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# Combination of low oxygen and high carbon dioxide treatments alters sprouting of white asparagus

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**Abstract:** A single cropping system with 1-year-old rootstocks by forcing culture has been proposed for white asparagus (*Asparagus officinalis*) production in Japan. To develop a method to control the sprouting period for such a cropping system, we investigated the effects of low oxygen ($O_2$), high carbon dioxide ($CO_2$), and a combination of these on the sprouting of asparagus spears, using a laboratory-scale test. We stored the rootstocks without soil for 6 weeks in six treatment conditions (20% $O_2$, control; 20% $O_2$ + 8% $CO_2$; 20% $O_2$ + 16% $CO_2$; 8% $O_2$; 8% $O_2$ + 8% $CO_2$; and 8% $O_2$ + 16% $CO_2$). After storage, we planted the rootstocks in pots with soil and investigated their yield performance for 20 weeks. Only the 8% $O_2$ + 16% $CO_2$ treatment applied during rootstock storage suppressed the sprouting of spears. The number of harvested spears and the yield of spears per plant in the 8% $O_2$ + 16% $CO_2$ treatment was higher than in control at the 16–20-week period, although the weight per spear did not differ. We suggest that the extension of sprouting after planting was due to the suppression of sprouting during rootstock storage. Our results suggest that controlling atmospheric $O_2$ and $CO_2$ concentrations around the rootstock during storage could be a new, effective method to alter the harvest period of white asparagus, including cultivation of fresh asparagus in a single cropping system with forcing culture.

**Key words:** *Asparagus officinalis*, controlled atmosphere, forcing culture, single cropping system

## 1. Introduction

A single cropping system by forcing culture with 1-year-old rootstocks has been proposed for white asparagus production in Japan (Jishi et al., 2008; Jishi and Araki, 2013). In such a system, controlling the sprouting period is important because the market price directly depends on the sprouting season. Our previous study demonstrated that a low oxygen ($O_2$) treatment applied to white asparagus rootstock delayed sprouting and suppressed the decrease in soluble solid content of rootstocks (Kitazawa et al., 2014). These findings could be applied to control the sprouting period in white asparagus production. We also suggested that sprouting of spears was delayed when the $O_2$ concentration around the rootstocks was 12.4%–14.4%. For plants, a hypoxic atmosphere reduces metabolic action due to respiration (Zabalza et al., 2009). Therefore, lowering $O_2$ to levels below those previously identified might further delay sprouting. However, the minimum $O_2$ concentration is limited because hypoxic conditions, e.g., partial $O_2$ pressure of 8–16 kPa or less, might induce damage to rootstock because of anaerobic respiration (Drew, 1983). It is also known that high carbon dioxide ($CO_2$) environments can inhibit the respiration of plants (Gonzàlez-Meler et al., 1996). Moreover, Qi et al. (1994) demonstrated that high $CO_2$ concentrations around the roots of Douglas fir (*Pseudotsuga menziesii*) inhibited respiration. Thus, a high $CO_2$ atmosphere may be used for controlling the sprouting period even if the $O_2$ concentration is not low.

In this study we investigated the effects of a combination of low $O_2$ and high $CO_2$ treatment during rootstock storage on the sprouting of spears using a laboratory-scale test. In the previous study, rootstocks were buried in soil during hypoxic storage (Kitazawa et al., 2014); thus, the hypoxic effect may be greater than in unburied rootstocks. Therefore, we used unburied rootstocks in this study.

