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Methodological advances: Using greenhouses to simulate climate change scenarios

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ABSTRACT

Human activities are increasing atmospheric CO_2 concentration and temperature. Related to this global warming, periods of low water availability are also expected to increase. Thus, CO_2 concentration, temperature and water availability are three of the main factors related to climate change that potentially may influence crops and ecosystems. In this report, we describe the use of growth chamber – greenhouses (GCG) and temperature gradient greenhouses (TGG) to simulate climate change scenarios and to investigate possible plant responses. In the GCG, CO_2 concentration, temperature and water availability are set to act simultaneously, enabling comparison of a current situation with a future one. Other characteristics of the GCG are a relative large space of work, fine control of the relative humidity, plant fertirrigation and the possibility of light supplementation, within the photosynthetic active radiation (PAR) region and/or with ultraviolet-B (UV-B) light. In the TGG, the three above-mentioned factors can act independently or in interaction, enabling more mechanistic studies aimed to elucidate the limiting factor(s) responsible for a given plant response. Examples of experiments, including some aimed to study photosynthetic acclimation, a phenomenon that leads to decreased photosynthetic capacity under long-term exposures to elevated CO_2, using GCG and TGG are reported.

1. Introduction

Human activities have increased the atmospheric CO_2 concentration from the beginning of the 19th century. CO_2 concentration has increased since pre-industrial period from 280 to 389–400 μmol mol^{-1} air (ppm) in the years 2012–2013. By the middle and the end of the century respectively, values as high as 550 and 700 ppm are expected [1]. CO_2 is the most important anthropogenic greenhouse gas, contributing the above-mentioned increases to global warming. If CO_2 emission continues at high levels, temperature is predicted to rise between 1.8 and 4.0 °C [2]. In line with these predictions, the warmest years of global surface temperature since 1850 have occurred from 1995 and beyond. Also, it is believed that climate change could decrease plant water availability and could increase the agricultural areas under drought, affecting crop production [3]. Therefore, crops in the future, and also some ecosystems, will face these three climate-related factors: elevated CO_2, elevated temperature and altered water availability.

Much of the research focused on experiments with elevated CO_2, which include predictions of global change effects, has been done in relatively small, fully controlled growth chambers, using day/night constant temperatures and lighting from electric lamps. Frequently, plant behavior in the field differs from that in controlled environments, due to differences in light quality and intensity, temperature and evaporative demand, among other factors. The use of growth chambers has also the disadvantage of size, limiting the number of plants and treatments. The more realistic the experimental conditions, the more likely it is that the predictions reflect the future. In the last 30 years, there have been approaches to simulate climate change scenarios: open top chambers (OTC) [4], free air CO_2 enrichment (FACE) [5], temperature gradient tunnels (TGT) [6–8] and free air temperature increase (FATI) [9] systems. The limitations and advantages of these approaches have been reported elsewhere [10]. In this paper, we report a new concept of greenhouse for plant research, the growth chamber – greenhouse (GCG) and an improved version of the temperature gradient tunnel, the

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Table 1
Some examples of experiments carried out in the growth chamber–greenhouses (GCG) and the temperature gradient greenhouses (TGG), including those aimed to study photosynthetic acclimation, where factors related to climate change were set as combined or in interaction among them (elevated CO_2, elevated temperature and drought), as single stress factors (UV-B light) or in interaction with other variables (CO_2 and arbuscular mycorrhizal fungi or N source, or CO_2, temperature and N_2 fixing strain).

Species	Growth in	Factors related to climate change	Source
Lettuce	GCG	Interactions of CO_2 and arbuscular mycorrhizal fungi	Baslam et al. (2012) [18]
Alfalfa	GCG	Interactions of CO_2 and arbuscular mycorrhizal fungi	Baslam et al. (2012) [19]
Alfalfa	GCG	Interactions of CO_2 and arbuscular mycorrhizal fungi	Baslam et al. (2014) [20]
Alfalfa	GCG	Interactions of CO_2 and N source	Kizildeniz (2013) [21]
Alfalfa	TGG	Interactions of CO_2, temperature and bacterial strain	Sanz-Sáez et al. (2012) [22]
Alfalfa	TGG	Interactions of CO_2, temperature and bacterial strain	Sanz-Sáez et al. (2013) [23]
Grapevine	GCG	Combined effects of CO_2, temperature and drought	Salazar-Parra et al. (2010) [24]
Grapevine	GCG	Combined effects of CO_2, temperature and drought	Salazar-Parra et al. (2012) [13]
Grapevine	GCG	Combined effects of CO_2, temperature and drought	Salazar-Parra et al. (2012) [25]
Grapevine	GCG	Interactions of temperature and irradiation intensity	Carbonell-Bejerano et al. (2013) [26]
Grapevine	GCG	Effects of UV-B radiation and duration exposure	Martínez-Lüscher et al. (2013) [15]
Grapevine	TGG	Interactions of CO_2, temperature and drought	Salazar-Parra (2011) [27]

temperature gradient greenhouse (TGG), both designed to simulate climate change scenarios.

