Multiple Effects of CO₂ Concentration and Humidity on Leaf Gas Exchanges of Sweet Pepper in the Morning and Afternoon

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In order to analyze the multiple effects of CO₂ enrichment and humidification on leaf gas exchanges (photosynthetic rate, transpiration rate, and stomatal conductance) in sweet pepper plant and their dependence on the time of day, we measured the steady-state leaf gas exchanges under different CO₂ concentrations (400 and 1000 μmol mol⁻¹) and relative humidities (20, 40, and 60%) in the morning (0900-1130) and afternoon (1230-1500) periods. Increasing CO₂ concentration significantly increased the photosynthetic rate but decreased transpiration rate and stomatal conductance during the both periods. Increasing relative humidity also significantly increased the photosynthetic rate due to improved stomatal conductance at both CO₂ concentrations in the morning. In the afternoon, a similar pattern was observed only at the lower CO₂ concentration, but not at the higher CO₂ concentration because stomatal conductance was extremely low. These results suggest that both CO₂ enrichment and humidification in greenhouses would be effective in the morning; however, in the afternoon, humidification would have little effect under CO₂ enrichment condition, during which photosynthesis was improved but stomata were almost closed.

Keywords: CO₂ enrichment, greenhouse, humidification, photosynthetic rate, stomatal conductance, water use efficiency

INTRODUCTION

Carbon dioxide (CO₂) concentration and humidity of the air are two extremely important environmental elements that influence photosynthesis in crops (i.e., growth). The difference in CO₂ concentration between air and leaf plays a significant role in the photosynthetic rate (Wall et al., 2011), whereas humidity affects photosynthesis through stomatal conductance (Yabuki and Miyagawa, 1970; Nonami et al., 1990).

These elements could become serious limiting factors for crop production in greenhouses. This occurs when the CO₂ concentration frequently drops below 400 μmol mol⁻¹, mainly because of continuous uptake by crops (photosynthesis) and insufficient inflow of CO₂ from outside to the greenhouse with ventilation (Yabuki and Imazu, 1965; Yasutake et al., 2014a), and when greenhouse air dries during daytime because of high air temperature inside the greenhouse (Yasutake et al., 2014b). Therefore, increasing not only CO₂ concentration but also humidity (i.e. CO₂ enrichment and humidification) are desired for increasing crop production with improved photosynthetic rate and water use efficiency. In particular, recent researches in relation to high-tech greenhouses and/or plant factories have focused on such environmental control technology with reference to multiple environmental elements (e.g., Suzuki et al., 2015; Hidaka et al., 2016).

However, crop responses to controlled multiple environmental elements and their mechanisms could be more complicated. For example, in the case of CO₂ enrichment and humidification, opposite effects on stomatal movement occurs, where the former induces stomatal closing and the latter induces stomatal opening (Kramer and Boyer, 1995). Furthermore, stomatal movement generally depends on the time of day (e.g., morning and afternoon) (Yoshimoto et al., 2005; Morandi et al., 2014), and therefore, the multiple effects of CO₂ enrichment and humidification would also change with the time. However, detailed information on these phenomena is not fully understood yet.

In this study, we conducted a fundamental experiment to measure steady-state leaf gas exchanges (photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency, etc.) in sweet pepper under different conditions of CO₂ concentration and relative humidity by using a leaf chamber system in the morning and afternoon in a greenhouse. The goals of this study were 1) to analyze the multiple effects of CO₂ concentration and humidity on leaf gas exchanges, 2) to analyze the difference in the multiple effects in the morning and afternoon, and 3) to show a strategy to control CO₂ concentration and humidity in greenhouses on the basis of the results.

MATERIALS AND METHODS

Experimental site and plant materials
Sweet pepper seeds (Capsicum annuum L. cv. Miogi) were sown in cell trays at the beginning of August 2013

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and after germination (10 d later) they were transplanted to plastic pots with a diameter of 12 cm. At the beginning of September, the plants were transplanted and cultivated until the end of June 2014 at a plant density of 0.92 plants m$^{-2}$ in an experimental greenhouse (20 m long and 7.5 m wide), which was located at the Kochi Prefectural Agriculture Research Center (N 33°35.5′, E 133°38.7′). The greenhouse soil was Gray Lowland with clay loam texture. Bark-based compost (3 kg m$^{-3}$), sugarcane straw (1.5 kg m$^{-3}$), and fertilizer (N: P:O: K =1:1:1; 32 g m$^{-3}$) were applied to the soil before beginning cultivation. Irrigation was conducted every few days to maintain soil water content within a P$	ext{F}$ range of 1.6–1.9. Organic nutrient solution (N as urea 9.8% and others (amino acid, sugar, etc.) 0.2%) (Tomiy Liquid Fertilizer Black, Katakura & Co-op Agri Corporation, Japan) was applied to the soil at 1 g N m$^{-3}$, once a week, as additional fertilizer.