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## 2. Materials and methods

### 2.1. Plant materials

Asparagus seeds (cv. UC157) were sown in 200-cell plug trays on 13 May 2014 and were grown in a glasshouse at the NARO Institute of Vegetable and Tea Science, Tsukuba, Japan. Commercial culture soil (pH 5.8–6.5, particle size: 0.5–3.0 mm, N: 200 mg $L^{-1}$, $PO_3$: 2500 mg $L^{-1}$, K: 200 mg $L^{-1}$, and Mg: 200 mg $L^{-1}$; Nippi Engei Baido No. 1, Nihon Hiryo, Tokyo, Japan) was used for cultivation. Seedlings were transplanted to black polyethylene pots (90 mm diameter and 360 mL volume) on 11 June 2014 and then to pots (10.5 mm diameter and 570 mL volume) on 12 September. To control for differences between male and female asparagus plants due to sprouting (Koizumi et al., 2002), the sex of the plants was determined by examining the flowers and/or by loop-mediated isothermal amplification method (Shiobara et al., 2011). Three male and 3 female plants were used for each treatment, although 2 treatments were applied to 4 male and 2 female plants.

### 2.2. $O_2$ and $CO_2$ treatments of rootstocks (experiment 1)

Shoots from the potted plantlets were cut just above the ground. The soil was then removed from the pots, and both rootstocks and pots were washed. The average weight of rootstocks was 19.4 g ± 1.8 of standard deviation. Six pots, each containing a rootstock without soil, were placed in a gas-tight acrylic chamber (340 × 380 × 375 $mm^3$; Figure 1) on 27 November 2014. Next, the six rootstocks in each chamber were used for replication, although we did not have replicate chambers.

Experimental gas treatments used in this study were 20% $O_2$ (high $O_2$; HO), 20% $O_2$ + 8% $CO_2$ (high $O_2$ and low $CO_2$; HOLC), 20% $O_2$ + 16% $CO_2$ (HOHC), 8% $O_2$ (low $O_2$; LO), 8% $O_2$ + 8% $CO_2$ (low $O_2$ and low $CO_2$; LOLC), and 8% $O_2$ + 16% $CO_2$ (LOHC). The HO treatment was considered the control, because it mimicked the ambient $O_2$ concentration (approximately 21%). Gases for each treatment condition were prepared by combining $O_2$, $N_2$ (nitrogen), and $CO_2$ gases in a gas mixer (MAP Mix 9001 ME, flow control type for the 3 gases; PBI-Dansensor A/S, Ringsted, Denmark). Each mixed gas was blown from the inlet port of the chamber, and the air remaining in the chamber was purged through an outlet port. The flow rate of each gas was approximately 7.5 L $min^{-1}$. A gas analyzer (Checkpoint $O_2$/$CO_2$; PBI-Dansensor A/S, Ringsted, Denmark) was used to monitor and adjust the $O_2$ and $CO_2$ concentrations in each chamber. The $O_2$ and $CO_2$ concentrations were also measured just prior to the evaluation of sprouting. The concentration of the gases in each chamber was adjusted to the initial concentration following the evaluation of sprouting.

The gas-tight chambers were opened at 2-day intervals for evaluation of sprouting. We defined sprouting as the length of the spear reaching 30 mm and recorded the number of sprouting spears. Spears were cut when the length reached 50 mm. Rootstocks were sprayed with approximately 1.8 mL of distilled water at 4-day intervals. Additionally, rootstocks were immersed in a fungicide (Topsin-M Wettable Powder, Nippon Soda, Tokyo, Japan; 1000 × dilution) for 5 s at 2-week intervals to prevent them from rotting. The chambers were maintained in a dark room at 20 °C for the duration of the experiment. The humidity in each chamber was approximately 100%. This experiment was conducted over 6 weeks (42 days).

