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Can higher CO₂ concentrations affect yield and quality parameters in lettuce and sugar beet crops?

O aumento de CO₂ pode afetar os parâmetros de rendimento e qualidade nas culturas de alface e beterraba sacarina?

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ARTIGO ABSTRACT Recebido: 14/08/2020 It has been suggested that the increase in CO_2 levels in the coming decades will have positive Aprovado: 30/11/2020 consequences on the nutritional content and yield of agricultural crops. However, the effects of the increase in CO₂ concentrations remain little known in Andean region. This study evaluated the effect of increased CO_2 on the protein content and growth of sugar beet and lettuce plants in the Andean region of Colombia. We used a Randomized Complete Block Design, strip 1 was open field, strip 2 was low tunnel with ambient CO_2 and strip 3 was low tunnel with 1000 Key words: ppm CO₂. The sugar beet experiment three harvest periods were evaluated. The results Protected crops indicated that CO₂ fertilization did not have a significant effect on the yield and head diameter Climatic change of the lettuce. However, biomass production tended to increase in the first sugar beet harvest Carbon fertilization but decreased significantly in the last two harvests, probably due to a negative effect caused by Vegetables acclimatization to CO₂ enrichment. The protein content was not affected by the increase in CO₂ Plant nutrition levels in any of the crops. The results suggest the increase in atmospheric CO₂ in the next years will not cause any benefit in lettuce or sugar beet grown in the Andean region. RESUMO Palavras-chave: A literatura sugere que o aumento dos níveis de CO₂ nas próximas décadas terá consequências positivas no conteúdo nutricional e na produtividade das culturas agrícolas. No entanto, os Cultivo protegido efeitos do aumento do CO₂ são pouco conhecidos nas regiões Andinas. Este estudo avaliou o Mudanças climáticas Fertilização com carbono efeito do aumento de CO_2 no conteúdo de proteína e no crescimento de plantas de beterraba e Vegetais alface na região andina da Colômbia. O estudo foi conduzido em um Delineamento Blocos Nutrição de plantas Casualizados onde a faixa 1 foi campo aberto, a faixa 2 foi o com concentrações de CO₂ ambiental e faixa 3 foi o túnel baixo com aumento da concentração de CO2 de 1000 ppm. As avaliações em beterraba foram realizadas em três safras. Os resultados indicam que a fertilização com CO2 não teve efeito significativo na produtividade e no diâmetro da cabeça da alface. Por outro lado, a produção de biomassa teve uma tendência a aumentar na primeira safra

de beterraba, mas diminui significativamente nas duas últimas safras, provavelmente devido às características de cumprimento curtas em altitudes mais elevadas que influencia as fixações de CO₂, como também das condições climáticas nestas regiões que podem alterar o crescimento das plantas. Os resultados sugerem que o aumento do CO₂ atmosférico devido às mudanças climáticas não trará nenhum benefício na produtividade das comodities agrícolas Andinas.



INTRODUCTION

For the last 650,000 - 800,000 years CO_2 concentrations in the atmosphere have not exceeded 280 ppm (LUTHI et al., 2008). However, with the industrial revolution, CO_2 levels started to rise dramatically and may reach levels close to 1000 ppm by the end of the 21st century (FUSS et al., 2014). Rising CO_2 levels is predicted to stimulate the yield of C3 crops by reducing stomatal conductance and by stimulating CO_2 uptake (GRAY et al., 2016). In lettuce, studies have shown that increasing CO_2 concentrations improves productivity (FURLAN et al., 2001; PÉREZ-LÓPEZ et al., 2013). In addition, several other factors also influence the effects of elevated CO_2 levels in crops, including genotype, exposure time and environmental conditions.

The short timescale and many influencing factors make it hard to predict how plants will respond to higher CO_2 levels based solely on the available scientific literature. For instance, several studies have found that the benefits of carbon fertilization are lost in the long term, when plants acclimatize to CO_2 . Lee et al. (2011) and Warren et al. (2011) showed how the effect of CO_2 fertilization on plants tends to decrease over time, eventually stabilizing at the same level of photoassimilate production as before CO_2 fertilization.

