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Leaf Carbohydrate Contents and Growth of *Chrysanthemum morifolium* in Response to Low Night Temperatures and High CO_2

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Summary

Decreasing the night temperature in greenhouse production of plants decrease the energy costs. However, problems may arise as low night temperature (LNT) is known to increase leaf starch contents and delay plant development. In this study it was investigated whether increased CO_2 concentrations can decrease the negative effects of LNT, when plants of vegetative *Chrysanthemum morifolium* 'Coral Charm' are grown under greenhouse conditions. Plants were grown under long day conditions (LD) with a photoperiod of 17 h in four compartments. The four compartments had a day temperature set point of 22 °C, and four combinations of night temperatures and CO_2 concentration during the day; the night temperature set points were 20 °C and 12 °C, and set points of CO_2 concentrations were, ambient CO_2 of 400 μl l^{-1} and high CO_2 of 900 μl l^{-1}. Plants were harvested after 3, 4 and 7 weeks.

The leaf carbohydrate contents of the chrysanthemums differed in relation to harvest time and the photon flux density (PFD) measured outside the greenhouse. Treatments with high CO_2 and low night temperature (LNT) increased leaf carbohydrate contents, but the effect was more pronounced at the 7 weeks harvest, where also the PFD was high. Generally, the treatment with high CO_2 increased growth in terms of increased shoot dry matter (DM) and the formation of leaves in plants grown at LNT. LNT had the opposite effect decreasing shoot DM production, stem length and leaf number, but only at ambient CO_2 concentrations. The results illustrate that when plants are grown in a combination with LNT and high CO_2, the accumulation of leaf carbohydrates increases, however plant growth is less limited by the LNT in comparison to plants grown in LNT at ambient CO_2 concentrations. It is suggested to include high CO_2 concentrations in greenhouse production of *Chrysanthemum morifolium*, when grown under LNT.

Key words. Leaf carbohydrate contents – temperature fluctuations – growth

Introduction

Dynamic climate control is a system which optimises greenhouse production of plants in order to save energy. Heat and CO_2 is supplied in periods with high irradiance, and energy is saved during the night and in periods with low irradiance (Aaslyng et al. 2003). Plant production is generally maintained at the same level as in more traditional systems, which tends to maintain temperatures as constant as possible. However, if the night temperature, and thereby also the mean temperature, is too low, the time to flowering may increase, and plant quality may decrease (Ottosen et al. 2003; Lund et al. 2006). Furthermore, it was recently shown that low night temperature (LNT) increase starch accumulation in the upper-most fully expanded leaves of *Chrysanthemum morifolium* when grown under controlled climate chamber conditions and in nutrient solution culture (Kjaer et al. 2007). LNT did not significantly affect plant dry matter (DM) production, however, plants tended to have fewer leaves and a smaller leaf area.

Results obtained in a controlled climate cannot be directly applied to greenhouse conditions because large fluctuations in the actual conditions will occur, although the set point of day and night temperature and CO_2 is fixed. This is due, not only to the natural fluctuations in the solar irradiance and the conditions outside the greenhouse, but also to the use of vents and screens.

Enhancing CO_2 concentrations is a common strategy in greenhouse production to optimise plant growth and photosynthesis at high PFD (Mortensen and Moe 1992; Ainsworth and Long 2005). However, high CO_2 may also contribute to an increased starch accumulation in the leaves (Kuehny et al. 1991; Katny et al. 2005). The consequences can be a lower amount of carbohydrates available to growth and even feed back inhibition of photosynthesis (Goldschmidt and Huber 1992).

The aim of the present study was to investigate the effect of CO_2 and night temperature on leaf carbohydrate contents and plant growth, when plants are grown under greenhouse conditions. The purpose was to clarify, whether negative effects of LNT in terms of fewer leaves

and a smaller leaf area could be avoided by increasing the concentration of CO_2.

Materials and Methods

Plant material and experimental design

Cuttings of *Crysanthemum morifolium* 'Coral Charm' were rooted in pots (11 cm in diameter). After three weeks, plants were pinched back to three leaves, and grown for seven weeks in a long day (LD) treatment in a greenhouse. The experiment was carried out from 3 January to 20 February, 2007 in four similar compartments each 9.9 × 7.6 m in a greenhouse located at The Department of Horticulture, University of Aarhus (Aarslev, Denmark, Lat. 55° N). Each compartment was equipped with four tables of 9.75 m^2 each.

