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CO$_2$ IMPROVES THE PERFORMANCE OF AN INVASIVE INSECT: a worrisome climate crisis scenario to native and agricultural ecosystems

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"I wish it need not have happened in my time," said Frodo.
"So do I," said Gandalf, "and so do all who live to see such times.
But that is not for them to decide.
All we have to decide is what to do with the time that is given us."

— J.R.R. Tolkien, The Fellowship of the Ring

Resumo

As emissões de dióxido de carbono (CO_2) desempenham papel central no fenômeno da Crise Climática (CC), cujas conseqüências afetam indiscriminadamente os ecossistemas naturais e antropogênicos. Entre as relações ecológicas, a interação planta-inseto é uma das mais impactadas pelo aumento da [CO_2]. Nesse estudo testamos a hipótese de que o aumento da [CO_2] afetaria a qualidade e as defesas do *Helianthus annuus* como espécie hospedeira; por sua vez, isso impactaria negativamente na preferência e performance das larvas de *Helicoverpa armigera*, um inseto invasor com distribuição mundial. *Helianthus annuus* e *H. armigera* se desenvolveram dentro de câmaras de topo aberto sob [CO_2] ambiente (~400ppm) e elevada (~800ppm). A maioria das nossas previsões sobre o desenvolvimento do girassol foram corroboradas, à despeito da aclimatação, que eliminou as diferenças iniciais no teor de nitrogênio foliar (N) e flavonóides (Flv). Os girassóis sob [CO_2] elevada cresceram mais (e.g. altura, biomassa foliar, número de folhas) mas eram de pior qualidade, principalmente durante seu desenvolvimento inicial. A despeito de não apresentarem preferência por nenhum dos tratamentos, as larvas de *H. armigera* tiveram uma performance melhor e num período de tempo mais curto sob condições do tratamento: e.g. maior taxa de crescimento e maior proporção de pupas (3x) e adultos (4x). Mesmo após os efeitos da aclimatação, as larvas de *H. armigera* sob elevada [CO_2] mantiveram maior consumo foliar ao longo de todo o experimento. Essa sobrecompensação comportamental possivelmente deveu-se às condições desvantajosas que as larvas sob elevada [CO_2] estavam submetidas durante os estágios de desenvolvimento iniciais/críticos (antes da aclimatação). Ao consumir mais material vegetal, essas larvas foram capazes de acessar maior conteúdo de N acumulado, que aumentou com a biomassa vegetal. A melhoria na performance das larvas de *H. armigera* sob elavada [CO_2] sugere um cenário agravado da CC. Especificamente, uma espécie invasora que já exerce grande impacto nos ecossistemas nativos e agrícolas seria favorecida por um aumento a taxa de consumo foliar e um aumento populacional.

Palavras-chave: praga agrícola, espécie invasora, dióxido de carbono, mudanças climáticas, lagarta do algodão, mudanças globais, traços foliares, girassol

Abstract

Carbon dioxide (CO_2) emissions are central in the Climate Crisis (CC) phenomenon, whose consequences indiscriminately affect natural and anthropogenic ecosystems. Among ecological interactions, plant-insect is one of the most impacted by the increase in [CO_2]. In this study we tested the hypothesis that increased [CO_2] would affect the host species *Helianthus annuus* leaf quality and defenses; in turn, this would negatively impact the preference and performance of *Helicoverpa armigera* larvae, an invasive insect currently distributed worldwide. *Helianthus annuus* and *H. armigera* developed inside open-top chambers under ambient (~400ppm) and increased [CO_2] (~800ppm). Our predictions concerning sunflower development were mostly met, despite the acclimation, which eliminated the early differences in leaf nitrogen (N) and flavonoids (Flv) content. Sunflowers under increased [CO_2] grew more (e.g. height, leaf biomass, number of leaves) but were of poorer quality, especially at early development/before acclimation. Despite showing no preference for either treatment, *H. armigera* larvae performed better within a shorter developmental time when reared with leaves from increased [CO_2]: e.g. higher growth rate and greater proportion of pupae (3x) and adults (4x). Even after the acclimation effects (by the 11th week), *H. armigera* larvae under treated conditions maintained a greater consumption. This behavioral overcompensation was possibly due to the disadvantageous conditions that larvae under increased [CO_2] were submitted during their earliest/critical developmental stages (before acclimation). By consuming more relatively more plant material, these larvae were able to access a greater N accumulation, which increased with plant biomass. The improvement in *H. armigera* larvae performance under increased [CO_2] suggests an aggravated CC scenario. Specifically, an invasive species that already exerts great impact to native and agricultural ecosystems would be favored with an increased consumption and a population increase.

Summary

Introduction

Climate crisis (CC) is caused by the emission of greenhouse gases, whose effects build-up and indiscriminately impact natural and anthropogenic systems (e.g. Figure S1) (Coviella & Trumble, 1999; Steffen et al., 2006). With time, human activities as a CC driver have been continuously proven and amplified, even by the IPCC (Intergovernmental Panel on Climate Change) conservative standard (Scherer, 2012; IPCC, 2014). Furthermore, CC is only one of the anthropogenic activities that comprise the Global Change phenomenon. Other examples are land-use change, habitat fragmentation, disruption of ecological interactions, biological invasions, and habitat poisoning (e.g. Jablonski, 1989; Ceballos et al., 2015; Díaz et al., 2019).

Among the ecological interactions, herbivory is severely impacted by anthropogenic activities such as the increase in atmospheric [CO_2e] (carbon dioxide equivalent). Hence, plant-herbivore ecology possesses a central role in Global Change studies, not only due to their relationship with agriculture, but also as an interaction *per se* (Massad & Dyer, 2010). Herbivores comprise about one-quarter of all living organisms (Strong et al., 1984) and consume up to 18% of the global plant biomass (White, 2005). Furthermore, herbivorous insects represent one of the largest and most diverse groups of animals (Speight et al., 2008), consuming ca. 5% of all the woody plant biomass (Kozlov et al., 2015). They provide valuable ecosystem services by regulating nutrient cycles and influencing plant community composition, competition, resource allocation, and survival (Losey & Vaughan, 2006; Weisser & Siemann, 2013). Overall, climate change initially affects plants, which later impact herbivores, and the cumulative effects reach parasites and predators (Coviella & Trumble, 1999).

Most of the impacts to herbivorous insects due to the increase in [CO_2] happen through host plant alterations. However, there are a few astonishing, yet less understood direct pathways such as

through alterations in insects immune function (apparently not related with leaf traits; Gherlenda et al., 2016), and also through their ability to search and choose host plants based on $[CO_2]$ (Coviella & Trumble, 1999). For instance, *Helicoverpa armigera* (Hübner) has been reported to detect fluctuations in CO_2 density as small as 0.14% or 0.5 ppm (Stange, 1992). Indirect $[CO_2]$ impacts may originate through numerous pathways, but mostly through alteration on the insects' host plants nutritional quality and defensive mechanisms (Scherber et al., 2013; Zavala et al., 2013). In addition, they could also occur because of cascade effects on multipartite interactions such as mycorrhizal fungi - host plants - herbivores (Zavala et al., 2013; Frew & Price, 2019).

In an environment with increased $[CO_2]$, plants (specially C3 species) undergo the CO_2 fertilization effect, stimulating plants' primary metabolism by enhancing their net photosynthesis rate (Wang et al., 2012; Tausz-Posch et al. 2020). Consequently, the synthesis increase in total nonstructural carbohydrates (TNC; e.g. starch and soluble sugars) alters the carbon to nitrogen ratio (C:N), increasing leaf toughness and diluting leaf nitrogen content (Zavala et al., 2013; Tausz-Posch et al. 2020). Furthermore, although resource allocation to plants primary metabolism is prioritized, there is a limit to how much plants are able to allocate the extra carbohydrates to growth (insufficient growth sink or metabolic assimilation; Reekie et al. 1998; Tausz-Posch et al. 2020). This leads to an accumulation of TNC in plant structures, which may trigger a plant acclimation (crops or non-crops) as well as further reducing leaf nutrition by proportionally decreasing its water content (Del Pozo et al., 2007; Tausz-Posch et al. 2020).

Increased $[CO_2]$ could also cause alterations in plant resource allocation and hormone production (e.g. jasmonic and salicylic acids). This may change the production of secondary metabolites (SM) (Zavala et al., 2013), with variable consequences to the ecology of plant-herbivores. SMs are organic compounds that are not involved in growth with allelochemical functions such as total phenolics, tannins, flavonoids, and terpenoids (Zavala et al., 2013; Sá et al., 2014; Rosenblatt &

Schmitz, 2016). Two important groups of allelochemicals are the flavonoids and sesquiterpenoids (ST), commonly found in Asteraceae species such as *Helianthus annuus* (Chou & Mullin, 1993; Prasifka et al., 2015). In fact, more than 2,500 flavonoids have been linked to Asteraceas, mostly flavones and flavonols (Bohm & Stuessy, 2001). Both flavonoids and STs are typically found in specialized trichomes (glandular) and they are known to negatively influence insect performance due to its toxic properties (Kim et al.,2000; Ambrósio et al., 2008). Furthermore, other complex array of SMs may be stored and secreted through these trichomes' glands with varying properties such as being adhesive and capable of turning plant tissue unpalatable, less nutritious, and even toxic (Stipanovic, 1983; Rogers et al., 1987; Fernandes, 1994; Rowe et al., 2012).

In addition to glandular trichomes, non-glandular are a plant mechanical defense that could also play a major antiherbivore role, as it does for certain pathogen infections (Ribeiro et al., 1994; Lake & Wade, 2009). Nevertheless, plant physical defenses have been relatively neglected in plant-herbivore experiments under CO_2 conditions. Non-glandular trichomes may act as a barrier that hamper insects' movement and foraging capabilities, which are especially effective against small insects or while on their early instars (Woodman & Fernandes, 1991; Robinson et al., 2012). The mechanisms by which trichome density is altered with the increase in $[CO_2]$ is still not clear, but best guesses have attributed to alterations in plant gibberellic and jasmonic acids and the expression of certain microRNAs (i.e. miR156) (Sun et al., 2016). Whether trichome density increases or decreases under elevated $[CO_2]$, the consequences may be significant for herbivorous insects' performance, depending on the group of insects and their developmental stage (Woodman & Fernandes, 1991; Sun et al., 2016).

