Research Article

Atmospheric CO_2 concentration affects the life cycle, yield, and fruit quality of early maturing edible legume cultivars

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Abstract

BACKGROUND: Elevated CO_2 usually reduces levels of proteins and essential micronutrients in crops. The adoption of early maturing varieties may minimize the deleterious effect of climate change on farming activities. Legumes stand out for their high nutritional quality, so the objective was to study whether the atmospheric CO_2 concentration affected the growth, yield, and food quality of early maturing cultivars of peas, snap beans, and faba beans. Plants grew in greenhouses either at ambient (ACO_2, 392 µmol mol^{-1}) or under elevated (ECO_2, 700 µmol mol^{-1}) CO_2 levels. Minerals, proteins, sugars, and phenolic compounds were measured in grains of peas and faba beans, and in pods of snap beans.

RESULTS: The effect of ECO_2 depended on legume species, being more evident for food quality than for vegetative growth and yield. The ECO_2 increased Fe and P in faba bean grains, and Ca in snap bean pods. Under ECO_2, grains of pea and faba bean increased levels of proteins and phenolics, respectively, and the sugars-to-protein ratio decreased in pods of snap beans.

CONCLUSION: Early maturing varieties of legumes appear to be an interesting tool to cope with the negative effects that a long exposure to rising CO_2 can exert on food quality.
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Keywords: atmospheric CO_2; faba bean; minerals; pea; proteins; snap bean

INTRODUCTION

The current agricultural system and food security are threatened by a combination of different events, including an increasing population, the need for more sustainable development, and the impacts of climate change.[1] According to a report published in 2016 by the Food and Agriculture Organization of the United Nations (FAO),[2] it has been estimated that global food production will need to increase by 60% to feed over 9.5 billion people by 2050. Moreover, the Intergovernmental Panel on Climate Change has estimated that the concentration of atmospheric CO_2 will reach values of 550 ppm by 2050 and it will continue increasing until it reaches ca. 700 ppm at the end of this century.[3] The predicted climate change will not only affect food availability but also food accessibility, food utilization, and the stability of food systems.[4] With these prospects of population increase in a context of climate change, several studies have reported that global increases in animal production and consumption are unsustainable, so that numerous experts have supported the idea of substituting foods of animal origin for others of vegetable origin such as legumes and pseudo-cereals, especially in developed countries.[5,6] Elevated CO_2, however, can cause significant decreases in the concentrations of proteins and some

micronutrients that are essential for human health (zinc and iron, for example) in the edible tissues and organs of C3 crops, which will reinforce the 'hidden hunger' that affects millions of people, including in developed countries.[7] Some of the causes of this would be the 'dilution effect' due to increased photosynthesis and thus carbon fixation and the decreased mineral absorption from the soil as a consequence of reduced transpiration levels.[8] As summarized in the title of the article published by Weigel,[9]

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therefore, results and conclusions from research performed in the last decade support the view that 'plant quality declines as CO_2 levels rise'.

One strategy to diminish the negative effects of climate change on agriculture is the adoption of climate-resilient crops, including heat, drought, and/or salinity tolerant varieties, and early maturing crops.[10] The adoption of early maturing crops or cultivars is an alternative that can reduce the effect of climate change on farming activities affected by shortened rainfall patterns, heat waves, erratic rainfalls, or drought. It has mainly been applied to cereals, such as maize[11] and winter wheat.[12]

Together with cereals, legumes are the most important C3 crop family because they have multiple applications, including animal forage, aquaculture feed and human food.[13] Moreover, legumes are very good candidates in a context of sustainable agriculture because they can fix atmospheric nitrogen, release high-quality organic matter in the soil, enhance water retention, and improve the circulation of nutrients in the soil. All these qualities make legumes beneficial for crop rotation and intercropping with cereals, so that the selection of the most adequate species and cultivars for the different cropping systems appears as a key point for future agriculture.[5,14]

The selection of early maturing legumes is a topic of increasing interest. In fact, together with yield, early maturity is a valuable pea trait for farmers and flowering time plays a major role in the adaptation of pea to different environments.[15] Malovichko et al.[16] tried to elucidate the mechanisms involved in early seed maturation in Pisum sativum and found enhanced expression of transposable element-associated open reading frames and altered expression of major maturation regulators in an early maturation pea cultivar. According to Etemadi et al.,[17] early maturity genotypes of faba bean are not necessarily inferior to late-maturity varieties and they may provide an opportunity for double cropping. A careful selection of the earliest maturing faba bean could allow its cultivation in regions with a growing season of less than 100 days, thus making this legume suitable for the boreal zone in Europe, Asia, and North America.[18] Under Mediterranean conditions, where drought and heat stress can compromise the viability, development and yield of common bean, the selection of early flowering and quick pod-setting varieties appears as suitable alternatives to avoid or diminish the negative effects of these abiotic stresses.[19]