This chrysanthemum experiment was located on one table in each of the four greenhouse compartments. The set points of the four treatments were a day temperature of 22 °C and four combinations of night air temperature and CO_2 during the day: Night temperature set points were 20 °C (NNT) and 12 °C (LNT). The set points for CO_2 concentrations were 900 µl l^{-1} (**h**igh CO_2) and 400 µl l^{-1} (**a**mbient CO_2) during the day time. Each treatment had three replicates with 30 plants each.

The photoperiod was 17 h, from 07:00 to 24:00 h. Whenever the photosynthetic flux density (PFD) fell below 198 µmol m^{-2} s^{-1} outside the greenhouse, supplementary light (high-pressure sodium lamps SON-T agro, 600 W; Phillips, Eindhoven, The Netherlands) provided 32 ± 2 µmol m^{-2} s^{-1} at the top of the plant canopy during the whole photoperiod. The heat was turned on during the beginning of the photoperiod, and the vents were open during the beginning of the dark period to reach the night or day temperature set points within one hour. Plants were fertigated by flooding of the tables for 20 min every second day, with a nutrient solution consisting of 5.7 mM $NaNO_3$, 12.6 mM NH_4NO_3, 8.9 mM KCl, 0.7 mM Fe-EDTA, 47.6 mM KNO_3, 0.4 mM $(NH_4)_2SO_4$, 3.4 mM $(NH_4)H_2PO_4$, 2.6 mM KH_2PO_4, 5.8 mM $MgSO_4$ and micronutrients.

Throughout the experiment, the air temperature, relative humidity (RH), and CO_2 concentrations were logged **at** one min intervals in each compartment just above plant canopy using a standard environmental greenhouse control computer (Completa; Senmatic, Søndersø, Denmark). Additionally the state of the supplementary light (on or off), and the position of the vents were logged. A weather station provided the data of global irradiance, air temperature, wind speed and direction.

Plant growth

The pinched plants developed three lateral branches. After 3, 4 and 7 weeks of the experiment 9 plants from each treatment (3 from each of the 3 replicates) were harvested for growth and carbohydrate analysis. The harvest took place just before the start of the photoperiod. Stem length was measured as the total length of the first lateral branch. Leaves were separated from lateral branches and counted, and leaf areas were measured on a LI-COR portable leaf area meter (LI-3000; Lambda Inst. Corp., Lincoln, NE, United States). Fresh mass (FM) of leaf and stem material was determined, before drying at 70 °C for 24 h for determination of dry mass (DM).

Carbohydrate analysis

At each harvest, the four upper-most fully expanded leaves were sampled from three plants in each of the three replicates to constitute a total of 9 plants from each treatment. The leaves were immediately frozen in liquid nitrogen and stored at −80 °C.

Prior to analysis, the leaves were freeze-dried and ground in liquid nitrogen. Concentrations of hexoses (glucose and fructose) and sucrose were determined on a HPLC (Hewlett Packard 1047A, Waldbronn, Germany) as described by Liu et al. (2004). Starch was determined in the remaining pellets after extraction of soluble sugars. The pellets were dried in a vacuum centrifuge and then gelatinised by boiling for 1 h. After centrifugation, the gelatinised starch was hydrolysed further and purified. The eluates were evaporated to dryness and glucose concentrations were analysed by HPLC as described by Kjaer et al. (2007).

Data analysis

Differences in means, maxima, sums and standard errors of PPFD, the status of supplementary light, temperature, relative humidity (RH), and CO_2 concentrations were calculated in relation to treatment and harvest time in SAS (SAS institute, V8.02, 1999). Differences across treatments in plant morphology and carbohydrate contents were analysed by the linear mixed effects model allowing for nested random effects using software package R release 2.4.1 (http://www.r-project.org).

Results

Temperature, irradiance and CO_2 concentrations in the greenhouse

The average day temperature was 22 ± 0.5 °C in all treatments and the average night temperature was 20 ± 0.8 °C and 13.6 ± 1.9 °C in the two treatments, respectively (Fig. 1). The average night temperature in the LNT treatment did not reach the set point of 12 °C due to high temperatures outside the greenhouse. The CO_2 treatments were significantly different from each other during the whole experimental period (Fig. 1B).

