (Mass of seeds from 10 siliques) Values = mean differences between ECO_2/ACO_2 and between sub-ACO_2/ACO_2 and mean differences.

Table 2: The average number of days ecotypes took to produce first flowers (FD) and mature (MD) under different CO_2 concentrations

Ecotypes and altitude (m)	**Number of days ecotypes took to flower and mature**					
	Sub-ACO_2		**ACO_2**		**ECO_2**	
	FD	**MD**	**FD**	**MD**	**FD**	**MD**
Col-0 100	84	99	60	77	49	64
Ksk-1 100	69	88	55	70	53	64
Su-0 100	138	158	84	104	60	79
Lan-0 200	85	99	55	76	50	68
Mt-0 200	74	95	50	78	43	63
Rsch-0 200	67	85	54	72	48	67
Mc-0 790	90	102	63	70	56	70
Can-0 1200	64	95	50	72	48	67

DISCUSSION

There was no significant effect of CO_2 variation on plant height. However, plants' floral stems in elevated CO_2 were observed to be bushier and were not prone to lodging suggesting that the plants could have produced more leaves in their floral stems instead of increasing their floral height. This suggests that for crops that are grown for leaf material, future global CO_2 increase may be of benefit. The stature and volume of plant in sub-ACO_2 were observed to be smaller than those growing under elevated CO_2. The upright habit shown by plants under elevated CO_2 could be due to stronger root system and greater capacity for increased turgor since CO_2 enrichment is reported to increase root growth [20].
Higher altitude ecotypes produced more branches than the lower altitude ones; this was significant under ECO_2.This response was also observed under ambient conditions. There are several reports that show positive response of both branches and stem heights to elevated CO_2; Radoglou and Jarvis [21] reported taller and more branches for four poplar clones *(Populus), Cenchrus ciliaris's* plant height increased by 43% [22]. Prichard *et al.* [23] in their review singled out *Rhizophora mangle* as a species in which branch number increased by 60%, while the number of tillers of *Agrostis capillaries* increased by 20% but *Castanea sativa* had no changes in height and branch numbers. Altitude of origin did not have an impact on the heights of plants. This is contrary to Woodward [24] findings on *Vaccinium myrtillus* where the plant heights declined with altitude.

There was a significant decrease in the number of inflorescences under elevated CO_2. These results contrast with that of Ainsworth [25] who showed that panicle number of rice increased under ECO_2 and that of Yang *et al.* [26] that showed increased panicle number in rice cultivars. The high stomatal density reported from another experiment conducted with the same set of plants suggested low water use efficiency [18]. This might explain why there was no significant growth rate in heights, and number of inflorescences under ECO_2. Eamus [27] explains that an increase in photosynthesis is reflected by increase in growth rate of the plants. Sub-ACO_2 did not significantly affect the inflorescence number, a response similar to that of stems and number of branches.

CO_2 enrichment generally enhanced reproductive plant fitness in this study. The percentage increase of seed mass under elevated CO_2 varied from as little as 7% to as great as 300% for these ecotypes. Silique length was also enhanced under elevated CO_2, and it partly explained the increased seed mass. Enhanced fitness suggests that photosynthates were partitioned to reproductive parts compared to vegetative parts. In addition ECO_2 tends to increase the time for seed filling [28]. An increased mass of seeds is a common response under CO_2 enrichment. Jablonski *et al.* [10] reported an increased seed mass for legumes and non-legumes in their study. Variation in the magnitude of increase in seed mass has been reported by Sicher *et al.* [29] among soybean cultivars under CO_2 enrichment. Sink capacity of the two cultivars was argued as the reason for the difference. Bunce [30] also found seed yield increased significantly under ECO_2 for varieties of *Phaseolus vulgare*. The enhanced plant fitness shown by these results signify some of the positive effects that changes in climate might bring. Both fitness variables assessed for ECO_2 were not significantly correlated with altitude of origin. But ecotypes from above 200 m altitudes seemed to have been more sensitive to CO_2 increase since they produced heavier seeds under CO_2 enrichment compared to ambient conditions

Sub-ambient CO_2 had a detrimental effect on plant fitness in terms of seed mass, silique length and silique number. This was consistent with reports of Ward and Kelly [17] and Ward and Strain [16] who found a reduction in growth, seed number and reproductive failure of Arabidopsis ecotypes under sub-ambient CO_2 concentration. Royer [31] attributed the decline in plant fitness under sub-ACO_2 to limited
photosynthesis.

Time of flowering was significantly hastened under ECO_2 compared to sub-ACO_2. The time for production of mature siliques or onset of maturation of fruits was advanced significantly under CO_2 enrichment in this study and delayed under sub-ACO_2. This agrees with Wullschleger et al. [20] findings that plants grow faster in CO_2 enrichment. CO_2 enrichment often alters development rate of plants and in most cases it often hastens time to reach floral stage [17] or advances time of flowering [32] and the onset of seed production [13]. The results showed that CO_2 enrichment had quickened the rate of maturation in the vegetative phase hence the earlier floral development [17].

The late initiation of flowering and maturing in sub-ACO_2 could suggest a limited supply of photosynthates under low concentration of CO_2 hence the longer time the plants took to reach the minimum size required for reproduction [16].The ecotypes Su-0 displayed winter annual life cycle of growth. It took a very long time after producing leaves to produce flowers and siliques compared to the rest of the ecotypes under both conditions; furthermore this time was even longer under sub-ACO_2. A change in CO_2 concentrations implies a shift in reproductive phenology which could have an effect on the future ecosystems.

CONCLUSION

A. thaliana ecotypes floral stem heights were not significantly affected by increased CO_2 concentration. Branch numbers of high altitude ecotypes was increased by increased CO_2. Inflorescence number was reduced under ECO_2 but not affected by sub-ambient CO_2. Elevated CO_2 enhanced plant fitness whereas sub-ACO_2 reduced it. Seed mass was exceptionally enhanced by CO_2 enrichment, and the magnitude of increase showed a great variation between the ecotypes even though this variation could not be explained by different altitudinal origins. Reproductive phenology was hastened by ECO_2 and delayed under sub-ambient CO_2. These results showed a limited variation in genotype response to changes in CO_2 concentrations. The positive reaction of plant fitness and reproductive phenology to elevated CO_2 suggests that the future climate changes especially increase of CO_2 might contribute to better yields. However growth of plants is controlled by different environmental factors that include temperature, water supply, humidity, and wind speed as well as soil factors [33]. The difficulty to recreate environmental features at high altitudes may have a bearing on the results found here. This highlights the necessity of monitoring plants in their natural environments if we are to fully understand the effects of climate change [34].

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CONFLICT OF INTEREST: None

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