From the nutritional point of view, legumes are an important source of proteins, fiber, carbohydrate, B vitamins, and mineral nutrients such as iron, copper, manganese, zinc, magnesium, and phosphorous. Their benefits for health include reducing the risk of developing type 2 diabetes, hyperlipidemia, and hypertension, and aiding weight control.[20] They also show anti-oxidant and anticarcinogenic activities due to the presence of resistant starch, soluble and insoluble dietary fiber, phenolic compounds and other compounds such as phytic acid, protease inhibitors and saponins.[21,22] Snap beans, peas, and faba beans are some of the most important legumes for human diet and animal feed.[23] The world production of snap beans, dry peas, and faba beans in 2019 achieved around 27, 14, and 5.5 million tons, respectively, which means increases in the production of around 30% and 53% in dry peas and faba beans in comparison with 1999, and the production of snap bean trebled that obtained 20 years ago.[24]

Taking into account all these precedents, the objective of the present study was to test the extent to which the CO_2 concentration in the atmosphere affects growth, yield, and food quality of early maturing cultivars of garden peas (Pisum sativum L.), snap beans (Phaseolus vulgaris L.), and faba beans (Vicia faba L.), commonly commercialized in Spain.

MATERIALS AND METHODS

Biological material and experimental design

Seeds of snap beans (cv. Nassau), peas (cv. Dulce de Provenza), and faba beans (cv. Muchamiel) (Semillas Battle, Molins de Rei, Barcelona, Spain) were sown in perlite on January 8, 2019. These three legumes and the selected varieties are widely commercialized for consumption in the human diet and have a short life cycle (around 2 months for P. vulgaris, and 3–4 months for P. sativum and V. faba according to commercial information). When seedlings had 2–3 fully developed leaves (January 16, 2019) they were transferred to 2 L pots (one plant per pot) filled with a mixture of vermiculite, sand, and peat (2.5:2.5:1, v/v/v). There were eight pots for each different legume. The substrate was previously sterilized at 100 °C for 1 h on 3 consecutive days to eliminate possible microorganisms mainly present in the peat. Properties of the peat (Floragard, Vilassar de Mar, Barcelona, Spain) were pH 5.2–6.0, nitrogen 70–150 mg L^{-1}, P_2O_5 80–180 mg L^{-1}, and K_2O 140–220 mg L^{-1}.

At transplanting, half of the plants from each type of legume were transferred to a greenhouse with ambient CO_2 concentration in the air (ACO_2, set at 392 µmol mol^{-1}, measured experiment mean 395 ± 20 µmol mol^{-1}); the other half of the plants from each type of legume were transferred to a greenhouse with elevated CO_2 concentration in the air (ECO_2, set at 700 µmol mol^{-1}, measured experiment mean 710 ± 22 µmol mol^{-1}). Plants were cultivated under different CO_2 concentrations in the air from January 16, 2019.

Atmospheric CO_2 concentrations were constantly monitored by using a 'Guardian Plus Infra-Red Gas Monitor' (Edinburgh Instruments Limited, Livingston, UK). All pots were switched between the greenhouses and the pots were randomized within treatments to eliminate edge effects.

All plants were grown at 25/15 °C (d/n) temperatures, 50/85% (d/n) relative humidity and received natural daylight supplemented with irradiation from sunlight-type Osram HQI-TS 400 W/D (OsramGmbH, Augsburg, Germany), which provided a minimum photosynthetic photon flux of around 500–600 µmol m^{-2} s^{-1} during a 14 h photoperiod. Moreover, all plants received 180–200 mL of complete Hoagland solution[25] once a week, alternated with distilled water (180–200 mL, twice a week).

Fruits (pods) were collected as they reached the final size according to the characteristics recorded in the label of the commercial product: fruits of 10–11 cm of length and around 9 seeds per fruit in the case of P. sativum, fruits of 17 cm of length for P. vulgaris, and fruits of 20–24 cm of length with 6–7 seeds per fruit in the case of V. faba. Water content (WC) in seeds of P. sativum and V. faba, as well as in pods of P. vulgaris was estimated at harvest as WC = FW − DW/DW × 100, where FW = fresh weight and DW = dry weight. Dry weight was calculated after drying plant material at 70 °C in an oven until reaching constant weight.

Plant height was recorded on days 7, 14, 21, 28, 35, and 42 after transferring plants to greenhouses.