Through the perspective of an herbivorous insect, the combination of those plant alterations due to $[CO_2]$ increase would lead to an unfavorable scenario. In other words, plants would possess an increased biomass availability, but with the cost of lower nutritional quality (leaf nitrogen and water

content), lower digestibility (leaf toughness and SMs), and greater plant mechanical (glandular and non-glandular trichome density) and chemical defenses (SMs such as flavonoids and sesquiterpenes) (Stiling & Cornelissen, 2007; Robinson et al., 2012; Zavala et al., 2013). Such disadvantageous scenario would potentially impact their performance by decreasing food conversion efficiency (ingested and digested), lowering growth rates and pupal weight, prolonging developmental time (larval and pupal), and lowering insect fecundity and survivorship (Massad & Dyer, 2010; Robinson et al., 2012; Li et al., 2016).

Under such circumstances herbivorous insects may adopt certain strategic mechanisms as a response to compensate for host plant lower quality and/or availability (Figure 1) (Nestel et al., 2016). Such responses may be physiological (e.g. temporary production of certain enzymes, alteration in gut morphology; Figure 1A) or behavioral (e.g. food switching, egg dumping; Figure 1B). For instance, insects may increase food intake of up to 80% when reared with leaf material from hosts grown under increased [CO_2] (Lincoln et al., 1993; Zavala et al., 2012). Nevertheless, compensatory behavior must not be taken as a standalone solution due to its inconsistent outcomes such as the ingestion of a toxic dose of leaf allelochemicals (Awmack & Leather, 2002; Nestel et al., 2016).

The goal of this study is to test the hypothesis that Helianthus annuus individuals grown under increased [CO_2] undergo changes in growth, nutrition, and defense; consequently, these changes impact Helicoverpa armigera larval preference and performance (Stamp, 2003, Lee et al., 2012). Hence, we ask the questions: 1) do H. annuus individuals differ in terms of growth, nutrition, and defense when grown under ambient and increased [CO_2]?; 2) Helianthus annuus from which treatment do H. armigera larvae prefer?; 3) Helianthus annuus from which treatment supports the greatest H. armigera larval performance?; 4) does the preferred H. annuus treatment also support the greatest larval performance? Our predictions are that H. annuus grown under increased [CO_2] will present greater height, number of leaves, stem diameter, fresh and dry biomass, leaf thickness, leaf

toughness, and trichome density (Robinson et al., 2012; Zavala et al., 2013). We also expect a decrease in leaf nitrogen and water content, and an increase in leaf flavonoids content. Furthermore, we predict that H. armigera larvae would prefer leaf material from ambient $[CO_2]$, which will positively correlate with greater larval performance (Chew, 1980; Robinson et al., 2012; Zavala et al., 2013). In other words, larvae reared with leaves from ambient conditions will present lower consumption rate and greater conversion efficiency, growth rate, and larval length. Within a shorter development time, individuals from ambient $[CO_2]$ will present a greater proportion of individuals that pupate and become adults.

Material and Methods

Host plant species

Helianthus annuus stands out for its historical, economical (19,4 Bi US\$ - (FAO, 2018) and nutritive importance. It has a profound history alongside humans, being domesticated and used for a variety of purposes by the Native North Americans 3,000 years ago (e.g. food source, dye, herbal medicine, and religious purposes) (Fick & Miller, 2015). Currently, sunflower is still a non-GMO crop produced in over 70 countries on every continent. It is the world's fourth-ranked oilseed in terms of production and the first among those exclusive for oil purposes (FAO, 2018). Sunflowers possess overall 40% of oil content (soybean has ca. 20%) and it is promoted for its health benefits (Wrigley et al., 2015). Some of the current *H. annuus* production serves the purposes of heat (hull, stem, and biomass), livestock and poultry food (meal), apiculture, and ornamental (Kaya et al., 2012; Wrigley et al., 2015). Future trends for oilseed production are to expand (Wrigley et al., 2015), but a negative outcome is expected for crops in general under the CC context and under standard agriculture practices (Streck, 2005).

A cultivar of *H. annuus* (BRS 323) was chosen for this study and obtained from the Brazilian Agricultural Research Corporation (EMBRAPA). This cultivar is a hybrid that has a uniform height (ca. 166 cm to 190 cm), a fast cycle (ca. 80 to 100 days), high oil content (ca. 40%), and a relatively greater vigor (Cvejić & Jocić, 2010; Carvalho et al., 2013). The combination of these characteristics makes BRS 323 one of the most important for Brazilian sunflower producers, as well as internationally.

Herbivore species

Helicoverpa armigera Hübner (Noctuidae), known as the old world bollworm or cotton bollworm (CABI, 2019), is a relatively large size, holometabolous, and multivoltine lepidopteran characterized by being one of the most destructive and widespread agricultural pests (Figure S2) (Fitt, 1989; Ávila et al., 2013). It is reported to feed on plant leaves, buds, inflorescences, fruits, and pods (Czepak et al., 2013), hence, profoundly damaging agricultural yields by rendering host plants unusable or with diminished quality (Karim, 2000). Global annual loss due to *H. armigera* is estimated to be ca. US$ 5 billion and, in China, 50% of insecticides target mainly *H. armigera* (Karim, 2000; Lammers et al., 2007). However, estimates vary greatly and are expected to be underestimated.

Helicoverpa armigera invasiveness success may be explained by four of the species ecophysiological and behavioral traits: i) polyphagy, ii) high mobility, iii) short lifecycle alongside a high fecundity, and iv) a facultative diapause (Fitt, 1989). Polyphagy enables *H. armigera* to rely on an indefinite number of hosts throughout crop rotations and seasons. Meanwhile, a short life cycle coupled with high fecundity promotes the possibility of individuals resistant to certain chemicals to pass their genes relatively fast to the next generation. Finally, high mobility increases the likelihood of gene transfer among distant populations at a fast rate. Nevertheless, if conditions are still harsh, they may enter diapause until more favorable circumstances are met. These ecophysiological and

behavioral traits not only sustain *H. armigera* invasiveness potential but also the reported resistance to numerous insecticides (Czepak et al., 2013).

Experimental design

Helicoverpa armigera eggs were obtained from the Brazilian Agricultural Research Corporation (EMBRAPA - Sete Lagoas, MG) for the preference and performance experiments. For this study, *H. armigera* larvae were individually separated from the beginning to prevent cannibalism, common in every developmental stage (Armes et al., 1992). The preference and performance experiments took place at the end of the sunflower growth period and anthesis beginning (ca. 50-70 days old), which is of the greatest interest due to its proximity to seed formation and harvest.

Sunflower cultivation and caterpillar development took place in Open-Top Chambers - OTCs (see details in Sá et al., 2014) located inside a greenhouse surrounded by a 30% light intensity reduction screen (Nortene®) in the Federal University of Minas Gerais (UFMG) (19°86'90.73"S; 43°96'65.60"W), Belo Horizonte, Brazil. The climate of the study area is known on the Köppen-Geiger classification as Tropical Savanna (*Cerrado* biome) (Alvares et al., 2013). Carbon dioxide concentration inside the OTCs was continuously monitored using an infrared gas analyzer (SBA-4 CO_2 Analyzer, PP SYSTEMS) as well as temperature and humidity. These parameters were automatically recorded every fifteen minutes with the Remote Integrated Control System (RICS 3.7, Evco). There were eight chambers randomly distributed in the greenhouse, four of them were designated as Control and four as Treatment. Carbon dioxide was continuously injected from pressurized cylinders (99% CO_2, 35.69 KgF.cm^{-2}) in Treatment chambers to maintain CO_2 levels equivalent to twice the ambient concentration, hence ambient [CO_2] was ca. 400 ppm and increased [CO_2] ca. 800 ppm. This was in accordance with one of the IPCC's intermediate scenarios

(Representative Concentration Pathways, RCP 6.0) from the latest report and represents a high confidence chance of global warming likely to exceed the threshold of 2º C by 2081-2100 (IPCC, 2014).

Helianthus annuus individuals were cultivated in high-density polyethylene (HDPE) pots (7L volume; 33cm height). There were 10 pots randomly distributed inside each of the eight chambers, hence 40 pots per treatment. Although every plant was cultivated under the same treatment conditions, only half of these pots were designated for the herbivory experiments. This was done to make sure that the analyzed leaf parameters would not suffer any interference due to leaf material removal for the herbivory experiments. Soil nutritional and chemical properties were analyzed in the Soil Department of the Federal University of Viçosa (UFV) and used as the basis for fertilization according to the agronomic recommendation for sunflower production in Minas Gerais (Ribeiro et al., 1999). Soil parameters were: pH, N, P (total and remnant), K, Ca^{2+}, Mg^{2+}, Al^{3+}, H + Al, B, cation exchange capacity (total and effective), base saturation index, Al saturation index, and organic matter. Sunflower pots were filled with appropriately fertilized soil that also had its acidity corrected. Before sowing, every pot was saturated with water and a near field capacity was maintained throughout the experiment duration. Four seeds were sown on each pot in June 2019 and two weeks after seedling emerged, the healthiest and uniform was selected per pot. The earliest seed emergence happened on June 17th/2019 (hereafter, week zero) and sunflowers remained inside OTCs until complete seed maturation/plant whiter. Sunflower pots were exchanged weekly among OTCs of the same treatment and rotated within the chambers (N-S/E-W) to avoid any chamber-specific and sunshade influence.

Host plant growth and nutrient quality

We evaluated if there was an increase in plant growth under the treatment condition (ambient vs. increased CO_2 concentration). Analyzed *H. annuus* were collected, stored and processed according to Pérez-Harguindeguy et al. (2016) through the following parameters: plant final height (measuring tape; cm), stem final diameter at the soil surface (digital caliper; cm), maximum number of leaves, leaf fresh mass (LFM; mg) and leaf dry mass (LDM; mg), specific leaf area (SLA), and development time until anthesis began (days).

Growth parameters were recorded weekly and the final measurements for plant height and stem diameter at soil surface were performed for each sunflower when anthesis began. This was done as vegetative growth tends to stabilize with plant anthesis, which purposely coincided with the chosen period for the beginning of the *H. armigera* larval performance experiments (9th week). Leaf fresh mass (LFM) was obtained through the removal of one disc ($\varnothing$ = 2.4 cm) from three different leaves per sunflower (N= 60/treatment; total= 120) at the 11th week. Leaf's main ribs were used as a reference, and discs were removed from their centermost region. Leaf discs were always from the bottommost and healthiest leaf to avoid any age- and position-specific variation. A high accuracy scale (0.00001 mg precision) was used to record leaf fresh and dry mass weight (after 72 hours in 65 ℃). SLA was calculated by dividing the leaf disc area by its dry weight.

