HortScience 47(9):1257–1260. 2012.

# $CO_2$ Balance of a Commercial Closed System with Artificial Lighting for Producing Lettuce Plants

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*Additional index words.* $CO_2$ utilization efficiency, operation ratio, $CO_2$ cost, *Lactuca sativa* L.

***Abstract*. The $CO_2$ balance of a commercial closed system with artificial lighting (CSAL), in which lettuce plants (*Lactuca sativa* L. 'Early Impulse', 'King Crown', and 'Cos Lettuce') were produced every day and $CO_2$ was added to the air by gas cylinders and workers' respiration, was analyzed. In the experiment, 95% of the $CO_2$ supplied from cylinders was apparently assimilated by the lettuce plants in the commercial CSAL, suggesting that the supplied $CO_2$ was used efficiently. The amounts of $CO_2$ assimilated by the lettuce plants and loss resulting from leakage, respectively, accounted for 78% and 22% of the total amount of $CO_2$ supplied. The amounts of $CO_2$ supplied by the cylinders and by the workers' respiration, respectively, accounted for 83% and 17% of the total amount of $CO_2$ supplied. Based on the analysis, a relatively high $CO_2$ utilization efficiency of 78% was observed in the experiment despite the operation rate of 33%, which is defined as the percentage of the culture beds with plants. If the operation rate could be increased to 100%, the $CO_2$ utilization efficiency would reach 92%. These results showed that $CO_2$ supplied by the workers' respiration helped to reduce the amount of $CO_2$ supplied by the cylinders and hence the $CO_2$ cost in a commercial CSAL.**

The CSAL is an airtight warehouse-like facility covered with thermally insulated walls and using artificial light as the sole light source for growing the plants. CSAL has been used to grow lettuce plants (Wheeler et al., 1994a), soybean and potato plants (Corey and Wheeler, 1992; Wheeler et al., 1994b), and wheat plants (Corey and Wheeler, 1992) and for producing transplants (Kozai et al., 2006).

The $CO_2$ balance of experimental CSALs has been investigated by several researchers (Wheeler, 1992; Yokoi et al., 2005; Yoshinaga et al., 2000) and most of the $CO_2$ supplied was assimilated by the plants (e.g., greater than 90%). In an experimental CSAL, the $CO_2$ was usually supplied into the CSAL 1) to prevent a decrease in $CO_2$ concentration to a compensation or suboptimal level as a result of the $CO_2$ assimilation by the plants; and 2) to keep the $CO_2$ concentration at high levels such as 1000 $\mu mol \cdot mol^{-1}$ to enhance the $CO_2$ assimilation by the plants (Kozai et al., 2002). However, maintaining elevated $CO_2$ concentrations will also increase the $CO_2$ loss resulting from leakage by increasing the difference in the $CO_2$ concentration between inside and outside the CSAL (Tingey et al., 2000).

The $CO_2$ balance of a commercial CSAL is expected to be similar to that of the experimental CSAL, but the human factor (i.e., $CO_2$ supplied by workers' respiration) needs to be considered. When workers enter a commercial CSAL, their respiration adds $CO_2$ to the air. This reduces the $CO_2$ required to be supplied from cylinders, thus decreasing the $CO_2$ cost. However, there is little information on this aspect. In this study, the $CO_2$ balance of a commercial CSAL, where lettuce plants are produced every day and workers in the facility respire $CO_2$ into the air, was analyzed.