Biochemical analysis

The extraction of starch, total soluble sugars (TSS), and total soluble proteins (TSP) from fresh samples (0.5 g FW) of seeds (P.

sativum and V. faba) and pods (P. vulgaris) was performed with potassium phosphate buffer (KPB) (50 mM, pH = 7.5). These extracts were filtered through four layers of cheesecloth and centrifuged at 28 710×g for 15 min at 4 °C. The pellet was used to determine starch after the iodine reaction.[26] The supernatant was collected and stored at 4 °C for TSS and TSP determinations. The TSPs were analyzed with the protein dye-binding method[27] and TSS with anthrone reagent[28] using, respectively, bovine serum albumin (BSA) and glucose as standards. Results were expressed as mg starch, TSS, or TSP per g (DW). For each sample, value of total carbohydrates was calculated as the sum of TSS and starch. Then the ratio of total carbohydrates to protein concentration was calculated.

Total soluble phenolic compounds were extracted as described by Chapuis-Lardy *et al.*[29] with some modifications. Samples of seeds (P. sativum and V. faba) and pods (P. vulgaris) (1.0 g of FW) were pulverized in liquid nitrogen, mixed with 20 mL of 80% methanol, and homogenized at room temperature for 1 min. After filtration, 0.5 mL of each sample was mixed with 10 mL of distilled water. Total phenolic content was determined from aqueous solutions by spectrophotometric analysis at 760 nm with Folin–Ciocalteau reagent.[30] Results were expressed as mg of gallic acid per g of DW. All chemicals were provided by Panreac Química SLU, Castellar del Vallès, Barcelona, Spain.

Mineral analysis

Vegetable samples (0.5 g DW, seeds in P. sativum and V. faba; pods in P. vulgaris) were dry ashed and dissolved in HCl as described by Duque.[31] Phosphorus, potassium, calcium, magnesium, manganese, zinc, iron, and sodium concentrations were determined using a Perkin Elmer Optima 4300 inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer Inc., Wellesley, MA, USA). The operating parameters of the ICP-OES were: radio frequency power 1300 W, nebulizer flow 0.85 L min^{-1}, nebulizer pressure 30 psi, auxiliary gas flow 0.2 L min^{-1}, sample introduction 1 mL min^{-1}, and three replicates per sample. Total nitrogen and carbon were quantified after combustion (950 °C) of dried fruit samples with pure oxygen by an elemental analyzer provided with a thermal conductivity detector (TruSpec CN, Leco, St. Joseph, MI, USA).

Statistics

A two-way analysis of variance (ANOVA) was performed in SPSS v.26 (IBM Corp., Armonk, NY, USA). The variance was related to the main treatments (CO_2 and species) and the interaction between them. The means ± standard error (SE) were calculated, and when the *F*-ratio was significant ($P < 0.05$), least significant differences were evaluated by the Tukey test. Significance levels were always set at 5%.

RESULTS

The height of pea and snap bean had an increasing trend during the first 4 weeks, growing in the greenhouse without being significantly affected by the CO_2 concentration in the air (Fig. 1). In contrast, the vegetative growth of V. faba was increased by the ECO_2 from the second week of cultivation in the greenhouse.

Fruits (pods) were collected when plants reached their reproductive maturity and this depended on both the legume species and the CO_2 treatment. Table 1 is showing the percentages of plants which began the reproductive stage along the weeks of growth in the greenhouse. The shortest life cycle corresponded
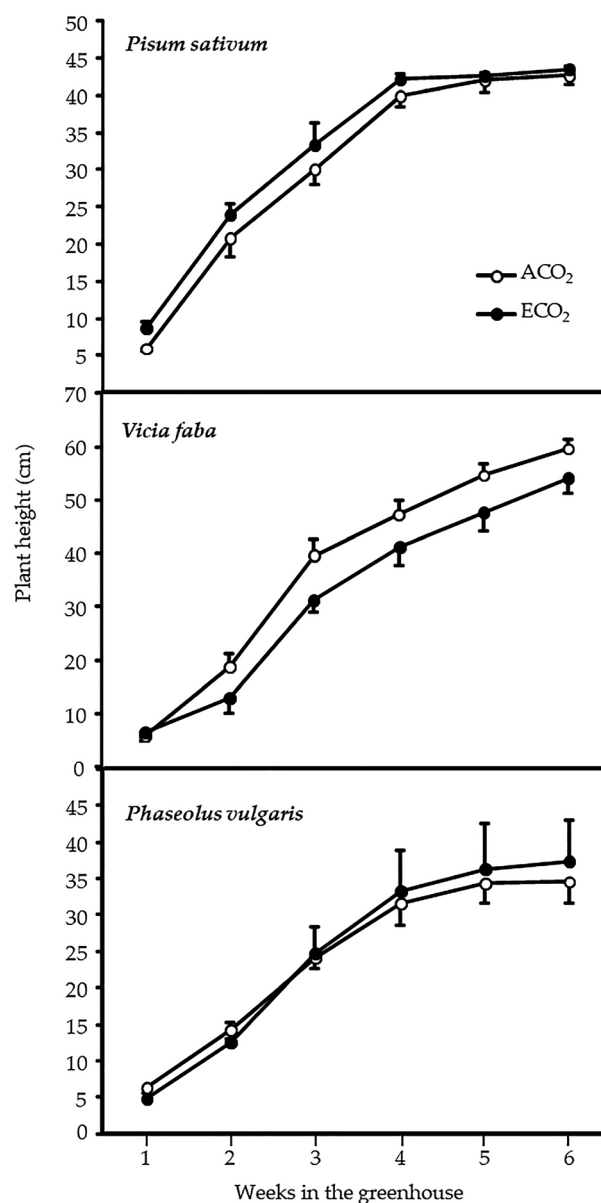