The chemical properties of rubisco are highly dependent on the CO₂ concentration in the environment. Under natural conditions, the oxygen concentration around the leaf is much higher (210 000 µmol mol⁻¹) than the CO₂ concentration inside the chloroplasts (415 µmol mol-1) (BUSCH, 2020). Increasing CO₂ concentrations increases carboxylation and suppresses the oxidation of rubisco, stimulating the photosynthetic rate. The CO₂ concentration around the rubisco can be affected by the resistance to CO₂ diffusion imposed by the membrane and cell wall of the stomata and mesophyll. These biochemical properties cause a progressive gradient from the CO₂ concentration in the environment to the chloroplasts (BUCKLEY, 2019). Changes in CO₂ concentrations can be strongly influenced by external conditions such as light intensity and quality, CO2 concentration, air humidity and the general water status of the plant (BUCKLEY, 2019).

Some studies have suggested food quality will decrease as CO_2 levels in the atmosphere increase. Bloom et al., (2014), Duval et al. (2013) and Weigel; Manderscheid (2012) showed that protein content in plants, as well as zinc, iron and sulfur content can decrease due to high CO_2 concentrations. However, there are few studies on the effect of increasing CO_2 on horticultural crops in the Andean region. This study evaluated the effect of elevated CO_2 concentrations on the protein content and yield of sugar beet and lettuce plants.

MATERIAL AND METHODS

The research was carried out at Botana Experimental Center, University of Nariño, located at 1° 10' latitude N and 77° 16' longitude W, altitude 2960 MASL. The center has an average temperature of 12 °C, annual rainfall of 900 mm, relative humidity 73%, solar brightness 1182 hours/year and average radiation between 4 and 4.5 kWh/m2/day (IDEAM, 2014). The

experiment was carried out in Vitric Haplustands soils. The relative humidity and temperature values were collected with CO_2 sensors (NDIR, non-dispersive infrared).

Culture conditions

Two experiments were performed to evaluate the effect of elevated CO_2 levels on sugar beet, a biennial plant, and lettuce, a short-cycle plants. The first experiment was conducted on 210 sugar beet plants of the Altissima variety. The seedlings were transplanted with 45 cm between each plant and planted in three beds 18 m long and 0.8 m wide. The second experiment was conducted with 245 Batavia lettuce of the Coolguard variety. Lettuce seedlings were also planted in three beds 18 m long and 0.8 m wide, but with only 40 cm between each plant.

This variety has a life cycle of more than two years and grows mainly in the tropical zone. The plants develop glabrous leaves and the large petiolate leaf at the base of the stem. The Coolguard variety has a compact head, along with thick and wavy leaves. The leaves form a closed head and protects of protecting the bud from mechanical damage.

Experimental design

The two experiments (lettuce and sugar beet) were conducted using a strip within Randomized Complete Block Design, with three treatments: strip 1 field planting, strip 2 low tunnel and strip 3 low tunnel with elevated CO₂ (around 1000 ppm). For each treatment, we used three randomized blocks.

CO₂ supply system in low tunnel

The low tunnel system was built with iron arches covered with plastic (polyethylene). Each low tunnel was 18 m long, 0.8 m wide and 0.6 m high. CO₂ was supplied by a tank with a storage capacity of 25 kg. The gas outlet force was controlled with a pressure regulator (ref: CO₂ TM 425-CD100-325) coupled to the tank valve. A stop valve located at the end of the regulator, controlled the CO₂ outflow. The high-pressure hose was extended from the stop valve to the experimental field. The hose was perforated every 5 cm to facilitate CO₂ diffusion inside the low tunnel. CO₂ enrichment was carried out daily from 10 am to 3 pm the concentration was recorded with a CO₂ sensor (NDIR, non-dispersive infrared, model CO210, accuracy \pm 50 ppm).

The variables in sugar beet and lettuce

In sugar beet we evaluated foliar biomass in three harvest periods. The first collection was made by cutting the leaves after 60 days. In the second collection, another leaf cut was made after 45 days. In the third and last collection, a leaf cut was made at 45 days. In lettuce, the evaluated parameters were days to harvest, number of leaves, head diameter, protein concentration and yield. The parameters were measured when more than 50% of the lettuces had formed their compact heads. The protein content was determined by means of a bromatological analysis. In the two experiments (lettuce and sugar beet), variance analysis was performed with the statistical software SAS, version 9.4. The treatments were compared using the Duncan test at significance level p < 0.05.