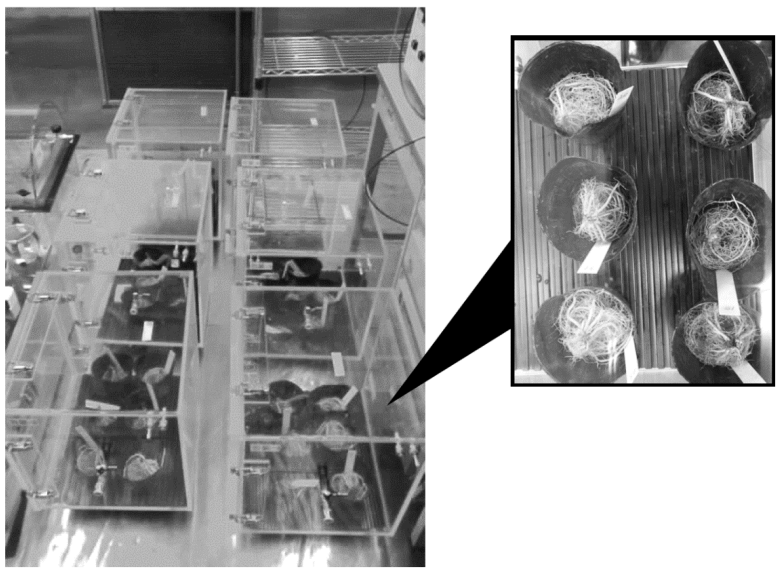

**Figure 1.** Gas-tight chambers and layout of pots with rootstocks and without soil.

**2.3. Yielding ability after storage at different $O_2$ and $CO_2$ concentrations (experiment 2)**

The rootstocks used in experiment 1 were planted on 8 January 2015 in the same pots with the same soil that was used to raise the plantlets. These pots were kept in a dark room (Figure 2) for 140 days at 20 °C and 80% humidity. Fifty mL of water was supplied every 4 days. The water not absorbed into the soil and/or the rootstocks was drained from a hole at the bottom of the pots and collected. The rootstocks stored with the HO treatment in experiment 1 were used as the control.

Spears were cut when their lengths were over 150 mm. To measure yielding ability, we recorded the number of harvested spears, the weight of the spears, and the yield of spears per plant at 35-day intervals. The spears differed in size; thus, for evaluation of weight, we normalized spear length by converting the measurement of all the spears to the same length (10 mm). The conversion was conducted as follows: the weight of spears per plant (mg 10 $mm^{-1}$) = [harvested spear weight (mg)/spear length (mm)] × 10. We only investigated yielding ability for HO (control) and the treatments that showed differences in sprouting ability compared to the control in experiment 1.

**Figure 2.** Appearance of the rootstocks buried in soil and sprouting.

**2.4. Statistical analysis**

Homogeneity of variance was not assumed in statistical analyses for comparisons between each treatment group and the control. Thus, we used Steel's test for many-to-one comparisons and/or Welch's t-test for pairwise comparison. The significance level (P) for both tests was set at 0.05. Steel's test was carried out using statistical software (Excel; Toukei 2012, Social Survey Research Information, Tokyo, Japan). Welch's t-test was carried out with a spreadsheet program (Excel 2013, Microsoft Japan, Tokyo, Japan). The primary purpose of this study was to clarify whether each gas treatment could control sprouting. Therefore, we investigated whether there was a significant difference between the control and each gas treatment and did not use the 2-factor factorial analysis of variance (2-way ANOVA) to confirm the interaction between $O_2$ and $CO_2$ concentrations.

## 3. Results

**3.1. Effect of $O_2$ and $CO_2$ on the sprouting of white asparagus during rootstock storage (experiment 1)**

**3.1.1. Changes in $O_2$ and $CO_2$ concentrations in the chamber**

The $O_2$ and $CO_2$ concentrations in each chamber during experiment 1 are shown in the Table. The average $O_2$ + $CO_2$ concentrations in the control, HOLC, HOHC, LO, LOLC, and LOHC treatments were 20.0% + 0.0%, 19.9% + 8.0%, 19.9% + 15.8%, 8.3% + 0.0%, 8.2% + 8.1%, and 8.3% + 15.8%, respectively. These were close to the expected values, and little variability was seen in the $O_2$ and $CO_2$