Figure 1. Plant height of *Pisum sativum*, *Vicia faba*, and *Phaseolus vulgaris* plants cultivated under either ambient (ACO_2) or elevated (ECO_2) CO_2. Values are means (n = 4) ± S.D.

to P. sativum; this legume started to produce buds 19 days after initiating its cultivation in the greenhouse and fruits were harvested over 10 days. There was, however, a clear difference between peas grown at ACO_2 or under ECO_2, because ECO_2 accelerated the beginning of the reproductive stage. Snap bean began its reproductive phase almost 10 days later than P. sativum, and ECO_2 slightly accelerated its life cycle. *Vicia faba* was the legume that entered the reproductive stage latest, almost 1 month after beginning its cultivation in the greenhouse, and the harvest of the mature fruits was completed 15–20 days later.

Plant yield parameters were, in general, slightly influenced by CO_2 (Table 2) ($P > 0.05$). In contrast, yield differed significantly among legume species ($P < 0.001$). In fact, faba beans produced fewer pods and total edible fruit DW per plant than peas or snap beans. *Phaseolus vulgaris* showed the highest edible fruit FW, DW, and WC, considering fresh pods as fruits. On the other hand,

Table 1. Percentage of plants showing buds (B), flowers (F) and/or pods (P) when grown either at ambient (ACO_2) or elevated (ECO_2) CO_2 concentration in the atmosphere

	Days after transferring to the greenhouse					
	19	23	27	30	34	42
Pisum sativum						
ACO_2	B: 50%	B: 100% F: 75% P: 50%	P: 75%			
ECO_2	B: 100%	B: 100% F: 100% P: 100%	P: 100%			
Vicia faba						
ACO_2			B: 25%	B: 75% F: 25%	B: 100% F: 75%	F: 100% P: 50%
ECO_2			B: 25%	B: 50% F: 25%	B: 75% F: 50%	F: 75% P: 50%
Phaseolus vulgaris						
ACO_2	—	—	B: 100% F: 25%	F: 100%	P: 100%	
ECO_2	—	—	B: 100% F: 75%	F: 100%	P: 100%	

Table 2. Yield components of *Pisum sativum*, *Vicia faba*, and *Phaseolus vulgaris* plants cultivated under either ambient (ACO_2) or elevated (ECO_2) CO_2

	Total edible fruit FW plant^{-1}	Total edible fruit DW plant^{-1}	Number of pods plant^{-1}	Number of seeds plant^{-1}	g FW edible fruit^{-1}	g DW edible fruit^{-1}	Edible fruit WC g H$_2$0 g^{-1} DW
Pisum sativum							
ACO_2	14.07 ± 1.16 b	4.21 ± 0.54 b	7.87 ± 0.65 a	35.50 ± 3.97 a	0.40 ± 0.02 c	0.12 ± 0.01 c	2.42 ± 0.28 b
ECO_2	15.87 ± 0.51 b	4.56 ± 0.19 b	10.00 ± 1.29 a	34.00 ± 2.48 a	0.48 ± 0.04 c	0.14 ± 0.01 c	2.49 ± 0.14 b
Vicia faba							
ACO_2	8.34 ± 0.67 b	2.12 ± 0.19 c	1.75 ± 0.14 b	3.75 ± 0.14 b	1.83 ± 0.26 b	0.47 ± 0.07 b	3.16 ± 0.20 b
ECO_2	10.93 ± 0.89 b	2.19 ± 0.10 c	2.38 ± 0.22 b	6.25 ± 0.48 b	1.38 ± 0.11 bc	0.28 ± 0.03 bc	3.67 ± 0.31 b
Phaseolus vulgaris							
ACO_2	51.60 ± 5.27 a	6.89 ± 0.47 a	8.50 ± 0.29 a	nd	6.99 ± 0.50 a	0.95 ± 0.10 a	6.52 ± 0.71 a
ECO_2	57.32 ± 2.47 a	8.02 ± 0.42 a	9.75 ± 0.85 a	nd	5.95 ± 0.35 a	0.83 ± 0.04 a	6.19 ± 0.36 a
CO_2	ns	ns	*	ns	*	*	ns
Species	***	***	***	***	***	***	***
CO_2 × species	ns	ns	ns	ns	ns	ns	ns

Edible fruits are seeds in *P. sativum* and *V. faba*, and pods in *P. vulgaris*. Values are means (n = 4) ± S.E. analyzed by the Tukey test ($P \leq 0.05$). Within each column means followed by the same letter are not significantly different. ANOVA: ns, not significant; FW, fresh weight; DW, dry weight; WC, water content; nd, not determined.
*$P \leq 0.05$.
*** $P \leq 0.001$.