The environmental elements such as solar radiation ($R_s$), air temperature ($T_a$), and air relative humidity ($R_H$) were measured around the center of the greenhouse, and their averages of 10 min were recorded on a data logger.

Further information relating to the experimental greenhouse set up and environmental observation is provided in Yasutake et al. (2014a).

**Measurement of leaf gas exchanges**

The measurement of gas exchange characteristics was carried out on young and fully expanded leaves ($n=3$), by using a portable leaf chamber system (LI-6400, Licor, USA), in the morning (0900–1130) and afternoon (1230–1500) on clear days (January 12, 13, 15, 16, and 17, 2014) at constant conditions of light intensity (photosynthetic photon flux density of 1000 μmol m$^{-2}$ s$^{-1}$) and air temperature (25°C) in the leaf chamber. The parameters recorded were photosynthetic rate ($P$) and transpiration rate ($T_r$) and their explanatory variables, stomatal conductance ($G_s$) and difference of CO$_2$ concentration between the air and leaf ($ΔC$) under steady-state. We used 2 CO$_2$ concentrations (400 μmol mol$^{-1}$ as an assumption of normal condition and 1000 μmol mol$^{-1}$ as CO$_2$ enrichment condition) and 3 relative humidities (20%, 40%, and 60%) in the chamber.

Water use efficiency (WUE) was also calculated as $P/T_r$.

**Statistical analysis**

Statistical analysis of leaf gas exchange characteristics ($P$, $T_r$, $G_s$, $ΔC$, and WUE) among different CO$_2$ concentrations and relative humidities were conducted using the Tukey-Kramer test at $P<0.05$, by using the statistical program R (version 3.2.4).

**RESULTS AND DISCUSSION**

**Greenhouse environment in the morning and afternoon**

Figure 1 shows boxplots for $R_s$, $T_a$, and $R_H$ in the greenhouse during the measurement periods (the morning and afternoon periods). Mean values of $R_s$ for both the morning and afternoon periods were almost same (approximately 250 W m$^{-2}$); however, scattering of $R_s$ was somewhat larger in the afternoon than that during the morning. $T_a$ showed large scattering in the morning because of low air temperature (nighttime to early morning), with a mean value of 29.9°C, while in the afternoon, $T_a$ was almost constant around 33–34°C. Stable $T_a$ in the afternoon was induced by ventilation, which was managed automatically. As a result of the varying $T_a$, $R_H$ was higher with larger scattering in the morning (mean value of 62.7%), while it was lower in the afternoon with smaller scattering (mean value of 57.6%).

Thus, environmental conditions in the greenhouse, i.e., outside the leaf chamber, were somewhat different between the measurement periods in the morning and afternoon because of diurnal changes.

**Effects in the morning and afternoon**

Figure 2-(a) shows the $P$, $T_r$, $G_s$, and $ΔC$ in the morning period. The increase in CO$_2$ concentration from 400 to 1000 μmol mol$^{-1}$ significantly accelerated $P$ under all humidity conditions. This was attributed to the increasing $ΔC$ but not to the $G_s$ behavior, which showed a decreasing trend with increasing CO$_2$ concentration. This decrease in $G_s$ with increase in CO$_2$ concentration is a typical stomatal response (Kramer and Boyer, 1995), which also induced a significant decrease in $T_r$ under all humidity conditions.

The increase in $R_H$ from 20 to 60% caused a significant increase in $P$ at both CO$_2$ concentrations. Bunce (1982) reported that humidity could be a strong limiting factor for photosynthesis and that higher humidity accelerated the photosynthesis of soybean by over 30%. In this study, the increase in $R_H$ induced a decrease in $T_r$ and therefore $G_s$, which is one of the important parameters of $P$, showed an increase trend with increase in $R_H$ though there were no significant differences for $T_r$ and $G_s$ at different $R_H$ conditions under the lower CO$_2$ concentration (400 μmol mol$^{-1}$). These behaviors of $T_r$ and $G_s$ in response to $R_H$ were consistent with the previous studies (e.g., Nonami et al., 1990). $ΔC$, another important parameter of $P$, did not change with $R_H$. These indicate that humidification should help to improve photosynthesis through increasing stomatal aperture at any CO$_2$ concentration in the morning.