**Table.** $O_2$ and $CO_2$ concentrations (%) for each treatment over 6 weeks.

| Treatment | Average | | Maximum | | Minimum | |
|---|---|---|---|---|---|---|
| | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ |
| HO (Control) | 20.0 ± 0.3[z] | 0.0 ± 0.1 | 20.9 | 0.3 | 19.4 | 0.0 |
| HOLC | 19.9 ± 0.3 | 8.0 ± 0.3 | 20.4 | 8.4 | 19.1 | 7.5 |
| HOHC | 19.9 ± 0.3 | 15.8 ± 0.4 | 20.4 | 16.4 | 19.0 | 14.8 |
| LO | 8.3 ± 0.4 | 0.0 ± 0.1 | 9.1 | 0.7 | 7.7 | 0.0 |
| LOLC | 8.2 ± 0.3 | 8.1 ± 0.3 | 8.8 | 8.6 | 7.4 | 7.5 |
| LOHC | 8.3 ± 0.4 | 15.8 ± 0.5 | 8.9 | 16.8 | 7.5 | 14.8 |

[z]Average ± standard deviation.

concentrations during the treatment. The small amounts of detected $CO_2$ in the control and LO treatments were a result of respiration of the rootstock, because the original gases in these treatments did not contain $CO_2$.

### 3.1.2. Number of sprouting spears

The average numbers of sprouting spears in the control, HOLC, HOHC, LO, LOLC, and LOHC treatments were 8.0, 8.7, 7.0, 6.8, 5.5, and 5.0, respectively (Figure 3). The average number of sprouting spears in the LOHC treatment was significantly lower than that in the control; however, there were no differences between any of the other treatments and the control. Based on these results we only evaluated yielding ability of rootstocks after storage under the control or LOHC treatment in experiment 2.

### 3.2. Yielding ability after storage at different $O_2$ and $CO_2$ concentrations

### 3.2.1. Number of harvested spears

The average number of spears at 1–5 weeks, 6–10 weeks, 11–15 weeks, and 16–20 weeks were 4.0, 4.3, 3.2, and 1.5, respectively, in control conditions (Figure 4) and 4.3, 4.2, 3.2, and 3.2, respectively, in the LOHC treatment condition. There was a significant difference between the 2 groups in the 16–20-week period but not in the 1–15-week period. The total numbers of harvested spears in the control and LOHC treatments were 13.0 and 14.8, respectively, and this difference was not significant.

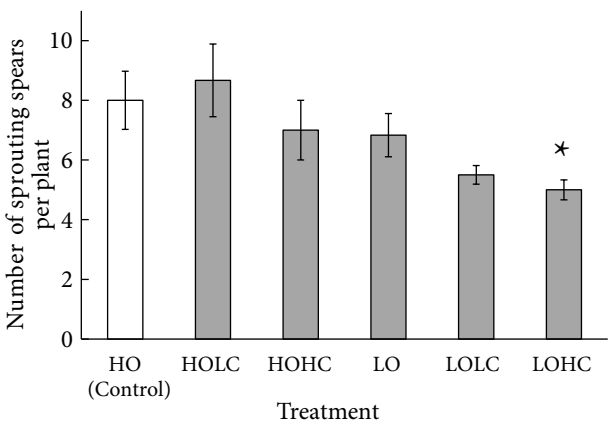


**Figure 3.** Effect of various oxygen and carbon dioxide concentrations on the sprouting of white asparagus during storage of rootstocks.
HO (control): 20% $O_2$, HOLC: 20% $O_2$ + 8% $CO_2$, HOHC: 20% $O_2$ + 16% $CO_2$,
LO: 8% $O_2$, LOLC: 8% $O_2$ + 8% $CO_2$, and LOHC: 8% $O_2$ + 16% $CO_2$.
Error bars show the standard error (SE) of each average value (n = 6).
Asterisk indicates treatment that differed significantly (Steel's test, $P < 0.05$) from the control.