Received for publication 24 Apr. 2012. Accepted for publication 3 July 2012.
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## Materials and Methods

*Commercial closed system with artificial lighting.* The cultural room of the commercial CSAL (hereafter referred to as commercial CSAL) developed by Mirai Co., Ltd. (Japan) was 21 m long, 15 m wide, and 4.5 m high. Nine basic modules with 10 shelves were installed inside the commercial CSAL. Each shelf was equipped with 31 or 47 fluorescent lamps and a culture bed (11 m long, 1.3 m wide, and 40 mm deep). In the culture beds, nutrient solution was circulated and Styrofoam boards were floated on the nutrient solution to support the lettuce plants. In the culture room, 90 culture beds were available in the commercial CSAL. In the present experiment, the operation rate, which is defined as the percentage of the culture beds with plants, was 33%. Six $CO_2$ cylinders were placed outside to supply $CO_2$ into the commercial CSAL. The $CO_2$ supply rate was adjusted by a controller to maintain the $CO_2$ concentration inside at a predetermined level. Seven heat pumps (total cooling and heating capacity: 154 and 145 kW; Daikin Industries Ltd., Japan) were installed in the commercial CSAL for controlling the air temperature.

*Lettuce plants and growth conditions.* Lettuce plants (*Lactuca sativa* L. 'Early Impulse', 'King Crown', and 'Cos Lettuce') were produced in the commercial CSAL for 35 d at a planting density of 133 plants/$m^2$ for the first 25 d and 33 plants/$m^2$ for the next 10 d. Photosynthetic photon flux (*PPF*) on the Styrofoam boards was 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the first 25 d and 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the next 10 d of lettuce plant growth with a photoperiod of 17 h (Table 1). *PPF* was adjusted by moving the lettuce plants to different shelves. The air temperature and the $CO_2$ concentration were set at 20 °C and 1800 $\mu mol \cdot mol^{-1}$ in the commercial CSAL, respectively. The electrical conductivity of the nutrient solution was maintained at 2.0 $dS \cdot m^{-1}$.

*Production procedure.* Four workers entered the commercial CSAL at 0915 HR and 1315 HR sequentially at intervals of 15 min and left together at 1200 HR and 1800 HR every day. During the period from 0915 HR to 0945 HR, the lettuce plants including 192 plants of 'Early Impulse', 192 plants of 'King Crown', and 768 plants of 'Cos Lettuce' were harvested at 35 d after sowing. After harvesting, the corresponding number of seeds in each cultivar was sown, so a series of lettuce plants of different ages between 0 and 34 d after sowing were grown at the same time in the commercial CSAL.

*$CO_2$ balance of the commercial closed system with artificial lighting.* In the present experiment, the $CO_2$ balance of the commercial CSAL was analyzed and used to calculate the $CO_2$ utilization efficiency.

The amount of $CO_2$ dissolved in the nutrient solution was constant during the experimental period because the amount of nutrient solution in the culture bed was not changed. Thus, the $CO_2$ balance of the commercial

CSAL was unaffected by the dissolution of $CO_2$ and expressed by:

$$A + S_C + S_W + I + L = 0 \qquad (1)$$

where $A$: the daily amount of $CO_2$ assimilated by the lettuce plants per unit area of the culture bed with plants ($mol \cdot m^{-2} \cdot d^{-1}$); $S_C$: the daily amount of $CO_2$ supplied by the cylinders per unit area of the culture bed with plants ($mol \cdot m^{-2} \cdot d^{-1}$); $S_W$: the daily amount of $CO_2$ supplied by workers' respiration per unit area of the culture bed with plants ($mol \cdot m^{-2} \cdot d^{-1}$); $I$: the daily amount of $CO_2$ increase in air per unit area of the culture bed with plants ($mol \cdot m^{-2} \cdot d^{-1}$); and $L$: the daily amount of $CO_2$ loss resulting from leakage per unit area of the culture bed with plants ($mol \cdot m^{-2} \cdot d^{-1}$).