We evaluated if there was a decrease in leaf nutritional quality (leaf nitrogen and water content) under treatments conditions (ambient vs. increased CO_2 concentration). Nitrogen Balance Index (NBI) and leaf water content (LWC; mg) were the chosen parameters to evaluate leaf nutritional quality from the analyzed sunflowers. NBI is a proxy to leaf nitrogen which was accessed using a dual excitation fluorimeter (Dualex® 4.5 Scientific, Force One CNRS-Lure, France) (Tremblay et al., 2012). This method was chosen due to its relative precision and practicality of a nondestructive

procedure. Dualex® readings were performed on both leaf sides (left/right) of three bottommost and healthy leaves per plant (N= 120/treatment; total= 240) on two occasions (7th and 11th week). The time span between these two dates was to evaluate if there was any physiological difference related to sunflower phenology (prior and after anthesis began). Leaf water content (LWC) was calculated using the difference between leaf fresh mass (LFM) and leaf dry mass (LDM). The collection of plant material for these procedures took place in the first week of the herbivory experiments, 11th sunflower week. This was done as caterpillar first instars are the most dependent/vulnerable to host plant traits (see Shelomi et al., 2010).

Host plant defense mechanisms

We evaluated if there was an increase in leaf anti-herbivore mechanisms (mechanical and chemical) under the treatment condition (ambient vs. increased CO_2 concentration), especially carbon-based defense structures. Sunflower leaf material was collected, stored and processed according to Pérez-Harguindeguy et al. (2016) and the following parameters were evaluated: leaf thickness (Mitutoyo® micrometer - 293 series; 0.0001 mm precision), specific leaf weight (SLW; calculated dividing 1 by SLA), leaf toughness (Chatillon Ametek® penetrometer; mg), and density of the non-glandular and glandular trichomes (mm²). Leaf thickness, SLW and leaf toughness were collected in the 11th week. The micrometer and the penetrometer were both used on two equidistant points of three healthy and fully expanded bottommost leaves per plant (N= 120/treatment; total= 240 for leaf thickness and 240 for leaf toughness). A penetrometer measures the required strength to drill a 0.5 mm diameter circle through the leaf (e.g. Loyola Jr & Fernandes, 1993). Trichome type and density were assessed between the 13th and 14th week. We counted separately non-glandular and glandular trichomes from each leaf side (abaxial and adaxial) of three fragments (leaf base, middle, and tip) per leaf from eight plants of each treatment (N= 48 focal points of 24 mm² per treatment;

total= 96) (Barker, 1990). Leaves were always the healthiest and bottommost. For leaf fragment diaphanization, fragments were immersed in a 5% diluted sodium hypochlorite solution (~30min.) for ca. 30 min. and later transferred to a solution containing 1% safranin (~30min.) to facilitate visualization in the microscope (Primo Star - Zeiss®; 10x magnification). A picture (Axiocam 105 color - Zeiss®) with a digital scale (500 µm) was taken of every focal point for later trichome counting (non-glandular and glandular). Finally, sunflower chemical defense was measured through flavonoids content, a simple phenolic compound with important antiherbivore properties. In addition, we chose this allelochemical as a proxy to evaluate the production of other secondary metabolites such as polyphenols under increased [CO_2]. The procedure was performed using a Dualex® on the same occasion and methods described for the NBI measurements (Meyer et al., 2006).

Herbivore preference test

The cafeteria method was used to evaluate *H. armigera* larval preference for *H. annuus* grown under the control and treated conditions (ambient vs. increased CO_2) on the 17th week (Pérez-Harguindeguy et al., 2003). After fasting for 20 hours, second instar caterpillars (8 days old; N= 30) were placed in the center of individualized Petri dishes containing a wet filter paper to maintain leaf turgor. Each half of the paper was labeled and received one leaf disc (∅= 3.0 cm) from each treatment (ambient vs. increased CO_2) with their abaxial side facing upwards. Leaf discs were randomly collected from sunflowers bottommost and healthiest leaves. The following method evaluated larval preference by considering caterpillars searching and feeding behavior through three steps: a) the first contact on one of the leaf fragments based on olfactory cues ("touched"); b) the act to start feeding on the leaf fragment after the initial assessment ("attacked"); and c) the sustained consumption on the preferred leaf fragment ("chosen") (Chew, 1980; Kerpel et al., 2006). Specifically, it was recorded which leaf disc the caterpillar first touched, and which fragment it started

feeding on. After that, caterpillars' location was recorded at intervals of 30 minutes for four consecutive hours, and the final assessment was performed 20 hours after the experiment started. At this time, leaf remains were photographed and consumption was assessed by comparing with the initial leaf area using the ImageJ software (Rueden et al., 2017). The preferred plant fragment/treatment was the one the caterpillars stayed the longest and kept feeding throughout the experiment (Chew, 1980). Total leaf consumption alone could not be used as an indicator of preference assuming there are differences in nutritional quality for *H. annuus* grown under ambient and increased [CO_2] (Cruz-Rivera & Hay, 2000).

Herbivore performance test

The larval performance evaluation started on the 9th week and considered larval development under control and treated conditions (ambient vs. increased CO_2 concentration). The cafeteria method was also applied (Pérez-Harguindeguy et al., 2003) with forty *H. armigera* larvae (N= 20/treatment; total= 40). Chosen larval performance parameters are well established in herbivore performance literature and were evaluated accordingly to the following parameters: relative consumption rate (RCR), ingested food conversion efficiency (CE; %), relative growth rate (RGR), larvae body length (LL; cm), development time until adult (DT; days), survival until pupae (SP, number of pupae), pupae success (PS, number of pupae), pupal weight (PW; mg), and number of adults emerging from chrysalises. Pupae success was used for caterpillars that entered the pre-pupal and did not abort in the first three days.

Helicoverpa armigera eggs were kept inside a Bio-Oxygen Demand Incubator (BOD) until larvae hatched (25±2 °C, 55±10% RH, and 12h photoperiod). Twenty transparent plastic pots (300mL) with a perforated plastic lid covered with a nylon fabric (to ensure aeration) were designated

to each treatment and distributed so that each OTC had five of them. A structure capable of vertically supporting five caterpillar pots was built in each OTC so that they would not have direct contact with the Chambers' surface (see layout Figure S3). To avoid any environmental influence, caterpillar pots daily rotated among the tower levels, within their OTCs (N-S/E-W positions), and among the OTCs of the same treatment (ambient vs. increased CO_2 concentration).

Once caterpillars hatched, each larva was designated to a plastic pot containing a wet filter paper and a hydrophilic cotton to maintain leaf turgor. Leaf fragments offered to them were daily collected from sunflowers (healthiest and bottommost leaves) grown under the respective treatment condition and placed on a designated larger pot containing water. Leaf fragments were randomly selected from these larger pots, photographed, and placed inside a caterpillar plastic pot. We always provided enough leaf fragments to last until the next day. Pots were daily sanitized, received a new filter paper, a piece of cotton and a fresh leaf fragment. Concomitantly, *H. armigera* larvae were gently weighted in a scale (0.00001 mg) for biomass gain and later were photographed for length measure. Larvae body length was recorded through photographs instead of the cephalic capsule since it has been reported to be sufficiently accurate for the species (Armes et al., 1992). Likewise the procedures for fresh leaf fragments, leaf remains were also photographed and their leaf area was obtained using the ImageJ software (Rueden et al., 2017). Leaf area consumption was obtained by subtracting the original fresh leaf area with the remaining area. Later, the consumed leaf area was multiplied by the SLW of the respective treatment to obtain the consumed leaf biomass (Kogan, 1986) used in the performance indexes. On the second day of pupa, survivability was recorded in case of abortion ("pupae success"), pupae were photographed, and their weight recorded for the same reasons as in the larval stage. Finally, the survivability and adult eclosion date from their chrysalis were used to calculate the success probability per development time in a time-to-event (survival) analysis. We

evaluated the results considering that an optimal scenario for *H. armigera* would be one that the greatest proportion of individuals survived (became adults) in the shortest time period.

We could not record instar change confidently, especially in the first instars, as caterpillars would consume their exoskeleton. Nevertheless, we used the wide literature data on *H. armigera* life-cycle as an approximation for the caterpillars in this study (2nd to 6th instar), in addition to the final average (Armes et al., 1992; Ali & Choudhury, 2009; Ávila et al., 2013). Furthermore, most of the larval performance parameters (RCR, CE, RGR, and LL) only considered larvae that started pupation as an indication of larval health.

Statistical analysis

All sunflower growth, nutritional quality, and defense parameters were compared between treatments (ambient vs. increased CO_2 conditions) using analysis of variance tests (ANOVA) through the construction of General Linear Models (GLM). They were: plant final height, stem final diameter at the soil surface, the maximum number of leaves, leaf fresh and dry mass, specific leaf area, sunflower time until anthesis, leaf water content, nitrogen balance index, leaf thickness, specific leaf weight, leaf toughness, density of non-glandular and glandular trichomes, and flavonoids content.

Larval preference (i.e. touched, attacked, and chosen) were individually compared between treatments using G-tests for goodness of fit (Chew, 1980). Larval performance parameters were also evaluated between treatments (ambient vs. increased CO_2 conditions) using ANOVA tests through the construction of GLMs. The following parameters were separately compared between treatments using the final averages and larval averages per instars: relative consumption rate, conversion efficiency of ingested food, relative growth rate, development time until pupation, and larval body length. Only the final averages were used to compare these parameters between treatments: survival

until pupae (pupae start), pupae success, pupal weight, and number of adults. Finally, a time-to-event or survival analysis was performed using the function "Regression for a Parametric Survival Model" from the "survival" package (Therneau, 2015) in R (R Core Team, 2019).

All constructed models were submitted to the most appropriate error correction according to Crawley (2012) (Table 2 & 3). Even though there was only one independent variable for each of the described analyzed parameters, the construction of GLMs were chosen for a few reasons. First, not all data followed a normal distribution and this method allowed us to deal better with this data (e.g. by adjusting the error distribution) than resorting to a non-parametric method. Also, the use of GLMs did not lead to a reduction in statistical power, rather the opposite in some cases.

A one-tailed null hypothesis was applied for larval performance parameters because of our directional hypothesis in which larvae preferred leaf material/treatment would positively correlate with a greater performance (Singer et al., 2004; Ruxton & Neuhäuser, 2010; Jing et al., 2012). In addition, leaf material with greater nutritional quality and/or lower defenses support greater larval performance. Standard errors were evaluated for superposition and p-values $\lesssim 0.05$ were considered statistically significant. All statistical analyses were performed on the R software (R Core Team, 2019).