Figure 2-(b) shows the results obtained in the afternoon: $P$ and $T_r$ recorded in the afternoon were lower than those recorded in the morning (Fig. 2-a). This can be attributed to the lower $G_s$ level in the afternoon than in the morning, similar to that frequently observed in various crops such as rice (Yoshimoto et al., 2005), corn (Yasutake et al., 2006), soybean (Nakano et al., 2015), and pear (orchard) (Morandi et al., 2014). Similar to the observations in the morning (Fig. 2-a), the increase in $R_H$ resulted in a significant increase in $P$ in response to an increase in $G_s$ under the lower CO$_2$ concentration (400 μmol mol$^{-1}$). The effect of $R_H$ on $P$ was not significant at higher CO$_2$ concentration (1000 μmol mol$^{-1}$). This could be because of the decrease in $G_s$ in the afternoon, owing to the cumulative transpirational water loss from the beginning of the day, higher evaporative demand in the afternoon (higher $T_a$ and lower $R_H$ as shown in Fig. 1) than in the morning, and increase in CO$_2$ concentration from 400 to 1000 μmol mol$^{-1}$, leading to stomatal closure. Therefore, stomata failed to respond to the change in $R_H$ under the higher CO$_2$ concentration.
Fig. 1 Boxplots for solar radiation ($R_S$), air temperature ($T_A$), and relative humidity ($RH$) in the greenhouse during the measurement periods: morning (0900–1130) and afternoon (1230–1500) on clear days. Open circles indicate mean values for the respective variables.

Fig. 2 Photosynthetic rate ($P$), transpiration rate ($Tr$), stomatal conductance ($G_s$), and difference in CO$_2$ concentration between the air and leaf ($\Delta C$) of sweet pepper leaf at different CO$_2$ concentrations (400 and 1000 µmol mol$^{-1}$) and relative humidities (20, 40, and 60%) under a photosynthetic photon flux density of 1000 µmol m$^{-2}$ s$^{-1}$ and air temperature of 25°C in the morning (a; 0900–1130) and afternoon (b; 1230–1500) on clear days. The means and standard error bars of 3 datasets are shown. Different small capital letters indicate significant difference at $P<0.05$ by Tukey-Kramer test.
CO2 concentrations, although no significant difference in increase in RH Tr morning and afternoon periods. In conclusion, both CO2 and humidity on leaf gas exchanges were elucidated in the concentration than at low CO2 concentration, because the increase in P or CO2 concentration induced an increase P but decreased Tr (Fig. 2) (e.g., Mortensen, 1987). Furthermore, the increase in RH showed a tendency to increase WUE at both CO2 concentrations, although no significant difference in WUE was noted among the 3 RH conditions in the morning. This WUE behavior could be attributed to the increasing P and decreasing Tr with the increase in RH in the morning, while only to the decreasing Tr in the afternoon (Fig. 2).

Thus, the leaf gas exchanges in sweet pepper were measured under different CO2 concentrations and relative humidities, and the multiple effects of CO2 concentration and humidity on leaf gas exchanges were elucidated in the morning and afternoon periods. In conclusion, both CO2 enrichment and humidification in greenhouses are effective for accelerating crop photosynthesis in the morning. In the afternoon, humidification is effective at the normal CO2 concentration, but it would have little effect under CO2 enrichment. However, in the present study, relative humidity conditions of only 60% and lower were assessed. Future studies involving higher humidity conditions must be conducted achieve a better understanding. In addition, further large-scale (whole plant or canopy) studies are also essential.

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Fig. 3 Water use efficiency (WUE) of a sweet pepper leaf at different CO2 concentrations (400 and 1000 μmol mol−1) and relative humidities (20, 40, and 60%) under a photosynthetic photon flux density of 1000 μmol m−2 s−1 and air temperature of 25°C in the morning (a; 0900–1130) and afternoon (b; 1230–1500) on clear days. The means and standard error bars of 3 datasets are shown. Different small capital letters indicate significant difference at P<0.05 by Tukey-Kramer test.

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