In the present experiment, the $CO_2$ utilization efficiency (*CUE*) was defined as the ratio of the amount of $CO_2$ assimilated by plants to the amount of $CO_2$ supplied into the commercial CSAL:

$$CUE = \frac{A}{S_C + S_W} \cdot 100\% \qquad (2)$$

The daily amount of $CO_2$ assimilated by the lettuce plants per unit area of the culture bed with plants ($A$) was estimated from the dry mass of the lettuce plants:

$$A = \frac{W}{m \cdot j \cdot B} \qquad (3)$$

where $W$: the increase in dry mass of the lettuce plants in the commercial CSAL ($g \cdot d^{-1}$); $m$: the molecular weight of $CO_2$ ($44\ g \cdot mol^{-1}$); $j$: the coefficient for converting the $CO_2$ assimilated by plants into dry mass ($0.68\ g \cdot g^{-1}$; Van Henten, 1994); and $B$: the area of the culture beds with plants ($434\ m^2$).

Assuming that 1) the growth rate of the lettuce plants was constant in the commercial CSAL; and 2) the dry mass of seeds was small compared with that of the harvested lettuce plants 35 d after sowing, the increase in dry mass of the lettuce plants in the commercial CSAL was estimated by:

$$W = \sum_{d=1}^{35} (w_d - w_{d-1}) = w_{35} \qquad (4)$$

where $w_d$: the dry mass of the lettuce plants "$d$" d after sowing (g).

The daily amount of $CO_2$ supplied by the cylinders per unit area of the culture bed with plants ($S_C$) was determined by:

$$S_C = \frac{\sum_{t=0}^{23} F_t}{B} \qquad (5)$$

where $F_t$: the $CO_2$ supply rate at time "$t$" ($mol \cdot h^{-1}$).

The daily amount of $CO_2$ supplied by the workers' respiration per unit area of the culture bed with plants ($S_W$) was estimated using a fixed respiration rate of workers and determined by:

$$S_W = \frac{R \cdot \sum_{t=0}^{23} \sum_{n=1}^{K} P_{n,t}}{B} \qquad (6)$$

where $R$: the respiration rate of the workers (0.95 mol/h/person; Japan Air Cleaning Association, 2000); $P_{n,t}$: the cumulative working time of worker "$n$" at time "$t$" (h); and $K$: the number of workers in the commercial CSAL.

The $CO_2$ increase in air describes the increase in the amount of $CO_2$ in air resulting from the increase in the $CO_2$ concentration. The daily amount of $CO_2$ increase in air per unit area of the culture bed with plants ($I$) was determined by:

$$I = \frac{k \cdot V \cdot \sum_{t=0}^{23} (C_{i,t+1} - C_{i,t})}{B} \qquad (7)$$

where $k$: the reciprocal of the molar volume of $CO_2$ ($41.4\ mol \cdot m^{-3}$); $V$: the volume of the CSAL ($1379\ m^3$); and $C_{i,t}$: the hourly average $CO_2$ concentration in the commercial CSAL at time "$t$" ($\mu mol \cdot mol^{-1}$).

The daily amount of $CO_2$ loss resulting from leakage per unit area of the culture bed with plants ($L$) was estimated by:

$$L = -(A + S_C + S_W + I) \qquad (8)$$

*Measurements.* The air temperature and relative humidity were measured in the commercial CSAL using sensors (RTR 53-AL; T&D Corp., Japan). The $CO_2$ concentration was measured by infrared gas analyzers (GMP 222; Vaisala Oyj, Helsinki, Finland) inside and outside the commercial CSAL. The $CO_2$ supply rate from the cylinders was measured by a flow meter (FD-A600; Keyence Corp., Japan). The hourly averaged data were recorded and used for analyzing the $CO_2$ balance of the commercial CSAL. The analysis of the $CO_2$ balance was repeated three times.

Three lettuce plants 35 d after sowing in each cultivar were sampled and dried at 60 °C for 7 d to determine the dry mass. Another three 'Early Impulse' lettuce plants were sampled 14, 24, 28, 32, and 35 d after sowing and their second leaf (14 d after sowing, counting from bottom) or third leaf (24, 28, 32, and 35 d after sowing, counting from bottom) were used for measuring the $CO_2$ assimilation rate using a portable photosynthesis system (LI-6400; LI-COR, Inc., Lincoln, NE). At the inlet of the leaf chamber, the $CO_2$ concentration was set at 200, 250, 300, 400, 600, 800, 1000, 1500, or 2000 $\mu mol \cdot mol^{-1}$. *PPF* and the leaf temperature were maintained at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 20 °C, respectively. The measurements were repeated three times.

