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# Effects of Elevated CO<sub>2</sub> and Temperature on Growth and Yield of Soybean in the Closed Plantation Experiment Facility

By

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K e y  $\,$  w o r d s : Atmospheric  $\,$  CO $_2$  concentration, biomass allocation, closed ecology experiment facilities, seed yield, temperature.

#### Summary

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An increase in the seed yield of vegetable-type soybean (Glycine max [L.] Merr., cv. Beer-friend) cultivated in the Closed Plantation Experiment Facility (CPEF) of the Closed Ecology Experiment Facilities (CEEF) is necessary for successful human habitation in the CEEF. It is well known that seed yield of soybean is markedly affected by atmospheric CO<sub>2</sub> concentration ([CO<sub>2</sub>]) and air temperature. To increase seed yield of soybean cv. Beer-friend in the CPEF, we examined the effects of [CO2] and air temperature on growth and seed yield in the CPEF. Day/night air temperatures of 22/15, 24/17, and 26/19°C were maintained at [CO<sub>2</sub>] of 350 and 700 μmol CO<sub>2</sub> mol<sup>-1</sup> air. At the beginning maturity stage (R7), plant dry weight increased only in response to increased [CO2] and not with temperature. In contrast, seed yield only rose in response to increasing day and night temperatures and not with increased [CO<sub>2</sub>]. This rise with increases in temperature was caused by increases in seed dry weight per pod and in the number of pods per plant. The absence of enhanced seed yield simultaneous with the increases in plant dry weight under elevated [CO<sub>2</sub>] was caused by a limited ability to increase either seed dry weight per pod or number of pods per plant. The resultant decrease in the fraction of the biomass allocated to seeds under elevated [CO<sub>2</sub>] was offset by an increase in the fraction of biomass allocated to leaves. These results indicated that increased air temperature was more effective in increasing seed yield of soybean cv. Beer-friend in the CPEF than a doubling of [CO<sub>2</sub>] within the range of air temperature of the present study.

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### Introduction

The Closed Ecology Experiment Facilities (CEEF) of the Institute for Environmental Sciences can be used as a test bed for Controlled Ecological Life-Support Systems (CELSS) which are being developed to support human life for extended periods in space. The CEEF's self-sufficient material circulation system is designed to be an artificial ecosystem that includes crop plants, animals, and two human inhabitants (TAKO & al. 2001, 2003). In the CEEF, the food source for the human inhabitants is restricted to crops cultivated in the Closed Plantation Experiment Facility (CPEF). Therefore, environmental conditions capable of producing increased crop yields should be determined in order to support long-term human habitation planned in the CEEF.

Vegetable-type soybean (*Glycine max* [L.] Merr., cv. Beer-friend) has been selected as a main crop in the CEEF because of its high protein level and usefulness for producing various food menus (KONOVSKY & al. 1994) that contribute to increases in the quality of life in the CEEF. It is well known that growth and seed yield of soybean both tend to increase in response to elevated atmospheric CO<sub>2</sub> concentrations ([CO<sub>2</sub>]) as a result of enhanced photosynthesis (AINSWORTH & al. 2002, JABLONSKI & al. 2002). Moreover, SIONIT & al. 1987 showed that the effect of elevated [CO<sub>2</sub>] on seed yield of grain-type soybean cultivated in controlled environment chambers increased with day/night air temperatures within the range from 18/12 to 26/20°C. However, little is known about the interactive effects of [CO<sub>2</sub>] and air temperature on the seed yield of vegetable-type soybean. In the present study, the effects of [CO<sub>2</sub>] and air temperature on the growth and seed yield of vegetable-type soybean cv. Beer-friend were examined using controlled environment chambers in the CPEF in order to ensure that the ecosystem can supply enough food for the human inhabitants.