ECO_2 did not significantly influence total edible fruit FW or DW per plant (CO_2, $P > 0.05$) although the weight per fruit was slightly reduced in the case of faba bean seeds.

The concentrations of mineral nutrients in fruits are shown in Tables 3 and 4. The level of CO_2 in the air exerted a significant influence on the accumulation of Ca ($P < 0.01$) and Mg ($P < 0.05$) in the edible part of fruits and significantly interacted with the species of legumes, affecting the levels of C, N, P, and Ca (Table 3). Elevated CO_2 reduced the C concentration in pods of snap bean and did not change the level of C in seeds of faba beans and peas ($P < 0.01$). Levels of P were enhanced by ECO_2 in the seeds of faba beans, but ECO_2 did not affect N and P concentrations in seeds of pea and pods of snap bean. Finally, the concentration of Ca increased in pods of snap bean under ECO_2, but it was similar in seeds of pea and faba bean cultivated under ACO_2 and ECO_2 (Ca; CO_2 × species, $P < 0.001$). The factor with the greatest influence on the mineral concentrations in fruits was the species of legume ($P < 0.001$). The lowest concentrations of C, N, and P and the highest levels of K, Ca, and Mg were found in the pods of snap beans (Table 3).

Table 4 shows the concentrations of some essential oligoelements and sodium in seeds of *P. sativum* and *V. faba*, and in pods

Table 3. Concentration of macronutrients in seeds of *Pisum sativum* and *Vicia faba*, and in pods of *Phaseolus vulgaris* cultivated under either ambient (ACO$_2$) or elevated (ECO$_2$) CO$_2$

	C	N	P	K	Ca	Mg
			(mg g^{-1} DW)			
Pisum sativum						
ACO$_2$	456.70 ± 4.37 ab	45.21 ± 2.07 a	6.37 ± 0.11 b	14.28 ± 0.42 c	0.66 ± 0.03 c	1.42 ± 0.04 b
ECO$_2$	464.25 ± 1.33 a	46.70 ± 1.33 a	6.30 ± 0.12 b	13.66 ± 0.25 c	0.56 ± 0.08 c	1.42 ± 0.02 b
Vicia faba						
ACO$_2$	464.87 ± 2.05 a	42.16 ± 2.02 a	6.60 ± 0.06 b	17.66 ± 0.68 b	0.46 ± 0.04 c	1.45 ± 0.05 b
ECO$_2$	463.37 ± 1.16 a	48.23 ± 2.24 a	7.45 ± 0.36 a	18.51 ± 0.57 b	0.82 ± 0.06 c	1.58 ± 0.06 b
Phaseolus vulgaris						
ACO$_2$	449.90 ± 1.98 b	23.11 ± 0.30 b	4.70 ± 0.12 c	23.48 ± 0.88 a	3.63 ± 0.33 b	2.76 ± 0.13 a
ECO$_2$	439.07 ± 1.85 c	20.20 ± 0.38 b	4.68 ± 0.13 c	24.36 ± 0.81 a	5.33 ± 0.38 a	3.11 ± 0.15 a
CO$_2$	ns	ns	ns	ns	***	*
Species	***	***	***	***	***	***
CO$_2$ × species	**	*	*	ns	***	ns

Values are means (n = 4) ± S.E. analyzed by the Tukey test ($P \leq 0.05$). Within each column, means followed by the same letter are not significantly different. ANOVA: ns, not significant. DW, dry weight.
*$P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.

Table 4. Concentration of micronutrients and Na in seeds of *Pisum sativum* and *Vicia faba*, and in pods of *Phaseolus vulgaris* cultivated under either ambient (ACO$_2$) or elevated (ECO$_2$) CO$_2$