The average PFD (µmol m^{-2} s^{-1}) was calculated from the data of global irradiance outside the greenhouse. The average PFD was below 60 µmol m^{-2} s^{-1} during the first three weeks and increased to approximately 150 µmol m^{-2} s^{-1} during the last four weeks (Fig. 1C). The condition (on/off) of the artificial light was measured inside the greenhouse, showing that the supplemental light was on during the most of the photoperiod.

Leaf carbohydrate contents

The carbohydrate content of the upper-most fully expanded leaves in the four treatments differed significantly between harvests (Fig. 2). Between week 3 and week 4, the leaf starch content decreased in all treatments, except for the treatment with LNT in combination with ambient

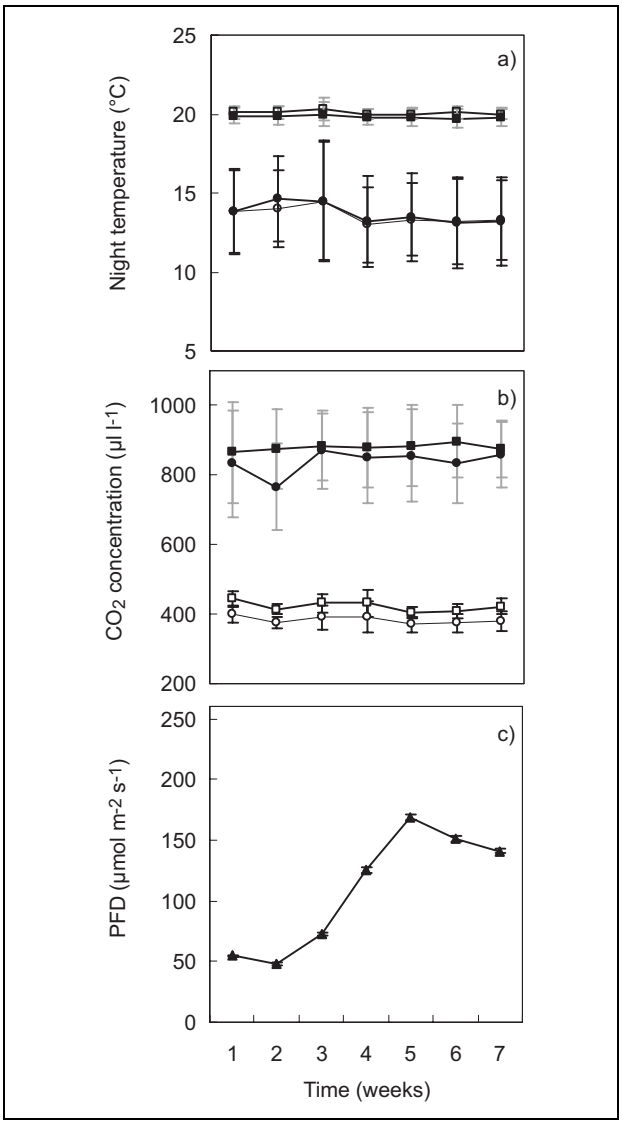


Fig. 1. Climatic conditions from January 3 to February 20, 2007 in each of four treatments in four identical greenhouse compartments. The data are the average for each of the seven weeks, in the experiment ± SD for each week. For (a) and (b), symbols represent the temperature set points of 20 °C (squares) and 12 °C (circles). Colour of symbols represents a high CO_2 of 900 µl l^{-1} (black) and ambient CO_2 of 350 µl l^{-1} (white). (a) The average night temperature. (b) The average CO_2 concentrations during the day. (c) The average PFD (dark triangles) measured outside the greenhouse.

CO_2. Between week 4 and week 7, the leaf starch content increased in all treatments, except for the treatment with LNT and ambient CO_2. At all three harvests, high CO_2 significantly increased leaf starch content (Fig. 2A). However, it was more pronounced, when the high CO_2 was combined with LNT. The alterations in the concentration of sucrose, glucose and fructose were similar in response to temperature and CO_2, and therefore the results were pooled and only the total concentrations of soluble sugars are presented. Also for the sugars, the leaf contents were lower in week 4 compared to values from week 3 and 7. The LNT increased the leaf contents of soluble sugars at the 7 weeks harvest, and in combination with high CO_2 at the 3 weeks harvest, whereas no effect was seen on the leaf contents of soluble sugars at the 4 weeks harvest. High CO_2 also increased the leaf contents of soluble sugars in NNT treatment at the 7 weeks harvest (Fig. 2B).