Results

Host plant growth and nutritional quality under increased CO_2

Individuals of *H. annuus* under increased [CO_2] grew more and for a longer period of time. They were bigger, had a greater stem diameter, and possessed more leaves. They also presented greater leaf biomass (fresh and dry) and water content but had a lower ratio of leaf area to leaf dry

mass (SLA). Finally, leaf nitrogen content was diluted in the beginning (7th week) but did not differ between treatments later (11th week).

Helianthus annuus final height, stem diameter at soil surface, and maximum number of leaves were 9.1%, 10.2%, and 8.4% respectively higher in plants grown under increased $[CO_2]$ (Figure 2ABC; Table 1). Treated sunflowers took, on average, ca. 4 days longer to begin anthesis, therefore a longer vegetative growth (Figure 2D; Table 1). Fresh leaf mass (FLM) and dry leaf mass (DLM) were, respectively, 6.2% and 6.8% higher in plants under increased $[CO_2]$ (Figure 3AB; Table 1). However, specific leaf area (SLA) was 6.2% lower (Figure 3C; Table 1), and leaf water content 6% greater among treated plants (Figure 3D; Table 1). Nitrogen balance index (NBI) varied between treatments depending on the analyzed period (Figure 5A; Table 1). It was 15.8% lower among sunflowers under increased $[CO_2]$ at the beginning of the experiment (7th week) and did not differ between treatments by the 11th week.

Host plant defense mechanisms under increased CO_2

Helianthus annuus individuals under increased $[CO_2]$ had thicker leaves with greater density of glandular trichomes. Nevertheless, their leaves were less tough than those under ambient conditions. Flavonoids content increased among treated plants at the experiment beginning (7th week) but reached the same level between treatments later (11th week).

Increased $[CO_2]$ had a positive effect on leaf thickness (Figure 3E; Table 1) and specific leaf weight (SLW) (Table 1). The average leaf thickness under increased CO_2 was 7.4% greater (0.409 mm) than in plant individuals under ambient conditions (0.381 mm). Concerning leaf toughness, an average force of 125.1 mg was required to drill a 0.5 mm hole through treated sunflowers leaves, 14.4% lower than the 143.1 mg required in control leaves (Figure 3F; Table 1). Plant individuals

under increased [CO_2] had, on average, 0.5 glandular trichomes per mm², 24.1% higher than the 0.4/mm² in control leaves (Figure 4; Table 1).

Concerning flavonoids content (Flv), the results also varied between treatments depending on the analyzed period (Figure 5B; Table 1). At the 7th week, the concentration was 9.8% higher in plants under increased CO_2 conditions (Figure 5B; Table 1) but did not differ statistically by the 11th week. The main results of *H. annuus* evaluated parameters have been summarized and contextualized with the two most recent meta-analyses on the effects of increased [CO_2] on plant-insects (Table S1A) (Stiling & Cornelissen, 2007; Robinson et al., 2012). By the 11th week, most of our results did not confirm what had been reported on these studies.

Herbivore preference and performance under increased CO_2

Helicoverpa armigera larvae did not show a preference for leaf material of either treatment, regardless of the larval preference step (touched, attacked, and chosen; Table 2). Nevertheless, *H. armigera* larvae performed better when reared with leaves grown under increased [CO_2] (Table 3). Larvae reared with treated leaves consumed more plant material and had a lower conversion efficiency of the ingested food. During the first instars, larvae under increased [CO_2] grew relatively less than those under ambient conditions, but this reverted during later larval instars. No difference between treatments was observed regarding larval pupal weight. Ultimately, larvae reared with leaves from increased [CO_2] were more successful since they had a greater proportion of individuals that started pupating and became adults in a shorter period of time.

Helicoverpa armigera larvae under increased [CO_2] presented a higher final relative consumption rate (RCR) (28.4%) (Figure 6A; Table 3). Similarly, the second (29.3%), fourth (48.8%), and fifth (25.6%) instars of larvae fed with treated leaves presented higher RCR (Figure 6A;

Table 3). On the other hand, they had a lower conversion efficiency rate of ingested food (CE) in their third (90.9%) and fourth (74.3%) instars (Figure 6B; Table 3) under increased [CO_2]. Also, *H. armigera* larvae had a lower relative growth rate (RGR) in their third (62.5%) and fourth (50.7%) instars, but higher RGR in their fifth (37%) and sixth (109%) instars (Figure 6C; Table 3). Consequently, larvae under increased CO_2 conditions presented shorter body length (28.9%) in their fourth instar, but longer in the sixth instar (24.9%) and final average (38.8%) (Figure 6D; Table 3).

The number of larvae that started pupating and successfully pupated (did not abort in the first three days) was greater among caterpillars under increased [CO_2] (Figure 7; Table 3). Hence, under increased CO_2 conditions, 50% of initial individuals became pupae (N=10), and 40% that successfully pupated (N=8). While 25% of initial individuals under ambient conditions became pupae (N=5), and 15% that successfully pupated (N=3). There were 4 times more adults emerging from pupae when their larvae were reared with leaves from increased [CO_2] (N= 8; 40% survival), than those fed with leaves from ambient conditions (N= 2; 10% survival) (Figure 7; Table 3). In addition, not only a greater proportion of individuals under increased [CO_2] survived (success probability), but they also completed their life cycle earlier than those under ambient condition (Figure 8; Table 3). The average length until larvae became adults under increased [CO_2] was 44.2 days, 5.2% earlier than those under ambient conditions (avg. 46.5 days). The time difference increases when we consider the time at which individuals of both treatments would have 50% of success probability (blue and red vertical lines; Figure 8). Specifically, 49.6 days for larvae under treated conditions and 54.02 days under ambient condition (ca. 9% earlier in increased [CO_2]). The main results of *H. armigera* larval preference and performance have been summarized and contextualized with the two most recent meta-analyses on the effects of increased [CO_2] on plant-insects (Table S1B) (Stiling & Cornelissen, 2007; Robinson et al., 2012). Overall, most of our results did not confirm what had been reported on these studies.

Discussion

Host plant growth, nutritional quality, and defenses under increased CO₂

Our predictions for *H. annuus* development under increased [CO_2] were mostly confirmed. Including the observed acclimation effects, analyzed plant traits under treated conditions suggests an overall unfavorable scenario for *H. armigera* larvae at early larval stages, but potentially advantageous later. Sunflower leaves under increased [CO_2] had lower nitrogen (N) content at early sunflower development (7th week). This may be explained as increased [CO_2] positively affects photosynthesis rates and cell division by positively regulating genes related with cell wall loosening (Wei et al., 2013; Thilakarathne et al., 2015). Consequently, the increase in production and accumulation of non-structural carbohydrates may cause a reduction in nitrogen uptake in relation to carbon gain. Altogether, these effects lead to leaves of lower nutritional quality due to the dilution of leaf [N] (Del Pozo et al., 2007). Nevertheless, an acclimation was later observed (11th week), which eliminated the [N] differences between treatments as well as of leaf flavonoids content. The acclimation under increased [CO_2] has been reported for crops and non-crops as plants may change their resource allocation throughout development and, most importantly, there is a limit to how much plants can allocate the extra carbohydrates to growth (sink strength limitation) (Long et al., 2004; Seneweera, 2011; Tausz-Posch et al., 2020). Finally, contrary to expectations, leaf water content increased in plants under increased [CO_2]. This may be explained due to increased [CO_2] induced effect of partially closing leaf stomatal pores, therefore increasing plant water-use efficiency, and decreasing overall water loss (Zavala et al., 2013; Nowak, 2017).

Our predictions for sunflower defenses were also confirmed, except for leaf toughness. Tougher leaves are an important hindrance for herbivorous insects, especially for early larval instars

whose feeding apparatus is not fully developed (Hochuli, 2001; Malishev & Sanson, 2015). Despite the known positive correlation of leaf thickness and leaf toughness (Cherret, 1968; Lincoln et al., 1993), leaves under increased [CO_2] were less tough than those under ambient conditions. This may be explained by the greater leaf water content (LWC) in treated plants, which has a strong negative correlation with leaf toughness (Cherret, 1968). For an efficient folivore such as *H. armigera* and specially on mature larval stages, plants from increased [CO_2] presenting greater biomass, higher nutritional return (i.e. leaf water content), and an easier digestibility (lower toughness) represent an overall advantage (Walters, 2011).

On the other hand, the increase in glandular trichome density coupled with higher flavonoids content (Flv) on plants under increased [CO_2] may have negatively affected *H. armigera* larvae for two reasons. First, these changes occurred when larvae were in their early/critical developmental stages, hence more vulnerable to host plant alterations (Hochuli, 2001). The second reason deals with the potential implications that an increase in leaf Flv represents. Flavonoids are biosynthesized via the phenylpropanoid (PAL) pathway and may negatively influence insect performance due to their prooxidant and palatability properties (O'Neill et al., 2010; Zavala et al., 2013). The PAL pathway demands high levels of carbohydrates to operate, hence, if Flv content increased, it is reasonable to infer that other allelochemicals may have increased as well (O'Neill et al., 2010; Robinson et al., 2012). Despite the later acclimation on the 11th week, some of these allelochemicals could have been allocated to the higher density of glandular trichomes, which may store and secrete them. Ultimately, the combination of these results may have further hampered herbivores performance under treated conditions (increased CO_2 concentration) (Stipanovic, 1983; Fernandes, 1994; Prasifka et al., 2015).

In summary, during early *H. annuus* development, while plants under increased [CO_2] had greater biomass availability that was more easily digestible due to greater water content; they were also nutritionally poorer and possessed greater mechanical and chemical defenses. It is true that the

sunflower acclimation affected two of the most important parameters on leaf quality (nitrogen and flavonoids content), however, it occurred later on sunflower and larvae development. Therefore, it certainly affected *H. armigera* larval preference and/or performance.

Herbivore preference and performance under increased CO_2

Helicoverpa armigera larvae did not show preference for *H. annuus* grown under ambient or increased CO_2 conditions. These results were not predicted due to the effects that the increased $[CO_2]$ had on treated plant individuals, even after the sunflower acclimation, when the preference test was performed (17th week). We propose two potential nonexclusive explanations for this finding.

First, at the occasion of the preference test, the observed sunflower acclimation had already taken place. Thus, despite the greater leaf water content in treated sunflowers, neither leaf [N] nor [Flv] differed between treatments. In fact, while sunflower leaves under ambient conditions were tougher, those from increased CO_2 had a greater density of glandular trichomes. Hence, the leaf trait differences between treatments could have not be sufficiently determinant to larval choice.