	Fe	Mn	Zn	Cu	B	Na
			(mg kg^{-1} DW)			
Pisum sativum						
ACO$_2$	76.19 ± 7.30 c	7.60 ± 0.24 b	52.20 ± 1.06 bc	1.54 ± 0.29 c	11.96 ± 0.65 b	73.91 ± 3.79 ab
ECO$_2$	101.26 ± 5.05 bc	7.79 ± 0.53 b	49.39 ± 0.86 cd	6.92 ± 0.13 bc	10.74 ± 0.28 b	65.35 ± 1.88 bc
Vicia faba						
ACO$_2$	124.71 ± 10.29 b	9.02 ± 0.95 b	66.28 ± 4.85 a	25.55 ± 5.70 a	10.45 ± 0.52 b	93.31 ± 5.83 a
ECO$_2$	195.32 ± 8.72 a	10.04 ± 1.16 b	61.58 ± 1.80 ab	20.01 ± 0.87 a	12.79 ± 0.60 b	91.24 ± 8.45 a
Phaseolus vulgaris						
ACO$_2$	81.84 ± 8.1 c	25.53 ± 2.33 a	40.84 ± 2.25 de	8.01 ± 0.25 b	19.21 ± 0.88 a	45.59 ± 2.38 c
ECO$_2$	72.00 ± 1.76 c	23.55 ± 0.79 a	38.38 ± 0.62 e	6.26 ± 0.19 bc	19.33 ± 0.61 a	52.29 ± 5.71 bc
CO$_2$	***	ns	ns	ns	ns	ns
Species	***	***	***	***	***	***
CO$_2$ × species	***	ns	ns	***	*	ns

Values are means (n = 4) ± S.E. analyzed by the Tukey test ($P \leq 0.05$). Within each column, means followed by the same letter are not significantly different. ANOVA: ns, not significant. DW, dry weight.
*$P \leq 0.05$.
*** $P \leq 0.001$.

of *P. vulgaris*. In any of the three species of legumes the concentration of CO$_2$ in the air affected the amounts of Mn, Zn, and Na. In contrast, ECO$_2$ significantly increased the level of Fe in seeds of faba bean but did not have a clear effect on Fe in pods of snap bean and seeds of pea (Fe; CO$_2$ $P < 0.001$, CO$_2$ × species $P < 0.001$). However, the most influential factor on the concentration of oligoelements in fruits was the species of legume ($P < 0.001$ for Fe, Mn, Zn, Cu, B and Na). While the highest concentrations of Fe, Zn, Cu and Na were found in seeds of *V. faba*, *P. vulgaris* accumulated the greatest content of Mn and B in pods.

The factor CO$_2$ influenced the protein concentrations in fruits, but only in interaction with the species of legume (Table 5) (CO$_2$ × species, $P < 0.001$), and the effect was strongly dissimilar depending on the species ($P < 0.001$). While ECO$_2$ favored the accumulation of soluble proteins in seeds of *P. sativum*, it caused no influence in amounts of proteins in the seeds of faba beans. The most influential factor on the levels of carbohydrates (TSS and starch) in fruits was the legume species ($P < 0.001$). In fact, the levels of TSS and starch in both seeds of faba bean and pods of snap bean were higher than in seeds of pea independently of

Table 5. Total soluble proteins (TSP), total soluble sugars (TSS), starch concentration and the ratio of total carbohydrates (TSS + starch) to protein concentration in seeds of *Pisum sativum* and *Vicia faba*, and in pods of *Phaseolus vulgaris* cultivated under either ambient (ACO_2) or elevated (ECO_2) CO_2

	TSP (mg g^{-1} DW)	TSS (mg g^{-1} DW)	Starch (mg g^{-1} DW)	Carbohydrates proteins^{-1}
Pisum sativum				
ACO_2	23.72 ± 2.12 bc	32.35 ± 2.88 b	5.82 ± 0.55 c	1.66 ± 0.05 c
ECO_2	53.17 ± 7.30 a	34.11 ± 3.41 b	8.15 ± 0.51 bc	0.80 ± 0.05 c
Vicia faba				
ACO_2	34.05 ± 3.39 b	56.75 ± 4.48 a	15.45 ± 0.82 a	2.08 ± 0.17 c
ECO_2	21.73 ± 3.09 bc	44.24 ± 4.95 ab	14.03 ± 1.42 a	2.57 ± 0.29 c
Phaseolus vulgaris				
ACO_2	10.48 ± 1.69 c	58.43 ± 6.10 a	14.38 ± 1.52 a	8.73 ± 0.89 a
ECO_2	11.59 ± 1.21 c	51.20 ± 6.10 ab	12.15 ± 0.92 ab	6.07 ± 0.64 b
CO_2	ns	ns	ns	*
Species	***	***	***	***
CO_2 × species	***	ns	ns	*

Values are means (n = 4) ± S.E. analyzed by the Tukey test ($P ≤ 0.05$). Within each column, means followed by the same letter are not significantly different. ANOVA: ns, not significant. DW, dry weight.
*$P ≤ 0.05$.
*** $P ≤ 0.001$.