Plant growth and morphology

Plant grew throughout the experiment (Fig. 3). However, in the treatments with high CO_2, shoot DM and stem length were significantly larger after 7 weeks, when compared with plants grown at ambient CO_2 (Fig. 3A and 3C). High CO_2 also increased leaf number, but only in combination with NNT (Fig. 3C), and no effect was seen on total leaf area (results not shown). The LNT decreased shoot DM and the number of leaves, but only in combination with ambient CO_2. Therefore, high CO_2 in part compensated for the LNT-mediated decrease in shoot DM and in the formation of leaves.

Discussion

Leaf starch contents in relation to dynamic growth conditions

Our results clearly demonstrated that high CO_2 and LNT increase leaf starch contents and that the effect of these parameters depended on the time at which the plants were harvested, which show that the effect of climatic parameters on starch accumulation in plants clearly depend on the light intensity and the developmental stage of the plants (Kuehny et al. 1991; Morin et al. 1992). The contribution of LNT to an increase in leaf starch contents was more or less the same at the 4 and 7 weeks harvest, when focussing on plants grown in either high CO_2 or ambient CO_2. This indicates that the LNT decreased the starch turnover during the night irrespective of the differences in leaf starch contents in plants grown in different concentrations of CO_2. The leaf starch contents in plants grown under greenhouse conditions was low compared to the leaf starch contents found in plants grown under climate chamber conditions (Kjaer et al. 2007). This was expected as the light intensity was much lower in the greenhouse experiment.

Leaf sugar contents in relation to dynamic growth conditions

The leaf contents of soluble sugars responded in a similar way as the leaf starch contents to changes in light intensity. However, LNT was shown to have a larger effect than high CO_2 in increasing the amount of soluble sugars in the leaves at dawn. This make sense as short term low temperature treatments are well known to increase sucrose synthase activity, which is reversed when the temperature is shifted back to warm temperature (Huner et al. 1993). The results indicate that the leaf content of soluble sugars in the chrysanthemum plants is related to the temperature, and that the CO_2 concentration has only little effect. However, as leaf sugar contents are known to vary in a diurnal pattern (Morin et al. 1992), and which may change in relation to night temperature (Kjaer et al. 2008), the effect of high CO_2 may have been more pronounced if the leaves were analysed for sugars at the end of the day.

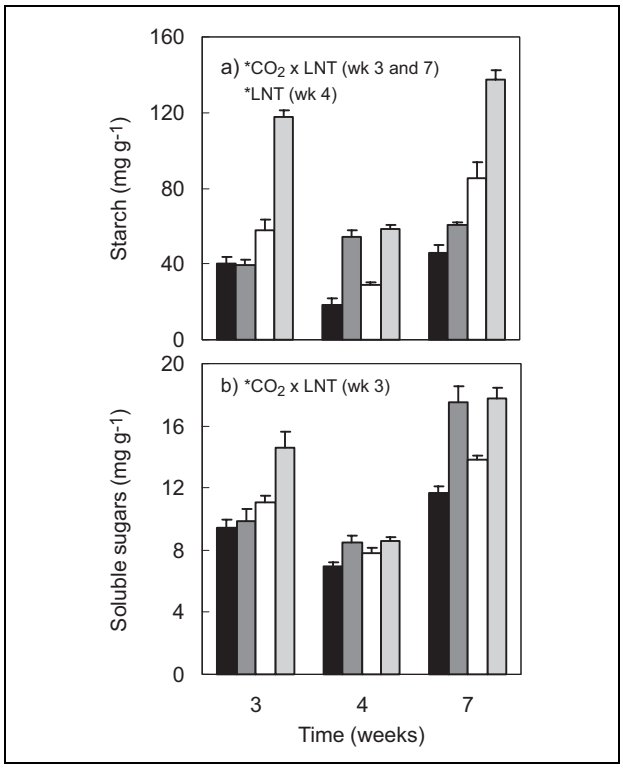


Fig. 2. Carbohydrate contents in the upper-most fully expanded leaves of *Chrysanthemum morifolium* after 3, 4 and 7 weeks growth in each of four treatments in four identical greenhouse compartments at a day temperature of 22 °C and 4 combinations of night temperature and CO_2 concentrations during the day. (a) Leaf starch contents. (b) Contents of soluble sugars including sucrose, fructose and glucose. The bars represent the four treatments: ■ NNT and ambient CO_2, ■ LNT and ambient CO_2, □ NNT and high CO_2, □ LNT and high CO_2. n = 9, *P < 0.05.