#### Material and Methods

Plant material and growth conditions

Soybean (Glycine max [L.] Merr., cv. Beer-friend) was cultivated hydroponically under two different [CO<sub>2</sub>] and three different temperature conditions using growth chambers with artificial lighting (high-pressure sodium lamps) in the CPEF (TAKO & al. 2002). Day/night air temperatures of 22/15, 24/17, and 26/19°C were maintained at daytime [CO<sub>2</sub>] levels of  $350 \pm 50$  and  $700 \pm 50$  µmol CO<sub>2</sub> mol<sup>-1</sup> air. These daytime air temperatures were near the temperature condition in the habitation module of the CEEF (25°C), since cultivation and harvesting of soybean will be conducted by human inhabitants in the CEEF and temperature changes can be a stressor for human inhabitants in such a closed environment (MORPHEW 2001). Maximum [CO<sub>2</sub>] at night was controlled to be less than 700 and 1000  $\mu$ mol CO<sub>2</sub> mol<sup>-1</sup> air for growth chambers with daytime [CO<sub>2</sub>] levels of 350  $\pm$  50 and 700  $\pm$ 50 μmol CO<sub>2</sub> mol<sup>-1</sup> air, respectively. For each set of growth conditions, a separate growth chamber was used, and plant cultivation was conducted once for 81 or 84 consecutive days in 2001, 2002, or 2003. The relative humidity during the day was maintained at  $65 \pm 5\%$ ,  $69 \pm 5\%$ , and  $69 \pm 5\%$  for daytime air temperatures of 22, 24, and 26°C, respectively. The relative humidity at night was maintained at 85 ± 5% for each set of growth conditions. The photoperiod was 14 h in each growth chamber, and photosynthetically active radiation at the top of the plant canopy was 466 µmol m<sup>-2</sup> s<sup>-1</sup> for the first 2 h of the photoperiod, followed by 930 µmol m<sup>-2</sup> s<sup>-1</sup> for 2 h, 1360 µmol m<sup>-2</sup> s<sup>-1</sup> for 6 h, 930

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 $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> for 2 h, and 466  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> for the last 2 h.

Each growth chamber included six  $5\text{-m}^2$  hydroponic cultivation beds, and 44 pots were placed in each bed. Three soybean seeds were sown in each 3-L pot (17 cm diameter × 19 cm depth), with approximately 2 L of porous clay balls (2–4 mm in diameter) used as the growing medium. The seedlings were thinned to a single vigorous plant per pot approximately 10 d after sowing. The seeds were not inoculated with leguminous bacteria before sowing. Each cultivation bed contained 330 L of nutrient solution (11.9 mol m<sup>-3</sup> NO<sub>3</sub>-, 1.2 mol m<sup>-3</sup> PO<sub>4</sub><sup>3-</sup>, 3.9 mol m<sup>-3</sup> K<sup>+</sup>, 3.9 mol m<sup>-3</sup> Ca<sup>2+</sup>, 1.5 mol m<sup>-3</sup> Mg<sup>2+</sup>, 1.8 mol m<sup>-3</sup> SO<sub>4</sub><sup>2-</sup>, 0.075 mol m<sup>-3</sup> Fe<sup>3+</sup>, plus micronutrients). The nutrient solution was renewed when the pH of the solution left the range of 5.0–6.6 or the electrical conductivity of the solution was less than 0.13 S m<sup>-1</sup>.

#### Growth measurements and biomass harvest

We destructively harvested the soybean 84 d after sowing for the 22/15 and 24/17°C growth temperatures and 81 d after sowing for the 26/19°C growth temperature. At the time of harvesting, plants from all growth conditions had reached growth stage R7 (beginning maturity, FEHR & CAVINESS 1977), and pods of the plants had started to turn yellow. Six plants were harvested from each growth condition and were separated into roots, stem, leaves, pods, and seeds. Leaf area was determined with a leaf area meter (AAM-8, Hayashi Denkoh, Japan). All plant parts were dried at 70°C for 72 h before weighing. Plant dry weight, seed yield (seed dry weight per plant), harvest index (ratio of total seed dry weight to plant dry weight), and leaf weight ratio (ratio of total leaf dry weight to plant dry weight) were determined for each harvested plant.

Seed yield can be expressed as the product of seed dry weight per pod and the number of pods per plant. Leaf weight ratio is the product of specific leaf weight (ratio of total leaf dry weight to total leaf area) and leaf area ratio (ratio of total leaf area to plant dry weight). These four parameters were also calculated to determine the mechanism responsible for changes in seed yield or in the fraction of biomass allocated to leaves under the different growth conditions.

All growth parameters were analyzed by means of two-way ANOVA with the factors of [CO<sub>2</sub>] and temperature and their interaction (ZAR 1998) with single pots (plants) as the experimental units. Statistical tests were conducted using StatView J-5.0 (SAS Institute Inc., Cary, North Carolina, USA).

#### Results and Discussion

In the CPEF, increased air temperature was more effective in increasing the seed yield of vegetable-type soybean 'Beer-friend' than a doubling of [CO<sub>2</sub>] with air temperatures set within the range suitable for the human inhabitants (Fig. 1b). The enhancement of seed yield with increasing temperature was caused primarily by an increase in the fraction of the biomass allocated to seeds (the harvest index, Fig. 2a) without a significant accompanying increase in plant dry weight (Fig. 1a). This increase in the harvest index resulted from increases in seed dry weight per pod and in the number of pods per plant at higher temperatures (Fig. 1c, d).