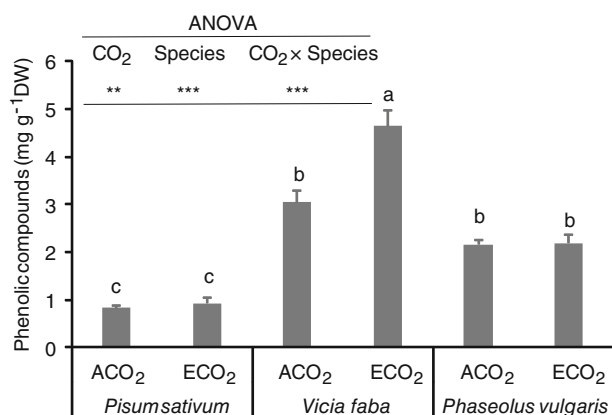**Figure 2.** Phenolic compounds in fruits of *Pisum sativum*, *Vicia faba*, and *Phaseolus vulgaris* plants cultivated under either ambient (ACO_2) or elevated (ECO_2) CO_2. Edible fruits are seeds in *P. sativum* and *V. faba*, and pods in *P. vulgaris*. Bars are means (n = 4) ± S.E. analyzed by the Tukey test ($P ≤ 0.05$). Means followed by the same letter are not significantly different. ANOVA: ns, not significant; *$P ≤ 0.05$; **$P ≤ 0.01$; ***$P ≤ 0.001$. DW, dry weight.

the air CO_2 concentration. When the ratio of carbohydrates to TSP was calculated, we observed its reduction in snap bean related to ECO_2 treatment ($P < 0.05$), with significantly higher values in this species in comparison with the other two legumes tested ($P < 0.001$).

At ACO_2 the highest concentration of phenolic compounds was observed in seeds of faba bean, whereas the lowest corresponded to seeds of pea (Fig. 2). Elevated CO_2 induced the accumulation of phenolics in *V. faba* seeds and had no effect on the levels of these secondary compounds in seeds of pea and pods of snap bean. Once more, the legume species was the most influential factor, in this case affecting the amount of phenolics in the fruits ($P < 0.001$).

DISCUSSION

The inclusion of legumes has been suggested as a strategy for mitigating the effects of climate change on agriculture because their cultivation allows the reduction of inorganic nitrogen fertilizers and CO_2 emissions.[14,32] The selection of early maturing varieties can allow harvest to occur before the arrival of high temperatures and the drought period. However, in a context of climate change, even the early maturing crops will be exposed to elevated concentrations of CO_2 in the air during their life cycle and this factor may affect growth, yield, the transition from the vegetative to the reproductive stage, and the quality of edible organs. The present study aimed to delve into the effect that the atmospheric CO_2 concentration itself can have on the development and characteristics of the fruit or grain in short-cycle varieties of three legumes consumed in the human diet.

Selecting cultivars with increasing or maintaining yield and nutrient content is crucial in a future ECO_2 environment. According to Palit *et al.*,[33] ECO_2 may augment photosynthesis in C3 plants toward greater shoot and root length, and sink utilization in legumes. Only our snap bean plants grown under ECO_2 treatment showed an increasing trend in shoot height (Fig. 1) together with a slightly enhanced total fruit DW production (Table 2). The failure of significant fruit yield increase in the legumes tested was probably not associated with a physical restriction to root growth because the volume of the containers was 2 L. However, in these short-cycle cultivars, the amount of time for which the plant is influenced by a changing climate scenario (ECO_2) may be reduced. In fact, long-term exposure to ECO_2 can cause photosynthetic acclimation[34] thus reducing the stimulation of vegetative growth in C3 crops. In addition, in legumes the response to ECO_2 is strongly altered by the incidence of other environmental stresses, such as temperature and drought.[35] Consequently, the utilization of early maturing varieties of legumes will shorten the time of exposure to ECO_2, thus avoiding or limiting the photosynthetic acclimation, and may allow harvest before the period of

high temperatures and water deficit. On the other hand, ECO_2 either did not affect or slightly accelerated the life cycle of the early maturing legumes tested (Table 1). Faba bean, the legume that showed the longer vegetative period, did not have a stimulatory response to ECO_2. Soares *et al.*,[36] who found an average change of only 5% in the seed yield response of snap bean to ECO_2, discussed the importance of selecting strong-responsive varieties in terms of yield and nutritional quality for future climate conditions. Similarly, Mousavi-Derazmahalleh *et al.*[1] bet on the utilization of the existing genetic variation among legumes to face the challenges of climate change. According to these last authors, genomic approaches can help the identification of genetic variations that can be used in crop-breeding programs.