Photosynthesis is one of the plant physiological processes most influenced by environmental variables and growth conditions, among others light quality and intensity, temperature, relative humidity, CO_2 concentration, water availability, mineral nutrition, pot size, etc. With respect to CO_2 concentration, effects of CO_2 on photosynthesis depend on exposure duration. C_3 plants require the enzyme Rubisco for CO_2 fixation. Since Rubisco activity is CO_2-limited and that the oxygenation activity of Rubisco is not inhibited under current atmospheric conditions, photosynthesis at twice ambient CO_2 concentration increases more than 50% in the short-term (minutes to hours) [11]. In the long-term (days to weeks), nevertheless, this initial stimulation is often followed by biochemical and molecular changes that result in a marked photosynthetic capacity decrease (acclimation processes) [12]. We summarize some of our reports on the effects of elevated CO_2 and photosynthetic acclimation using GCG and TGG. Species investigated so far include alfalfa, grapevine and lettuce, studying the interactions of CO_2 with arbuscular mycorrhizal fungi, N source, temperature and bacterial strain, and temperature and drought. Also, the interactions of temperature and irradiation intensity, and the effects of UV-B radiation and duration exposure, have been investigated (see Table 1 and further discussion below).

2. Growth chamber – greenhouse (GCG). A new concept of greenhouse for plant research

Our aim was to design and construct two research greenhouses that could also be used as growth chambers. This was achieved by using disassembling panels of steel and 35 mm width insulator polyurethane. Fig. 1 shows pictures of the growth chambers (left, with panels) and the greenhouses (right, without panels). As mentioned above, one of the disadvantages of the growth chambers is their limited working space. Our GCGs are 9.3 m long, 3.4 m height and 3.15 m width. Inside each GCG, there are two series of galvanized steel tables (where plants grow; culture surfaces are placed 76 cm from the GCG ground) and a central corridor. This generates an area and volume for growing plants of 15.6 m^2 and 23.45 m^3, respectively.

One of the key characteristics of the GCG in order to simulate climate change is the possibility of supplying CO_2. The CO_2 concentration can be set in the GCG in the range of ambient (ca. 400 ppm) to 3000 ppm using an infrared gas Guardian Plus sensor (Edinburgh Sensors, Edinburgh Instruments Ltd., Livingston, UK) and an Omron E5CK controller (Kyoto, Japan). In most experiments where effects of climate change are assessed, one GCG is set at ambient CO_2 concentration and the second one at the IPCC predicted concentration for the end of the century, 700 ppm (Fig. 2). Because of the greenhouse effect of the CO_2, the second important parameter that requires fine control in the GCG is temperature. Cold water and heaters are used in the GCGs for refrigeration and heating, respectively. Temperature is measured with Pt-100 sensors, connected to an Omron E5CK controller. When used as growth chamber, working temperatures may be in the range 15–40 °C or 5–40 °C ± 1 °C, during the day or night (with lights on or off) respectively. When used as greenhouse, temperatures may range 20–35 °C/15–35 °C (day/night) during the summer, and 10–30 °C/10–25 °C (day/night) during the winter. A typical set of temperature conditions, when using GCG as greenhouse and simulating climate change, is 24/14 °C (day/night) in the greenhouse set at current conditions, and 28/18 °C (day/night) (+4 °C, following IPCC predictions for the end of the century) in the greenhouse that simulates future climate conditions [13]. A representative set of temperature data recorded in the GCG is shown in Fig. 3. The third important stress factor related to climate change is water availability. In all experiments, usually half of the plants of each GCG are optimally irrigated whereas the other half are water-stressed, the level of stress being controlled by placing water sensors in the pots. All plants are automatically fertirrigated.

Light and relative humidity are two other basic variables that can be controlled in the GCG. The GCGs have three alternative light sources, two as sources of photosynthetic active radiation and a third one as a source of UV-B light. Photosynthetic active light sources are used when the GCG is used as growth chamber or, when used as greenhouse, as supplementary light that increases light intensity in cloudy days (electric lamps can optionally be switched on when sunlight out of the GCG decreases below a certain threshold, now fixed at 900 μmol photons m^{-2} s^{-1}) or that extends the photoperiod if required. Quantum sensors are installed outside (Apogee SQ-200, Apogee Instruments Inc., Logan, USA) and inside (LI-190SA, LI-COR, Lincoln, Nebraska, USA), used to give the signal of switching on and off, and to check the proper functioning of the artificial lighting respectively. Within the photosynthetic active radiation range GCG uses sunlight-type Osram HQI-TS 400W/D (Osram GmhH, Augsburg, Germany) and/or orange-enriched high-pressure sodium lamps (SON-T Agro Phillips, Eindhoven, Netherlands). These lamps give light intensities of 500–600 and 400 μmol photons m^{-2} s^{-1} at 80 cm from the culture table, respectively. Predicted scenarios of climate change over the next decade include enhanced levels of incident UV-B radiation because of fluctuations in cloud patterns [14]. The GCG are constructed with aluminum and glass, the latter filtering solar UV-B light when used as greenhouse. Supplemental UV-B is applied using Philips TL 100W/01 tubes (311–313 nm spectrum peaking, Philips, Eindhoven, The Netherlands) suspended 0.35 m above the canopy [15]. Spectra of all four types of light sources GCG can use are shown in Fig. 4.