Second, the choice between leaf fragments of the same species may not be as important for *H. armigera*. This is supported by the species oviposition behavior, which prefer flowered plants for oviposition, but oviposit indiscriminately over large areas in their absence (Parsons, 1940; Fitt, 1989). This behavior is sustained through generations when we consider *H. armigera* larval traits. For instance, the combination of polyphagy and high mobility (even in larval stages) enable them to search for other host species, possibly more nutritious (Ávila et al., 1986; Pedgley et al., 1987; Fitt, 1989). And if the conditions remain unfavorable, there is always the possibility to adopt one or some of the compensatory responses (Figure 1; Nestel et al., 2016). Altogether, these ecophysiological and

behavioral traits may lessen the importance of larval host choice/preference for this species (Singer, 1986; Price et al. 1990).

Our initial expectations for *H. armigera* larval performance were that, under increased CO_2 conditions, larvae would present lower conversion efficiency of ingested plant material (CE), higher relative consumption rate (RCR), and lower relative growth rate (RGR) and larval body length (LL) (Lincoln et al., 1993; Bale et al., 2002; Robinson et al., 2012; Nestel et al., 2016). CE and RCR predictions were confirmed, but not if we consider the observed acclimation. In other words, RCR was expected to be higher, but only when treated plants were of poorer leaf quality (before the acclimation). However, a greater RCR prevailed throughout the experiment, even when the differences between two of the most important leaf traits did not persist (i.e. lower N and greater Flv). This could be explained by the poorer conditions in which larvae under increased [CO_2] were subjected in the earliest stages. Hence, they behaviorally overcompensated by consuming relatively more leaf material throughout the rest of their development (Nestel et al., 2016). This unforeseeable overcompensation further explains the results for larval growth (i.e. RGR and LL). Thus, RGR and LL were greater among larvae under control conditions at early larval instars but reversed when they were more mature.

The greater performance of larvae reared on plants under increased [CO_2] reflected on the higher proportion of individuals that started pupating, that successfully pupate, and that emerged from their pupae as adults. Thus, despite the lack of larval preference, *H. armigera* performed better when reared with leaves from increased [CO_2] and this positively correlated with greater developmental success in a shorter time. This contradicts our predictions and the most recent meta-analysis (Stiling & Cornelissen, 2007; Robinson et al., 2012), confirming that the compensatory strategy of prolonging larval stage to compensate for a disadvantageous diet/condition was not adopted, on the contrary (Figure 8).

Although leaf N was lower among treated plants earlier and statistically the same later, plant total nitrogen (N) accumulation is expected to increase alongside plant biomass, just not at the same rate (Lakshmi et al., 2017; Tausz-Posch et al., 2020). This further supports the results of greater larval success under increased [CO_2]. By consuming relatively more plant material due to the disadvantageous diet in earliest larval stages, *H. armigera* was able to access the greater accumulation of total N (Mattson 1980; Zavala et al., 2013).

It is true that *H. armigera* larvae mortality rates were high, especially under the control condition. However, this does not diminish the relevance of our results; on the contrary, the low number of larvae at the final stage of the experiment reduces the likelihood of finding any significant effect, which we did. In addition, a study using the *H. armigera - Cajanus cajan* L. (pigeon pea) system reported significantly longer development time and lower survival when larvae were reared exclusively with leaf material. While 75-90% of larvae fed with pigeon pea pods reached their pupal stage, only 5-55% did when reared with a leaf-only diet (Sison & Shanower, 1994). Although the pigeon pea and sunflowers are phylogenetically unrelated species, the significantly high mortality found for *H. armigera - C. cajan* study further supports our results, crossing out the possibility of any non-measured/not-predicted factors, evidencing the positive effects of increased [CO_2] on larval performance.

At the most straightforward interpretation, *H. armigera* improved performance under an increased CO_2 atmosphere dismisses an optimistic scenario for agriculture in which crops would benefit from a greater productivity due to CO_2 fertilization. Nevertheless, the implications of these results are much more pervasive and may aggravate an already worrisome Climate Crisis scenario. Historically, the success of *H. armigera* as an invasive species is irrefutable (Fitt, 1989; Emiljanowicz et al., 2017). In addition, humans inadvertently favor *H. armigera* by providing the resources and eliminating the biotic constraints in the mainstream industrial agriculture model (Struik & Kuyper,

2017). Under increased [CO_2], *H. armigera* larvae not only performed better, but also supported a greater number of pupae and adults within a potentially shorter developmental time. In the long term, *H. armigera* populations are likely to continuously grow, threatening native and anthropogenic ecosystems by proportionally increasing the damage to cultivated and wild plants and displacing or eliminating indigenous species. This scenario questions the support for inefficient agriculture models and fossil fuel-based technology. Concomitantly, our results highlight the emergency to adopt preventive measures to deal with biological invasions and implement integrated management practices for post-entry challenges. Inertia would mean a serious threat to food security and the adoption of radical measures that could trigger positive feedback loops such as poisoning natural environments beyond restoration.

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APPENDIX I – Tables and captions

Table 1. Sunflower growth, nutritional quality, and defense mechanisms grown under ambient and increased CO_2 conditions.

Parameters	Ambient CO_2 ($\bar{x} \pm SE$)	Increased CO_2 ($\bar{x} \pm SE$)	P-value	D.f.	Errors D.
Sunflower growth and nutritional quality					
Final height (cm)	75.222 ± 1.196	82.056 ± 2.816	**0.0322**	34	gaussian
Stem diameter (cm)	7.521 ± 0.190	8.290 ± 0.288	**0.0324**	34	gaussian
Number of leaves	17.778 ± 0.563	19.278 ± 0.491	**0.0454**	34	quasipoisson
Time until anthesis (days)	68.158 ± 1.132	71.790 ± 1.133	**0.0234**	36	quasipoisson
Fresh leaf mass (mg)	83.420 ± 1.247	88.553 ± 1.822	**0.0218**	118	gaussian
Dry leaf mass (mg)	16.690 ± 0.306	17.823 ± 0.363	**0.0186**	118	gaussian
Specific leaf area (SLA)	2.761 ± 0.047	2.599 ± 0.052	**0.0229**	118	gaussian
Leaf water content (mg)	66.730 ± 1.148	70.730 ± 1.654	**0.0493**	118	gaussian
NBI 7th week	21.058 ± 0.313	18.186 ± 0.243	**5.60E-12**	238	gaussian
NBI 11th week	26.679 ± 0.809	26.799 ± 0.809	0.9165	238	gaussian
Sunflower physical and chemical defenses					
Leaf thickness (mm)	0.381 ± 0.005	0.409 ± 0.006	**0.0003**	238	gaussian
Specific leaf weight (SLW)	0.369 ± 0.007	0.394 ± 0.008	**0.0186**	118	gaussian
Leaf toughness (mg)	143.108 ± 7.581	125.092 ± 5.582	**0.0569**	238	gaussian
Non-glandular trichome density (mm²)	0.434 ± 0.026	0.477 ± 0.039	0.3645	93	gaussian
Glandular trichome density (mm²)	0.437 ± 0.026	0.543 ± 0.039	**0.0272**	93	gaussian
Flavonoids 7th week	1.348 ± 0.016	1.480 ± 0.018	**5.69E-08**	238	gaussian
Flavonoids 11th week	1.041 ± 0.026	1.015 ± 0.022	0.4480	238	gaussian

Table 2. *Helicoverpa armigera* larval preference of sunflower leaf material from ambient or increased CO_2 concentration. Larvae first touched, attached, and chose *H. annuus* leaf fragments.

	Preference steps		
Treatment	**(a) Touched**	**(b) Attacked**	**(c) Chosen**
Ambient CO_2	15	11	16
Increased CO_2	14	10	13
None	0	8	0

Caption: a) touched: the treatment of the first leaf fragment reached and touched by a larva; b) attacked: the treatment of the first leaf fragment that the larva started feeding within the first five-ten minutes after it touched; c) chosen: the treatment of the first leaf fragment the larva stayed/consumed the longest.

Table 3. *Helicoverpa armigera* larval performance parameters under ambient and increased CO_2 conditions.

Parameters	Ambient CO_2 ($\overline{x} \pm SE$)	Increased CO_2 ($\overline{x} \pm SE$)	P-value	D.f.	Errors D.
Relative consumption rate (mg/mg/day)	0.451 ± 0.017	0.579 ± 0.043	**0.0308**	13	gamma
2nd instar	0.623 ± 0.086	0.805 ± 0.052	**0.0418**	11	gamma
3rd instar	0.533 ± 0.069	0.629 ± 0.051	0.1405	12	gamma
4th instar	0.396 ± 0.037	0.589 ± 0.070	**0.0374**	12	gamma
5th instar	0.334 ± 0.033	0.419 ± 0.022	**0.0242**	13	gamma
6th instar	0.363 ± 0.042	0.365 ± 0.011	0.4755	13	gamma
Conversion efficiency of ingested food (mg/mg/day)	189.124 ± 21.314	188.091 ± 37.940	0.4787	12	gaussian
2nd instar	60.907 ± 23.943	58.971 ± 14.226	0.4210	12	gaussian
3rd instar	264.490 ± 39.680	138.542 ± 24.791	**0.0074**	12	gaussian
4th instar	385.664 ± 40.850	221.262 ± 38.319	**0.0160**	12	gaussian

5th instar	268.956 ± 103.756	456.912 ± 156.893	0.2343	12	gaussian
6th instar	78.481 ± 35.749	124.989 ± 17.735	0.0973	12	gaussian
Relative growth rate (mg/mg/day)	0.423 ± 0.037	0.347 ± 0.045	0.2736	13	gamma
2nd instar	0.775 ± 0.170	0.704 ± 0.224	0.3867	13	gamma
3rd instar	1.030 ± 0.226	0.634 ± 0.055	**0.0045**	13	gamma
4th instar	0.557 ± 0.096	0.370 ± 0.044	**0.0352**	13	gamma
5th instar	0.255 ± 0.025	0.349 ± 0.033	**0.0263**	13	gamma
6th instar	0.076 ± 0.015	0.159 ± 0.019	**0.0036**	13	gamma
Body length (cm)	1.297 ± 0.040	1.442 ± 0.044	**0.0270**	13	gaussian
2nd instar	0.361 ± 0.025	0.356 ± 0.012	0.4179	13	gaussian
3rd instar	0.689 ± 0.056	0.669 ± 0.052	0.4093	13	gaussian
4th instar	1.382 ± 0.075	1.072 ± 0.065	**0.0061**	13	gaussian
5th instar	1.862 ± 0.126	1.783 ± 0.097	0.3204	13	gaussian
6th instar	2.154 ± 0.065	2.691 ± 0.077	**0.0001**	13	gaussian
Time until adult (days)	46.5 ± 1.061	44.2 ± 0.955	**0.0191**	37	weibull
Pupae (%)	0.250 ± 0.099	0.500 ± 0.115	**0.0500**	38	binomial
Pupae success (%)	0.150 ± 0.082	0.400 ± 0.112	**0.0363**	38	binomial
Pupal weight (mg)	185.437 ± 33.014	207.461 ± 7.670	0.1754	9	gaussian
Adults (%)	0.100 ± 0.069	0.400 ± 0.112	**0.0122**	38	binomial