## Results and Discussion

The $CO_2$ concentration and the air temperature inside the commercial CSAL were 1800 ± 56 $\mu mol \cdot mol^{-1}$ and 20 ± 0.4 °C, respectively. The relative humidity inside ranged from 68% to 80%. The amounts of $CO_2$ assimilated by the lettuce plants and loss resulting from leakage, respectively, accounted for 78% [= 0.27·100%/(0.29 + 0.06)] and 22% [= 0.08·100%/(0.29 + 0.06)] of the total amount of $CO_2$ supplied (Table 2). The amounts of $CO_2$ supplied by the cylinders and by the workers' respiration, respectively, accounted for 83% [= 0.29·100%/(0.29 + 0.06)] and 17% [= 0.06·100%/(0.29 + 0.06)] of the total amount of $CO_2$ supplied. The amount of $CO_2$ increase in the air was less than one-hundredth of the other amounts.

The air can leak out of the commercial CSAL through small gaps and opening of the door when workers enter and leave the commercial CSAL (Acock and Acock, 1989). This process can be affected by the variation in environmental conditions such as air temperature (Wheeler, 1992). As a result, part of the $CO_2$ was lost because of leakage.

Table 1. Light environment in commercial closed systems with artificial lighting for producing lettuce plants.

| Days after planting | *PPF*[z] ($\mu mol \cdot m^{-2} \cdot s^{-1}$) | Photoperiod ($h \cdot d^{-1}$) | Time of day (HR) |
|---|---|---|---|
| 0–24 | 100 | 17 | 1600–0900[y] |
| 25–29 | 130 | 17 | 1300–1600[y] |
| | | | 1600–1700 |
| | | | 1900–2000[y] |
| | | | 2000–0900[y] |
| 30–34 | 130 | 17 | 2000–1300[y] |

[z]*PPF* was measured on the surface of Styrofoam boards.
[y]Half of the lettuce plants were illuminated at 1600 HR to 1700 HR and the other half of the lettuce plants were illuminated at 1900 HR to 2000 HR to give the same cumulative photoperiod.
*PPF* = photosynthetic photon flux.

Table 2. $CO_2$ balance and $CO_2$ utilization efficiency of commercial closed systems with artificial lighting for producing lettuce plants.[z]

| Item | Value ($mol \cdot m^{-2} \cdot d^{-1}$) |
|---|---|
| Daily amount of $CO_2$ supplied by the cylinders per unit area of culture beds with plants ($S_C$) | 0.29 ± 0.01 |
| Daily amount of $CO_2$ supplied by the workers' respiration per unit area of culture beds with plants ($S_W$) | 0.06 ± 0.00 |
| Daily amount of $CO_2$ assimilated by the lettuce plants per unit area of culture beds with plants ($A$) | 0.27 ± 0.00 |
| Daily amount of $CO_2$ loss due to leakage per unit area of culture beds with plants ($L$) | 0.08 ± 0.01 |
| Daily amount of $CO_2$ increase in air per unit area of culture beds with plants ($I$) | 0.00 ± 0.00 |
| $CO_2$ utilization efficiency = $A/(S_C + S_W) \cdot 100\%$ | 78% |

[z]Mean ± SE.