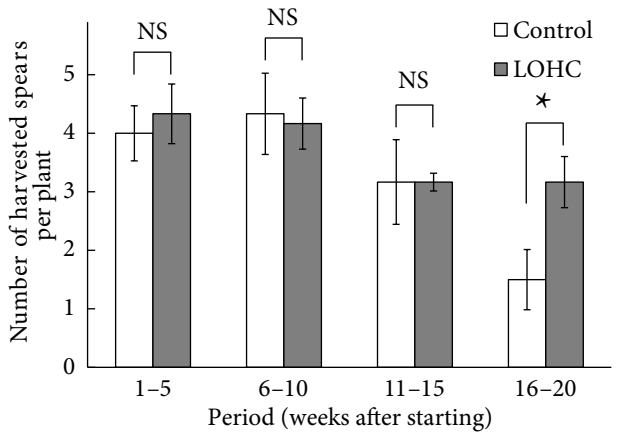


**Figure 4.** Effect of the LOHC treatment during storage of rootstocks on the number of harvested spears per plant after planting.
Control: 20% $O_2$ and LOHC: 8% $O_2$ + 16% $CO_2$.
NS = not significant, and asterisk indicates significant difference by Welch's t-test ($P < 0.05$) within each period.
Each error bar shows the SE of each average value (n = 6).

### 3.2.2. Weight of spears per 10 mm

In the control treatment, the average weight of spears per 10 mm at 1–5 weeks, 6–10 weeks, 11–15 weeks, and 16–20 weeks were 20.9, 21.0, 16.4, and 12.8 mg, respectively (Figure 5). The average weights for the LOHC treatment at the same periods were 23.9, 31.2, 26.2, and 15.7 mg, respectively. There was no significant difference between the control and the LOHC treatment in any of the experimental periods.

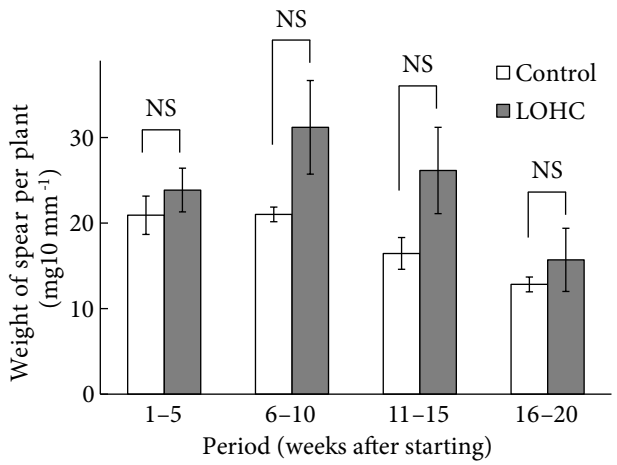


**Figure 5.** Effect of the LOHC treatment during storage of rootstocks on the weight of spears per plant after planting.
Control: 20% $O_2$ and LOHC: 8% $O_2$ + 16% $CO_2$.
NS indicates no significant difference by Welch's t-test ($P < 0.05$) within each period.
Each error bar shows SE of each average value (n = 6).

### 3.2.3. Yield of spears

The yields for the control at 1–5, 6–10, 11–15, and 16–20-week periods were 1.3, 1.5, 0.8, and 0.3 g, respectively (Figure 6). The yields for the LOHC treatment at these periods were 1.6, 2.0, 1.4, and 0.7 g, respectively. The LOHC treatment showed a significantly higher yield than the control at 16–20 weeks; however, there was no significant difference during the 1–15-week period.

## 4. Discussion

Sprouting of spears was delayed when the $O_2$ concentration in the gaseous phase of the soil around the rootstocks was 12.4%–14.4% (Kitazawa et al., 2014). However, in this study, sprouting of spears did not decrease under an 8% concentration of $O_2$ (LO and LOLC treatments). In this study, rootstocks were not buried in the soil during storage. In other words, rootstocks contacted only the gaseous phase. Therefore, the differences in reaction to $O_2$ concentrations may be attributed to the lack of distribution of liquids and solids around the rootstocks. Our results also suggest that lower $O_2$ concentrations are required to control sprouting of spears when the rootstocks are stored without soil.