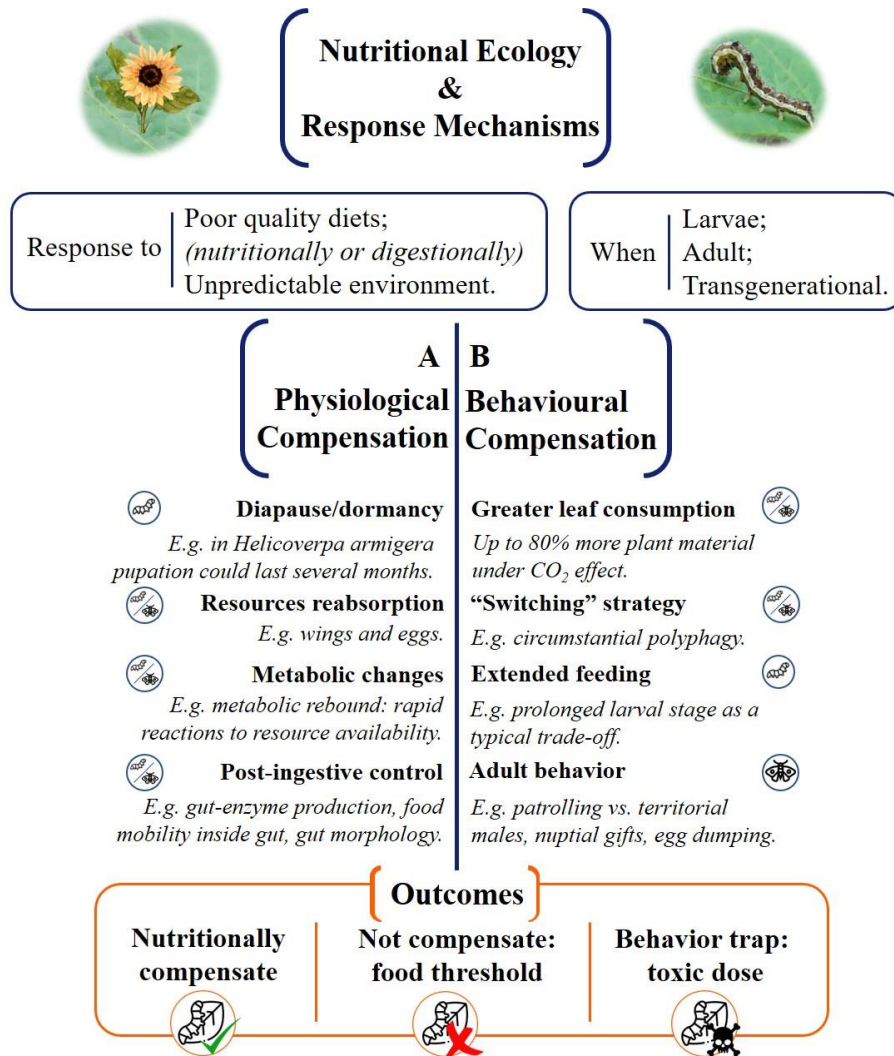# APPENDIX II – Figures and captions

Figure 1. *Infographic of insect herbivores nutritional ecology, compensatory mechanisms with examples, and potential outcomes. Larval and adult compensation strategies may be A) physiological and B) behavioral. Small blue circles identify at which Lepidoptera stage the exemplified compensations may happen. Adapted from Slansky & Wheeler, 1992; Cruz-Rivera & Hay, 2000; Nestel et al., 2016.*
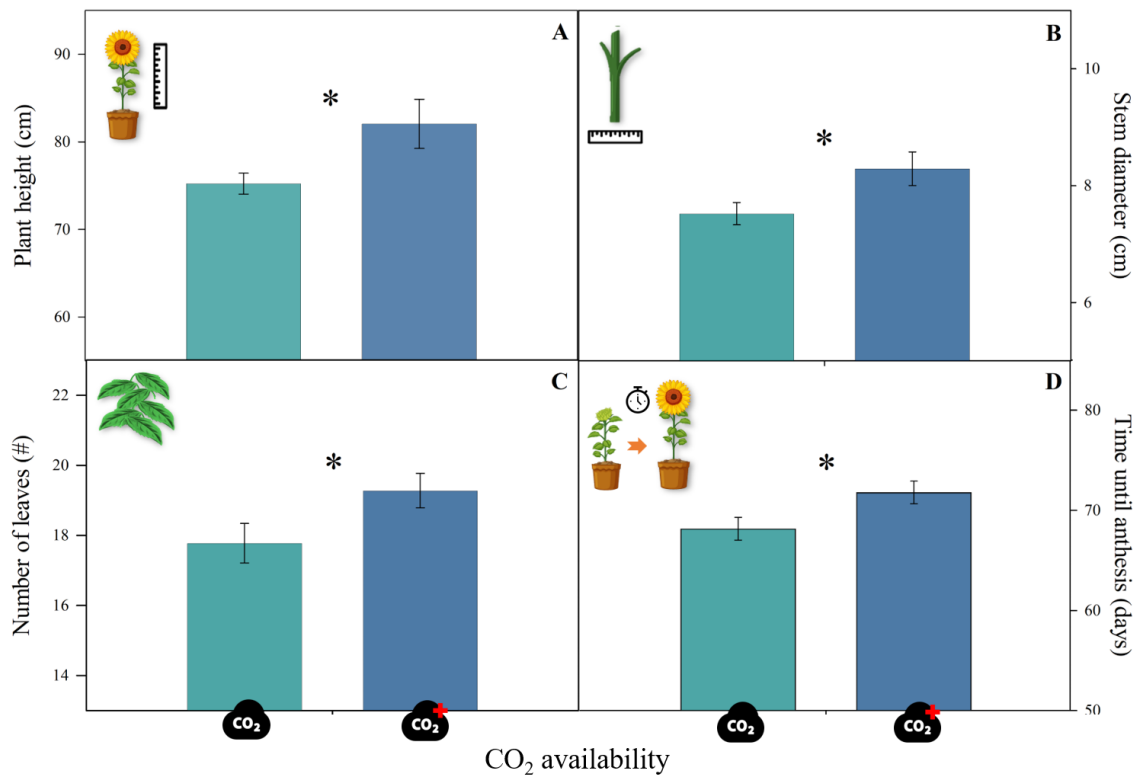
Figure 2. *Sunflower response to CO₂ enrichment in open-top chamber experiment. (A) Height ($\bar{x} \pm SE$); stem diameter at the soil surface ($\bar{x} \pm SE$); (C) maximum number of leaves per plant ($\bar{x} \pm SE$); (D) sunflower development time until anthesis began ($\bar{x} \pm SE$. * indicates a statistically significant difference between the groups.*
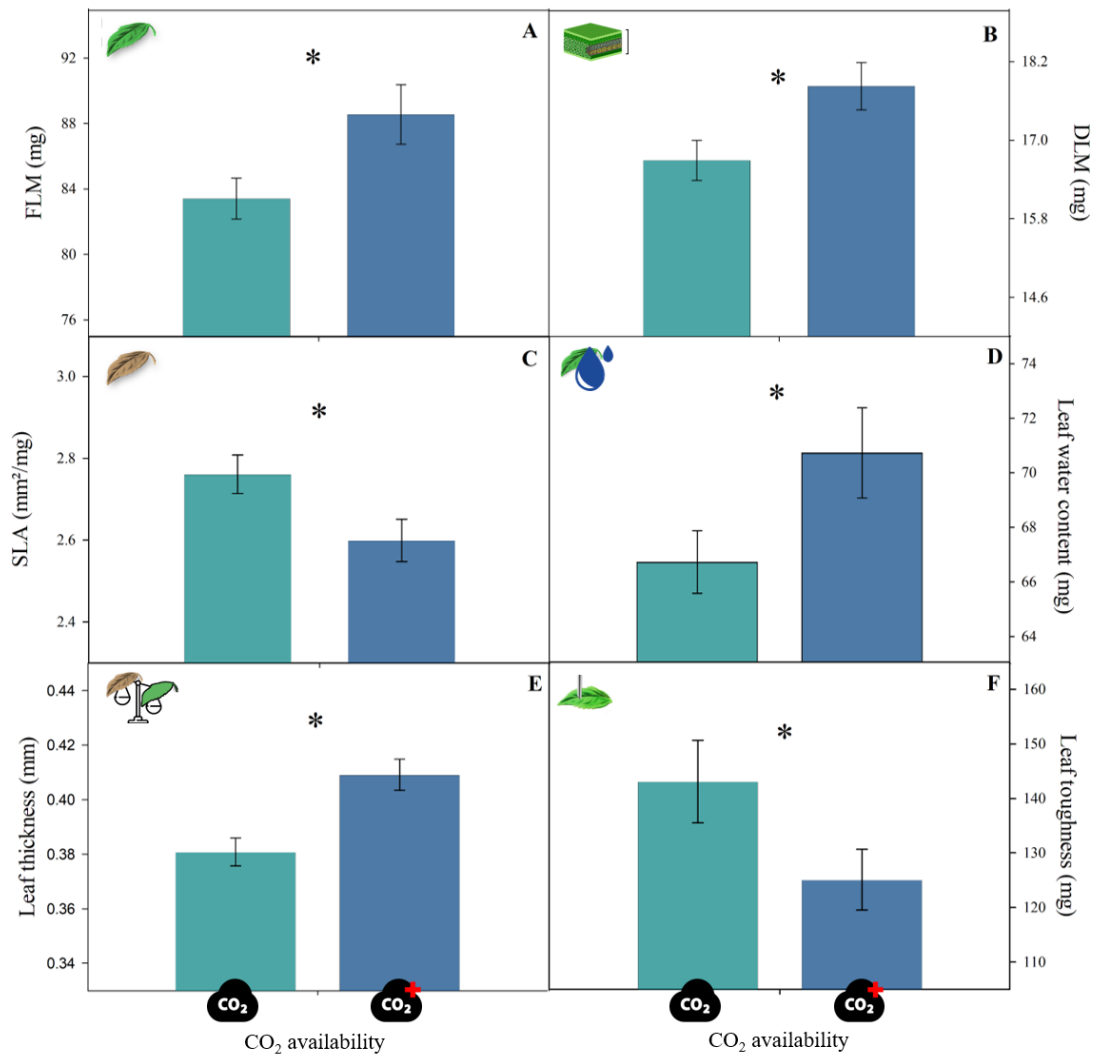
Figure 3. *Sunflower leaf responses to CO_2 enrichment experiment. (A) Fresh leaf mass (FLM) ($\overline{x} \pm SE$); (B) dry leaf mass (DLM) ($\overline{x} \pm SE$); (C) specific leaf area ($\overline{x} \pm SE$); (D) leaf water content (LWC) ($\overline{x} \pm SE$); (E) leaf thickness ($\overline{x} \pm SE$); (F) leaf toughness ($\overline{x} \pm SE$) * statistically significant difference between the groups.*
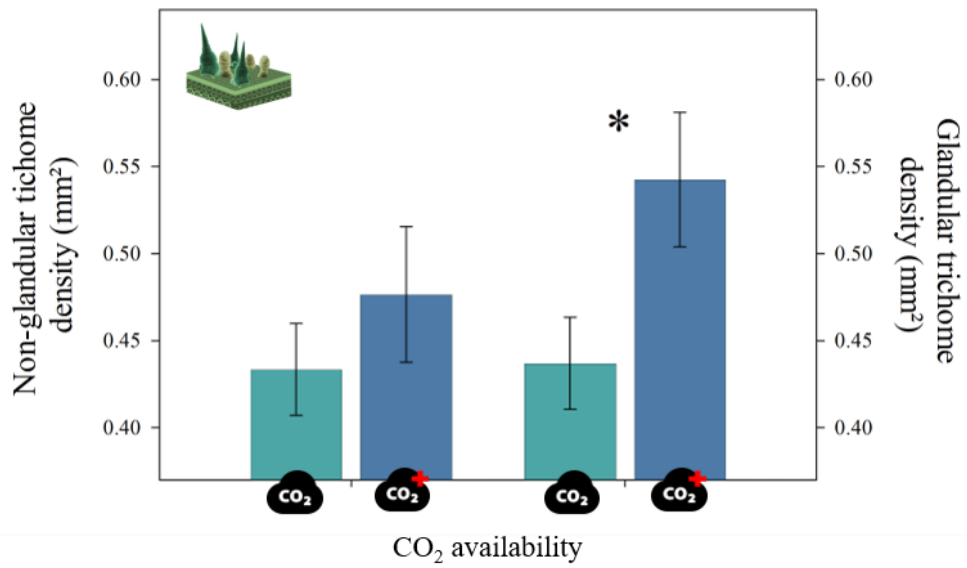
Figure 4. *Sunflower non-glandular and glandular trichome density ($\bar{x} \pm SE$) under CO_2 enrichment experiment. * statistically significant difference between the groups.*
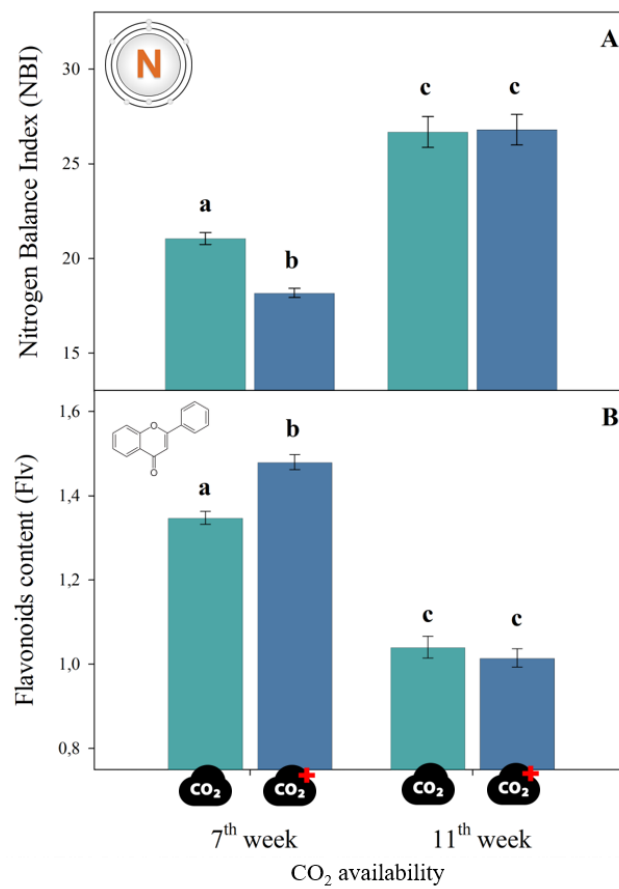