In previous studies, the $CO_2$ concentration in the CSAL was first increased as a result of the plant respiration during the dark periods and decreased quickly to the $CO_2$ concentration set point because of the plant $CO_2$ assimilation at the start of the photoperiod (Wheeler, 1992; Yoshinaga et al., 2000). As a result, the amount of $CO_2$ increase in air increased during the dark period and decreased during the start of the photoperiod in response to the variation in $CO_2$ concentration in the CSAL. In this study, the $CO_2$ concentration in the commercial CSAL ranged from 1750 to 1900 μmol·mol$^{-1}$ during the experimental periods because the photoperiods were staggered. The daily amount of $CO_2$ increase in air is just 5% of the total amount of the $CO_2$ supplied, assuming that the $CO_2$ concentration in the commercial CSAL was increased by 150 (1900 to 1750) μmol·mol$^{-1}$ during a day. Besides, both the increase and decrease in the $CO_2$ concentration in the commercial CSAL during a day were included in the analysis of $CO_2$ balance. Thus, the daily amount of $CO_2$ increase in air was small and can be neglected.

In the present experiment, 94% of $CO_2$ supplied by the cylinders was apparently assimilated by the lettuce plants in the commercial CSAL, suggesting that the $CO_2$ supplied into the commercial CSAL was used efficiently. In the previous studies, all of the $CO_2$ supplied was from the cylinders because there were no workers in the experimental CSAL. However, in the present experiment, there were two $CO_2$ supply sources: the cylinders and the workers' respiration. If no workers entered the commercial CSAL, the amount of $CO_2$ supplied by the cylinders would be increased by 21% to obtain the same daily amount of $CO_2$ assimilated by the lettuce plants. This suggests that workers' respiration can replace part of the $CO_2$ supplied by the cylinders and thus decrease the $CO_2$ cost.

The $CO_2$ utilization efficiency of the commercial CSAL was 78%, which was lower than that reported by Yoshinaga et al. (2000). This was mainly because the operation rate was 33% as a result of supply and demand for the lettuce plants. In other words, only 30 culture beds were used for growing lettuce plants in the commercial CSAL. If all the culture beds were occupied with plants, the $CO_2$ utilization efficiency would be 92%, which is comparable to that reported by Yoshinaga et al. (2000). Increasing the $CO_2$ utilization efficiency by increasing the operation rate will decrease the $CO_2$ cost for producing lettuce plants in the commercial CSAL. In addition, the $CO_2$ utilization efficiency is 100% and is unaffected by the $CO_2$ concentration if the CSAL is completely airtight, although this is difficult to achieve in practice (Baker et al., 2004).

The $CO_2$ assimilation rate of a single leaf of a lettuce plant increased with the increase in the ambient $CO_2$ concentration (Fig. 1), indicating that the amount of $CO_2$ assimilated by the lettuce plants also increased with the increase in the $CO_2$ concentration in the commercial CSAL. Simultaneously, the increasing $CO_2$ concentration in the commercial CSAL results in an increase in $CO_2$ loss resulting from leakage, which may reduce the $CO_2$ utilization efficiency of the commercial CSAL. If the set point of $CO_2$ concentration in the commercial CSAL decreased from 1800 in the present study to 1000 μmol·mol$^{-1}$, the amount of $CO_2$ assimilated by the lettuce plants would decrease by 22% according to measurements of the $CO_2$ assimilation rate of single leaves of lettuce plants (Fig. 1). At the same time, the amounts of $CO_2$ loss resulting from leakage and $CO_2$ supplied by the cylinders also decreased by 44% and 32%, although the amount of $CO_2$ supplied by the workers' respiration was constant. Therefore, the $CO_2$ utilization efficiency of the commercial CSAL will be 6.5% higher than that obtained in the present experiment. However, for the commercial production of lettuce plants in CSAL, promoting the growth of the lettuce plants by increasing the $CO_2$ concentration is occasionally more important than increasing the $CO_2$ utilization efficiency. Although the $CO_2$ assimilation rate of single leaves can be scaled up to canopy level, this scaling-up is not straightforward and may cause errors (Bugbee, 1992). In future studies, the canopy $CO_2$ assimilation rate should be measured for estimating the effects of $CO_2$ concentration on the $CO_2$ balance of the commercial CSAL.