Culture system	Sugar beet			Lettuce		
	Temperature (°C)	Relative humidity (%)	CO ₂ concentration (ppm)	Temperature (°C)	Relative humidity (%)	CO ₂ concentration (ppm)
Field planting	18.3	61.2	406.7 ±7.1 sd	17.2	68.5	404.9 ±4.3 sd
Low tunnel	27.2	54.9	392.5 ±4.2 sd	25.8	59.9	383.6 ±2.3 sd
Low tunnel with elevated CO ₂	28.6	55.2	1097.7 ±224.1 sd	27.1	57.1	$1027.9 \pm 184.2 \text{ sd}$

Table 1. Mean temperature, relative humidity and atmospheric concentration of CO_2 in the three culture conditions (field planting, low tunnel and low tunnel with elevated CO_2) in sugar beet.

RESULTS AND DISCUSSION

For sugar beet in low tunnel, CO_2 fertilization led to a nonsignificant increase of 3,4% in the first harvest, while in the second and third harvest, yield decreased significantly by 12% (Fig. 1). Considering all three harvests, the yield of the sugar beet plants grown in low tunnel was 8.6% lower when subject to CO_2 fertilization.

Figure 1. Effect of three types of culture conditions (field planting, low tunnel and low tunnel with elevated CO_2) on sugar beet yield in the three harvests. There was no biomass production in the open field in the first harvest. Different letters indicate a significant difference.



Elevated CO_2 concentrations did not significantly affect any of the evaluated variables in lettuce plants. The increase in CO_2 concentrations tended to increase the yield, but not significantly (Fig. 2). Bromatological analysis suggested that CO_2 fertilization did not affect dry matter and protein percentage values in sugar beet or lettuce (Fig. 3-4). **Figure 2.** Effect of three types of culture conditions (field planting, low tunnel and low tunnel with elevated CO_2) on sugar beet yield in the three harvests. Different letters indicate a significant difference.







Figure 4. Average protein and dry matter percentage for lettuce produced in low tunnel systems with and without elevated CO₂ levels. Different letters indicate a significant difference.



Average air temperature in low tunnel was 9.6 °C higher for sugar beet and 9.2 °C higher for lettuce (Table 1) compared to open field planting. In both species, air humidity was approximately 10% lower in the low tunnel. These conditions affected both precocity and foliar biomass in sugar beet plants. The low tunnel microclimate affected the growth rate of both sugar beet and lettuce. Sugar beet grown under the warmer temperatures of the low tunnel were harvested 30 days earlier. Thus, the low tunnel sugar beet culture produced three harvests during the five months of the experiment, while field planting produced only two. As a result, open field planting yielded 39.9% less foliar biomass overall than low tunnel planting. In lettuce, low tunnel conditions increased the number of leaves and head diameter by 25.4% and 27.9% respectively, compared to field planting (Fig. 5-6). In addition, the lettuce plants grown in low tunnel were harvested 10 days earlier than in open field conditions.

Figure 5. Effect of three different culture conditions (field planting, low tunnel and low tunnel with elevated CO₂) on head diameter of lettuce. Different letters indicate a significant difference.



Figure 6. Effect of three types of culture conditions (field planting, low tunnel and low tunnel with elevated CO_2) on the number of lettuce unfolded leaves. Different letters indicate a significant difference.



In our study, CO₂ fertilization did not lead to higher yields in sugar beet and lettuce. Sugar beet yields actually dropped significantly in the last two harvests with CO₂ enrichment. This yield reduction can be explained by the sugar beet plants acclimatizing to higher CO₂ levels. Changes in the ultrastructure of chloroplasts in sugar beet in response to long periods of elevated CO₂ levels have been well documented. The elevated CO₂ levels reduced the proportion of total thylakoids and increased chloroplast stroma (TIAN et al., 2020). Biochemical changes can also occur in prolonged periods of high CO₂ exposure. The accumulation of assimilates that are not used in respiration or storage can activate changes in the chloroplast biochemistry to stop the synthesis of photosynthetic components. Studies of long-term exposure to elevated CO₂ levels in five C3 species indicate that the rubisco activation state was reduced after prolonged exposure to high CO₂ concentrations (IÑIGUEZ et al., 2020). Thus, biochemical and morphological changes in chloroplasts induced by elevated CO₂ levels could explain why shoot biomass decreased in the last two harvests compared to control.