Figure 5. *Sunflower leaf responses to CO_2 enrichment experiment measured on the 7th and 11th week after seedling emerged. (A) Nitrogen Balance Index (NBI) ($\bar{x} \pm SE$); (B) flavonoids content*

(Flv) ($\overline{x} \pm SE$). Different lowercase letters indicate a statistically significant difference between the groups.
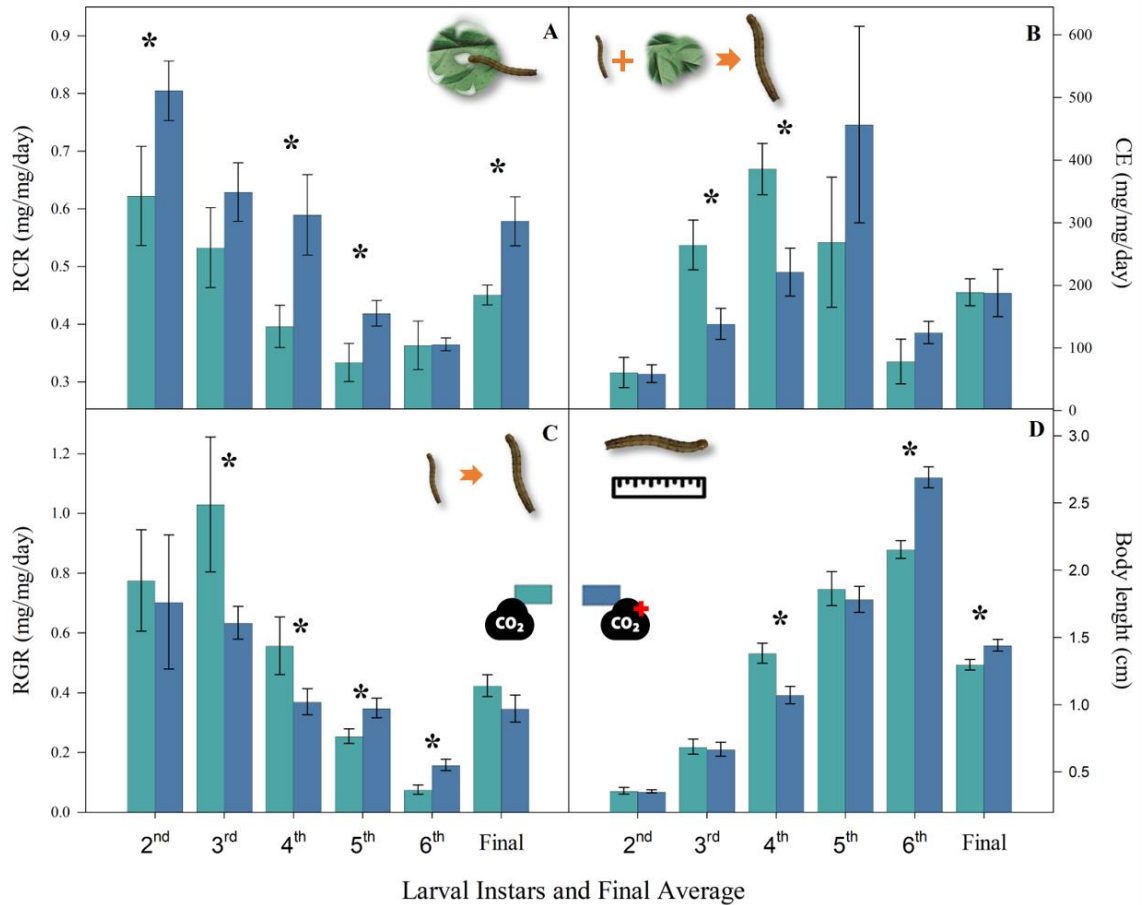

Figure 6. *Larval performance parameters ($\overline{x} \pm SE$) of H. armigera during development (second to sixth instar and final average) when treated with sunflower leaves from ambient condition (control) and increased CO_2 condition (treatment): relative consumption rate (RCR) in mg/mg/day (A); conversion efficiency of ingested food (CE) in mg/mg/day (B); relative growth rate (RGR) in mg/mg/day (C); and larval body length in cm (D). * indicate a statistically significant difference between the groups.*
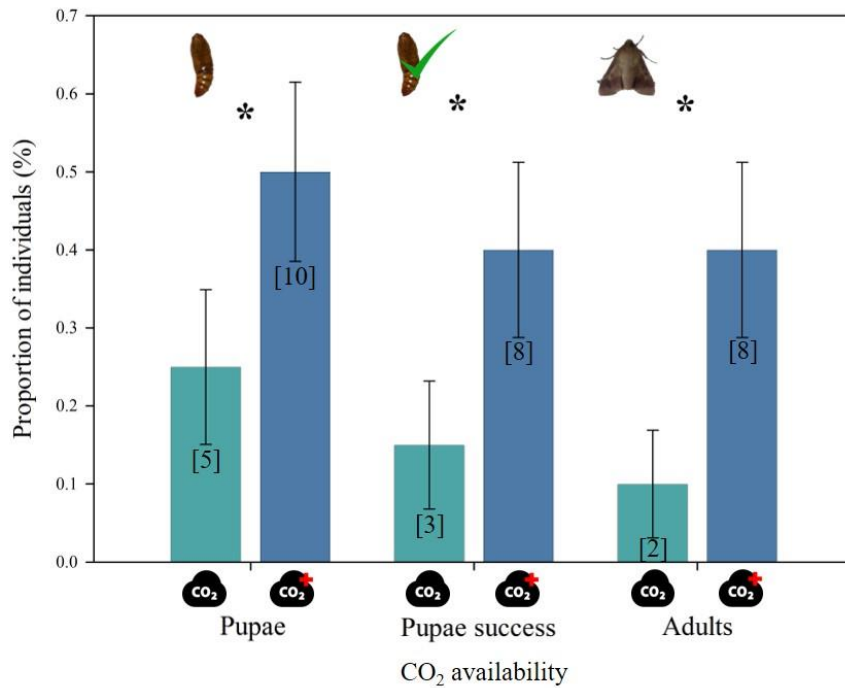
Figure 7. *Proportion ($\bar{x} \pm SE$) of H. armigera larvae treated with sunflower leaves from ambient and increased CO_2 condition that started pupation ("pupae"), pupated without aborting ("pupae success"), and the number of adults ("adults"). The number of larvae/pupae are in brackets on top of each bar. * indicate a statistically significant difference between the groups.*
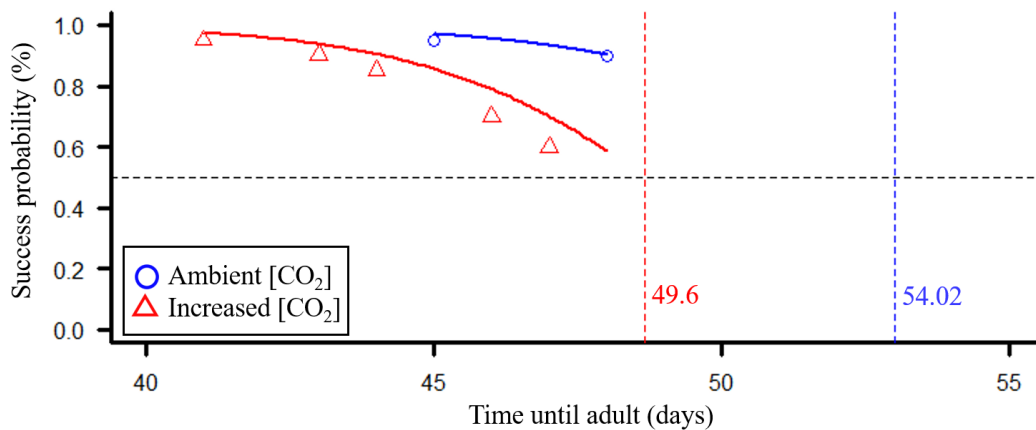