According to the American Conference of Governmental Industrial Hygienists (1991), the indoor $CO_2$ concentration should be lower than 5000 μmol·mol$^{-1}$ for an 8-h working day to guarantee the workers' health. In the present experiment, the $CO_2$ concentration in the commercial CSAL was below 2000 μmol·mol$^{-1}$ and the working hour in each day is less than 8 h. Thus, the impacts of high $CO_2$ concentration on workers' health can be neglected. However, the accumulation of other air pollution in the commercial CSAL should be a concern, because the air exchange was minimized.

In the present experiment, we assumed constant values for the workers' respiration rate for analyzing the $CO_2$ balance of the commercial CSAL. The workers' respiration rate was determined based on interviews about their work, which mainly consisted of seeding, transplanting, and harvesting the lettuce plants in the commercial CSAL. The respiration rate ranged between 0.67 mol/h/person when seated quietly and 2.9 mol/h/person during heavy work (Japan Air Cleaning Association, 2000). In future work, the workers' respiration rate should be precisely determined for obtaining the $CO_2$ balance of the commercial CSAL more accurately. On the other hand, the $CO_2$ concentration below 20,000 μmol·mol$^{-1}$ does not elevate the workers' respiration rate significantly (Rice, 2003). Thus, the respiration rate measured under normal conditions was used to estimate the daily amount of the $CO_2$ supplied by the workers' respiration in this study.

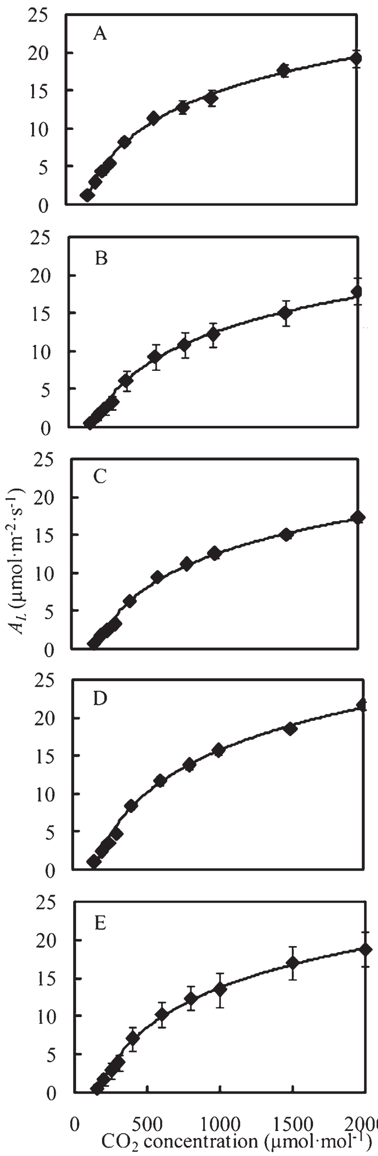


Fig. 1. $CO_2$ assimilation rate ($A_L$) of the second leaf of 'Early Impulse' lettuce plants 14 d after sowing (**A**), the third leaf of the lettuce plants 24 (**B**), 28 (**C**), 32 (**D**), and 35 d after sowing (**E**) as affected by the $CO_2$ concentration at the photosynthetic photon flux (*PPF*) of 200 μmol·m$^{-2}$·s$^{-1}$. Mean ± SE.

## Conclusions

In conclusion, 94% of $CO_2$ supplied by the cylinders was apparently assimilated by the lettuce plants in the commercial CSAL. This was because the amount of $CO_2$ supplied by the workers' respiration was almost equal to that of $CO_2$ loss resulting from leakage (Table 2) and thus contributed to the relatively high $CO_2$ utilization efficiency (78%). Our results showed the important contribution of the $CO_2$ supplied by workers' respiration to decreasing the amount of $CO_2$ supplied by the cylinders and hence the $CO_2$ cost in a commercial CSAL.

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