Another important point is that the ECO_2 is closely related to increased demand for nutrients and water, resulting from increased plant growth.[37] Many authors have described a dilution effect of minerals associated with this plant growth enhancement together with reductions of mass flow of nutrients due to decreases in transpiration rates[32] or physical restriction of root growth, which alters nutrient acquisition.[36] Pods of *P. vulgaris* showed a decrease in tissue C, an increase of Ca, and no difference in other nutrients due to ECO_2 conditions (Table 3). By contrast, faba beans enhanced their level of P in seeds under ECO_2 treatment, with no differences in the other macronutrients. This suggests that ECO_2 changed the equilibrium among carbon metabolism and mineral nutrients in interaction with the legume species. As suggested by Soares *et al.*,[36] mineral changes in the species are distinct from each other, suggesting that the mechanism involved is more complex than growth dilution alone.

Legume seeds are typically rich in micronutrients such as Fe and Zn.[38] Regarding micronutrients (Table 4), no significant reductions in Fe and Zn were observed in our legume species due to ECO_2, previously described in works of Loladze[39] and Myers *et al.*[40] In fact, ECO_2 improv

ed the Fe content of seeds of faba bean. According to Duval *et al.*,[41] CO_2 effects on plant nutrient status depended on the nutrient elements, plant functional groups, and plant tissues, suggesting that the expected lower plant nutrient concentrations via carbohydrate dilution is not a general response. The fact that mineral concentrations varied among legume species supports the need of selecting varieties whose nutrient levels are not negatively affected by ECO2 and therefore keep being nutritious for consumers even under climate change scenarios.

Medek *et al.*[42] advised that the increase in atmospheric CO_2 will decrease the intake of proteins in several countries and around 15% of the world population will be at risk of protein deficiency, a percentage that will increase if the concentration of CO_2 in the air keeps rising. In legumes, the decrease in tissue N or grain protein concentration under ECO_2 is absent or less than in cereals.[43] Early maturing cultivars of the legumes tested, maintained the N content in the edible parts (Table 3) although they were apparently not associated with N_2 fixing bacteria. According to the results shown in Table 5, the effect of ECO_2 on proteins in fruits and grains depended strongly on the legume species, at least in the early maturing varieties that were tested. However, the prediction by Medek *et al.*[42] could not be applied to these cultivars. In fact, the concentration of proteins in pea was higher in grains from plants subjected to ECO_2 than in those harvested from plants grown at ACO_2. Pea proteins are considered of high quality due to their well-balanced profile of amino acids and high proportion of lysine, and they constitute a good alternative to soybean and animal proteins in functional food applications.[44] Proteins in

fresh pods of snap bean were less abundant than in grains of faba beans and peas, regardless of the concentration of CO_2 in the air. This is an expected result because, even within *P. vulgaris* species, the protein content is significantly higher in grains consumed as dry beans than in the fresh pods of snap beans.[45] However, snap bean was the only legume species whose carbohydrate-to-proteins ratio in fruits decreased under ECO_2, which favors the nutritional quality of pods, widely consumed either fresh or processed.

Surprisingly, changes in the protein levels caused by the increase of CO_2 in the air were not correlated with the total nitrogen (N); in fact, pea was the unique legume that accumulated higher amount of proteins under ECO_2 than at ACO_2 without changes in the N concentration. This suggests that, under ECO_2, an important part of the total N in seeds of pea was reduced in other compounds that were different from soluble proteins.

The high activity of the secondary metabolism in faba bean was corroborated by the accumulation of phenolic compounds in seeds under both ACO_2 and ECO_2 conditions (Fig. 2), reaching levels always greater than those found in the seeds of pea and pods of snap bean. Moreover, the concentration of these secondary compounds in grains of faba bean significantly increased under ECO_2. Kwon *et al.*[46] identified 22 different phenolic compounds in five genotypes of *V. faba* and observed that the total amount of phenolics depended on the genotype. Taking into account that there was a positive relationship between the content of total phenolics and the antioxidant capacity, we can suggest that ECO_2 could enhance the antioxidant activity in seeds of *Vicia faba* cv. Muchamiel. According to Liu *et al.*,[47] faba beans are better dietary sources of phenolic antioxidants than peas.

CONCLUSIONS

The effect of ECO_2 on the development and food quality of the early maturing cultivars of pea, faba bean, and snap bean was strongly dependent on the species of legume and it was more evident in terms of grain or pod quality than in terms of vegetative growth and yield. Those effects of ECO_2 on the quality of grains or pods were usually positive as rising CO_2 in the air induced the accumulation of Fe and P in the grains of the faba bean, as well as Ca in the pods of the snap bean. Moreover, when exposed to ECO_2, grains of pea showed higher levels of proteins, the sugars-to-protein ratio decreased in pods of snap bean, and grains of faba bean accumulated greater amounts of phenolic compounds.

Our results therefore suggest that the utilization of early maturing varieties of legumes is an interesting alternative to cope with the negative effects that a long exposure to rising CO_2 (often aggravated by concurrence with other abiotic stresses) can exert on food quality.

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