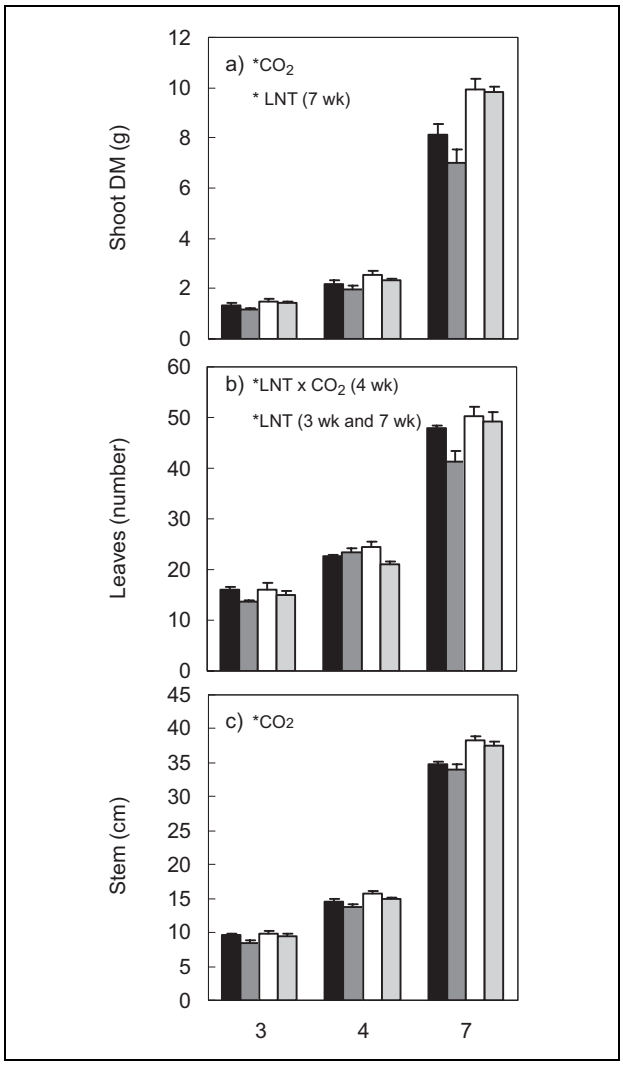


Fig. 3. Morphological parameters of *Chrysanthemum morifolium* grown in each of four treatments. (a) Shoot dry matter (DM), (b) leaf number and (c) stem length of plants grown at a day temperature set point of 22 °C, and four combinations of night temperature and CO_2 concentrations during the day. The bars represent the four treatments: ■ NNT and ambient CO_2, ■ LNT and ambient CO_2, □ NNT and high CO_2, □ LNT and high CO_2. n = 9, *P < 0.05.

Plant growth and carbohydrate dynamics

The increase in leaf contents of soluble sugars and starch in response to high CO_2 and LNT seen in this experiment is well known (Kuehny et al. 1991; Katny et al. 2005; Kjaer et al. 2007). When more carbohydrates are accumulated in the leaves of plants, it is normally associated with an increase in DM% of the leaves. This relation was not significant in this study, due to generally low leaf carbohydrate contents. Furthermore, it has been reported that the increased leaf starch contents decrease the amount of carbohydrates available for the formation of new leaves (Kjaer et al. 2008). However, this effect could only be suggested for plants grown at LNT at ambient CO_2, suggesting that high CO_2 both increase growth and leaf carbohydrate contents, whereas LNT decrease growth on the expense of increased leaf carbohydrate contents.

This study illustrates large-scale effects on vegetative chrysanthemum, when combining LNT and CO_2. The overall conclusion is that leaf carbohydrate contents increase more when plants are grown in high CO_2 combined with LNT in comparison to plants grown at high CO_2 with NNT. It further illustrates that when plants are grown at high CO_2, the negative effects of LNT on growth parameters are decreased. From this study it is concluded that it is possible to save energy on heating during the night without major losses on plant vegetative growth, if the CO_2 concentration is high during the daytime.

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