The sprouting of spears in the LOHC treatment was significantly lower than in the control. The metabolism of plants under dark conditions is suppressed under high $CO_2$ levels, compared to normal atmospheric conditions (Mathooko, 1996a, 1996b; Robredo et al., 2011). Mathooko (1996b) suggested that $CO_2$ directly affected the glycolytic pathway in the metabolism of stored fresh produce. We suggested that the suppression of sprouting was related to the suppression of the decrease in soluble solids of the rootstocks (Kitazawa et al., 2014). Thus, the sprouting of spears in the LOHC (16% $CO_2$ concentration) treatment might decrease because of the suppression of carbohydrate metabolism in the rootstocks under high $CO_2$ concentrations. Further studies are needed to clarify this point. Sprouting in the LOLC treatment (8% $CO_2$ concentration) did not differ from that in the control, suggesting that a $CO_2$ concentration of at least 16% is necessary to suppress sprouting. Our future work will focus on determining the ideal ratio of $O_2$ and $CO_2$ to suppress sprouting during rootstock storage.

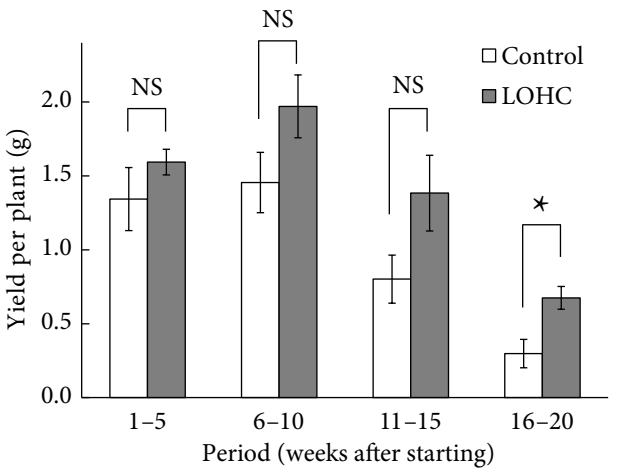


**Figure 6.** Effect of the LOHC treatment during storage of the rootstock on the yield per plant after planting.
Control: 20% $O_2$ and LOHC: 8% $O_2$ + 16% $CO_2$.
NS = not significant, and asterisk indicates significant difference by Welch's t-test ($P < 0.05$) within each period.
Each error bar shows SE of each average value (n = 6).

Our previous study (Kitazawa et al., 2014) suggested that yield ability after cessation of altered $O_2$ treatments was not influenced if sprouting ability during the treatments did not change relative to control. Hence, in this study, we estimated that yield ability after cessation of HOLC, HOHC, LO, and LOLC treatments was not changed relative to that of the control. The number of harvested spears and yield in the LOHC treatment at 16–20 weeks was significantly higher than in the control. On the other hand, there was no significant difference between the two groups in any of the experimental periods in terms of average weight of spears. Thus, the higher yield during the 16–20-week period in the LOHC treatment was likely due to the number of sprouting spears and not the weight per spear. There was no significant difference in the total number of harvested spears after planting between the LOHC treatment and the control. Thus, we suggest that the combination of low $O_2$ and high $CO_2$ had the greatest effect during storage, and combination effects remain as an extension of sprouting after planting.

The results of this study suggest that a combination of low $O_2$ and high $CO_2$ in the rootstock environment during storage could alter the harvest period of white asparagus after planting. According to our findings, controlling atmospheric $O_2$ and $CO_2$ concentrations could be a new and effective method for altering the harvest period of white asparagus, including a single cropping system with 1-year-old rootstocks by forcing culture.

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