Environmental conditions also play an important role in how plants respond to CO_2 fertilization. Temperature and light intensity can influence how efficiently carbon dioxide is utilized, which affects the photosynthetic rate of plants. Pérez-López et al. (2013) found that stoma closure leads implies low CO_2 fixation. Iñiguez et al. (2020) showed that increases in temperature in C3 plants limit the production of rubisco which can result in low photosynthetic rates.

 CO_2 enrichment increases net photosynthetic rates, and thus has frequently been demonstrated to increase plant productivity and yield (LONG et al., 2004; LIU et al., 2017; DONG et al., 2018). However, other studies have found no effect from CO_2 fertilization, or a fleeting effect that disappears when plants acclimatize to higher CO_2 levels (GRAY et al., 2016; IÑIGUEZ et al., 2020). Photosynthetic rate models have indicated that lighting is more important than CO_2 enrichment for increasing lettuce yield in greenhouses (JUNG et al., 2018). Besides, some studies demonstrate that higher light intensity increases the concentration of nitrate reductase in lettuce, which could affect the photosynthetic process (SIGNORE et al., 2020).

More studies modeling photosynthetic rates in different temperatures, light conditions and CO_2 concentrations will be necessary in order to predict how plants will respond to higher CO_2 levels. Atmospheric CO_2 concentrations will likely double during the 21st century, and it is vital to know how this increase in CO_2 will affect future food production (FUSS et al., 2014).

Simulations with global climate models (GCMs) suggest that the projected increase in CO_2 will alter global and local climatic conditions in other ways, leading to increasing temperatures, changing precipitation patterns, and more severe droughts- and floods. These diverse effects on local growing conditions also need to be calculated to evaluate the net effect of higher CO_2 concentrations on food production (BEACH et al., 2019). However, more studies are necessary to elucidate the effect increasing carbon dioxide concentrations will have on agricultural crops.

Elevated CO_2 levels did not have a significant effect on the protein and dry matter percentage in our study. The effect of elevated CO_2 levels on protein content depends on other factors, such as genotype and type of fertilizer. For instance, Baslam et al. (2012) showed that the effect of increased CO_2 concentrations on protein accumulation in tissues differed between the two lettuce cultivars "Batavia Rubia Munguía" and "Maravilla de Verano". Bloom et al. (2014) found that protein concentrations can be reduced in C3 plants subject to higher CO_2 concentrations when an ammonia fertilizer is used. In our study we used an ammonia fertilizer that may have facilitated nitrogen assimilation to proteins. Nevertheless, there is no substantial evidence that increasing CO_2 levels can influence food quality. Our results suggest that lettuce can retain its protein quality while subject to CO_2 concentrations of 1000 ppm.

Interestingly, low tunnel conditions increase the precocity and yield of sugar beet and lettuce. The favorable effect of low tunnel on the growth rate of plants has also been well documented in other studies (JÚNIOR et al., 2004; KUMAR et al., 2018). Low tunnel accelerates the physiological processes in plants mainly due to an increase in temperature. In some cases, the low tunnel system can increase the number of harvests per year, as was demonstrated in lettuce by (VELASQUEZ et al., 2014). Recently, physiological models have been developed to predict the effect of temperature on crop development (HE et al., 2012; KUMUDINI et al., 2014), and the molecular mechanisms involved in how plants respond to temperature changes are being elucidated (BROWN et al., 2013; KINMONTH-SCHULTZ et al., 2018).

CONCLUSIONS

The protein content and yield of lettuce and sugar beet were not affected by elevated CO_2 levels. The sugar beet yield increased due to the enrichment of CO_2 in the first harvest but then decreased significantly in the last two harvests. The Lettuce and sugar beet crops will probably not be affected by increased CO_2 concentrations in the coming years. The effects CO_2 fertilization has on the photosynthesis of crop plants will depend on several factors and more studies are necessary for carbon fertilization to be used in agricultural productions.

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