Figure 8. *Probability success (%) per time (days) of Helicoverpa armigera larvae becoming adults. Blue circles are for larvae under ambient [CO_2] and red triangles for those under increased [CO_2]. Blue and red vertical lines are for the calculated time when individuals of both treatments would have a 50% success probability of becoming adults. p=0.0191.*
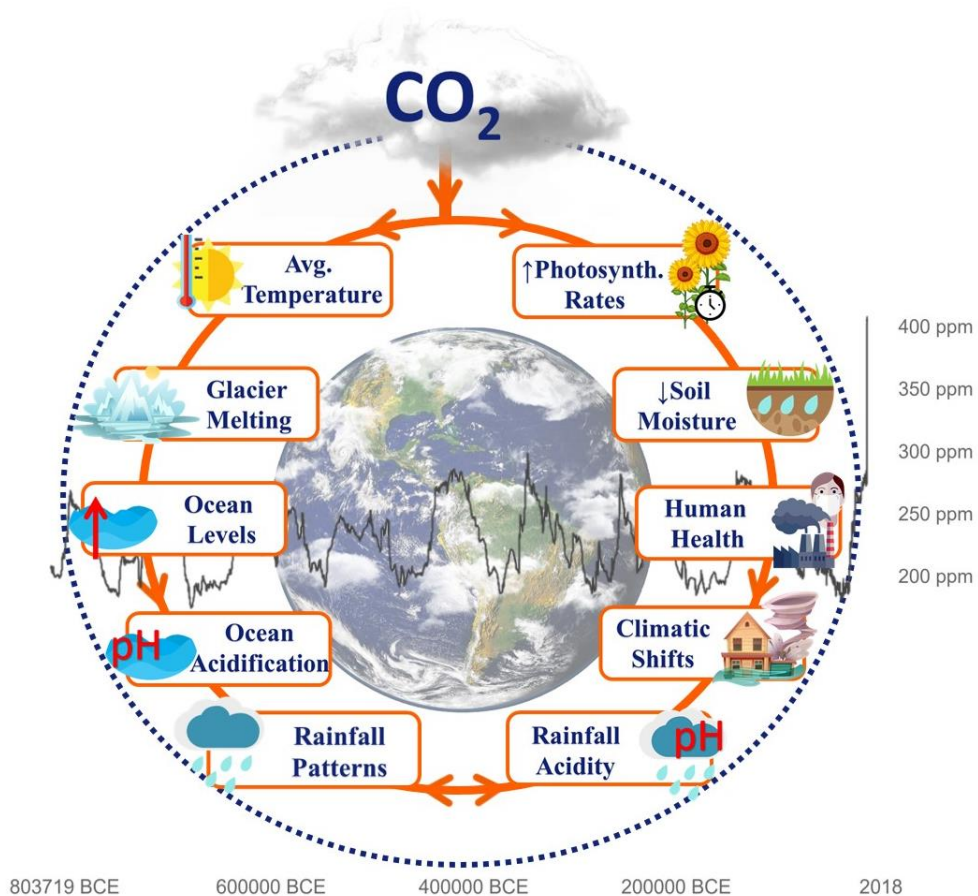
APPENDIX III – Supplementary material

Figure S1. *Infographic of CO_2 direct and indirect effects on the Earth System and human populations (orange boxes) (adapted from Coviella and Trumble, 1999). In the background, an open-access chart with the historical carbon dioxide data stored in ice cores from 803,719 BCE to 2018 (Ritchie & Roser, 2020).*
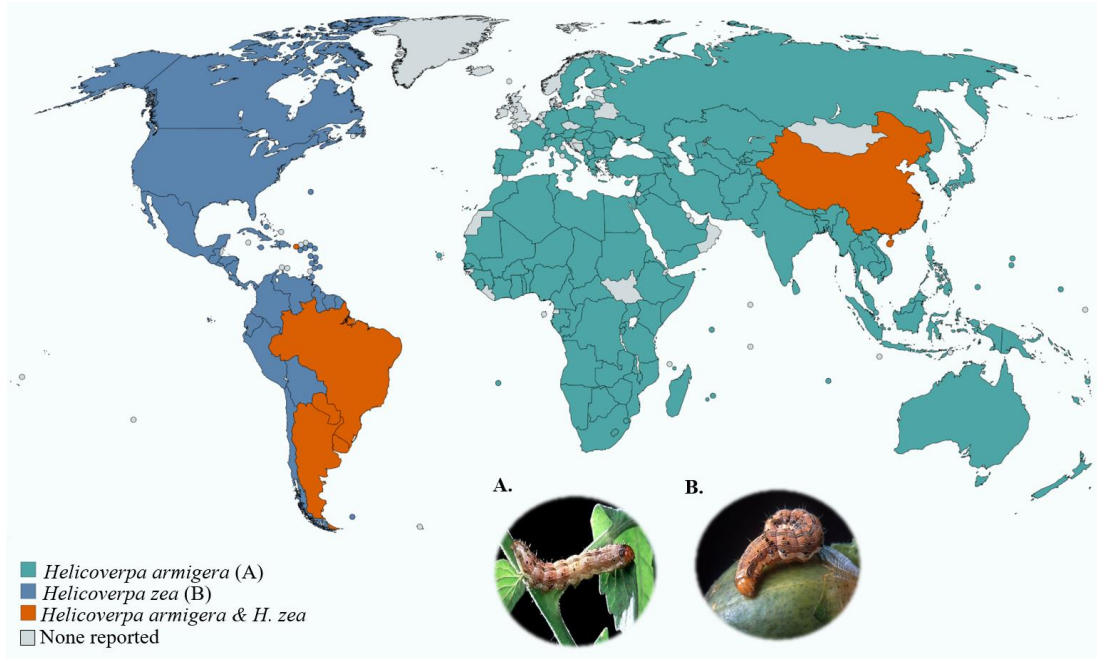
Figure S2. *Global distribution of Helicoverpa armigera (A) and H. zea (B) per country according to the Global Invasive Species Database (GISD) (ISSG, 2020). Helicoverpa sp. images are from the Global Invasive Species Database.*
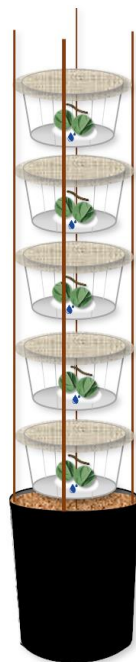

Figure S3. *Layout of one of the constructed structures ("towers"), and five transparent plastic pots with a perforated lid covered with a nylon fabric. Each pot had one H. armigera caterpillar and*

each tower supported five pots that daily rotated among their levels. There was one tower per Open-top Chamber (OTC) that also rotated among OTCs of the same treatment.

Table S1. A contextualization between this study results and the two most recent meta-analyses on the topic. (A) Plant responses to elevated CO_2; (B) Insect responses to elevated CO_2.

(A) Plant response	CO_2
Nitrogen content (NBI)	Expect ↓ at increased CO_2
References	SC07; RRN12
We found	Confirmed (7th week).
	Opposite (11th week).
Leaf toughness	Expect ↑ at increased CO_2
References	RRN12
We found	Opposite.
Flavonoids	Expect ↑ at increased CO_2
References	RRN12
We found	Confirmed (7th week).
	Opposite (11th week).
SLA	Expect − at increased CO_2
References	SC07; RRN12
We found	Opposite.
SLW	Expect ↑ at increased CO_2
References	RRN12
We found	Confirmed.
General toughness - thickness	Expect ↑ at increased CO_2
References	RRN12
We found	Confirmed.

(B) Insect response	CO_2
Relative growth rate (RGR)	Expect ↓ at increased CO_2
References	SC07; RRN12
We found	Confirmed (3rd and 4th instar).
	Opposite (5th and 6th instar).
Relative consumption rate	Expect ↑ at increased CO_2
References	SC07; RRN12
We found	Confirmed.
Conversion efficiency - ingested	Expect ↓ at increased CO_2

References	SC07; RRN12
We found	Confirmed.
Development time	Expect $\uparrow$ at increased CO_2
References	SC07; RRN12
We found	Opposite.
Pupal weight	Expect $\downarrow$ at increased CO_2
References	SC07; RRN12
We found	Opposite.
Survival	Expect $-$ at increased CO_2
References	SC07; RRN12
We found	Opposite.

Blue text indicates that our study results (H. annuus - H. Armigera system) confirmed the meta-analyses reports (Stiling & Cornelissen, 2007 = SC07; and Robinson et al., 2012 = RRN12). $\uparrow$: increase; $\downarrow$: decrease; $-$: no change.

Supplementary material references

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