Increases in the growth and seed yield of soybean resulting from  $CO_2$  enrichment have been documented in a number of studies (AINSWORTH & al. 2002, JABLONSKI & al. 2002). In the present study, however, seed yield did not increase with increasing  $[CO_2]$  despite increases in plant dry weight (Fig. 1a, b). Similar results have been reported by FISCUS & al. 1997 and ZISKA & al. 1998. The difference in the response of seed yield to elevated  $[CO_2]$  may be related to differences in cultivars (ZISKA & al. 1998) and/or in growth temperatures (SIONIT & al. 1987) among the studies.

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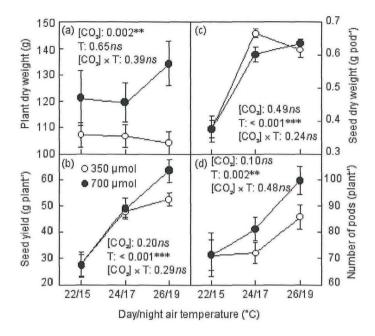


Fig. 1. Plant dry weight (a), seed yield (b), seed dry weight per pod (c), and number of pods per plant (d) of soybean grown at atmospheric  $CO_2$  concentrations of 350 and 700  $\mu$ mol  $CO_2$  mol<sup>-1</sup> air and at day/night air temperatures of 22/15, 24/17, and 26/19°C. Vertical bars represent one standard error of the mean of six plants. P-values for two-way ANOVAs for the effects of  $CO_2$  concentration ([ $CO_2$ ]), air temperature (T), and their interaction ([ $CO_2$ ] × T) on these parameters are shown (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, ns not significant).

In the present study, the lack of enhanced seed yield simultaneous to the observed increases in plant dry weight under elevated  $[CO_2]$  was related to the limited ability to increase seed dry weight per pod and the number of pods per plant in response to elevated  $[CO_2]$  (Fig. 1). The resultant decrease in the fraction of the biomass allocated to seeds (the harvest index) under elevated  $[CO_2]$  was offset by an increase in the fraction of biomass allocated to leaves (the leaf weight ratio; Fig. 2a, b). The increase in leaf weight ratio in response to increased  $[CO_2]$  was not caused by an increase in leaf area ratio, but rather by an increase in specific leaf weight (Fig. 2c, d), which is known to be associated with starch accumulation in soybean grown under elevated  $[CO_2]$  (SIMS & al. 1998). Therefore, the lack of enhancement of seed yield observed in the soybean cultivar "Beer-friend" grown under elevated  $[CO_2]$  in the CPEF may have been caused by the inability of the plant to increase the amount of photoassimilate transported from the leaves into the seeds due to a limited plasticity of the seed dry weight per pod and of the number of pods per plant in response to elevated  $[CO_2]$ .

Although CO<sub>2</sub> enrichment had no significant effect on seed yield of the soybean cultivar 'Beer-friend' within the range of temperature conditions of this

study (p = 0.20), this cultivar exhibited the highest average value of seed yield under elevated  $[CO_2]$  and day/night air temperature (26/19°C) (Fig. 1b). This growth condition has been already selected for cultivation of soybean in the CPEF, and it has been demonstrated that seed yield of the cultivar under this growth condition is sufficient to meet the requirement of human inhabitants in the CEEF (MASUDA & al. 2005).

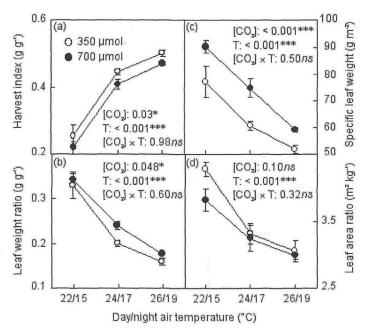


Fig. 2. Harvest index (a), leaf weight ratio (b), specific leaf weight (c), and leaf area ratio (d) of soybean grown at atmospheric CO<sub>2</sub> concentrations of 350 and 700  $\mu$ mol CO<sub>2</sub> mol<sup>-1</sup> air and at day/night air temperatures of 22/15, 24/17, and 26/19°C. Vertical bars represent one standard error of the mean of six plants. P-values for two-way ANOVAs for the effects of CO<sub>2</sub> concentration ([CO<sub>2</sub>]), air temperature (T), and their interaction ([CO<sub>2</sub>] × T) on these parameters are shown (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, ns not significant).

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