Ethylene reduces gas exchange and growth of lettuce plants under hypobaric and normal atmospheric conditions
Chuanjiu He\textsuperscript{a}, Fred T. Davies Jr\textsuperscript{a,*} and Ronald E. Lacey\textsuperscript{b}

\textsuperscript{a}Department of Horticultural Sciences and Interdisciplinary Program of Molecular and Environmental Plant Sciences (MEPS), Texas A&M University, College Station, TX 77843 2133, USA

\textsuperscript{b}Department of Biological and Agricultural Engineering, Texas A&M University, College Station, TX 77843 2117, USA

Elevated levels of ethylene occur in controlled environment agriculture and in spaceflight environments, leading to adverse plant growth and sterility. The objectives of this research were to characterize the influence of ethylene on carbon dioxide (CO\textsubscript{2}) assimilation (C\textsubscript{A}), dark period respiration (DPR) and growth of lettuce (\textit{Lactuca sativa} L. cv. Buttercrunch) under ambient and low total pressure conditions. Lettuce plants were grown under variable total gas pressures of 25 kPa (hypobaric) and 101 kPa (ambient) pressure. Endogenously produced ethylene accumulated and reduced C\textsubscript{A}, DPR and plant growth of ambient and hypobaric plants. There was a negative linear correlation between increasing ethylene concentrations [from 0 to around 1000 nmol mol\textsuperscript{-1}/ppb] on C\textsubscript{A}, DPR and growth of ambient and hypobaric plants. Declines in C\textsubscript{A} and DPR occurred with both exogenous and endogenous ethylene treatments. C\textsubscript{A} was more sensitive to increasing ethylene concentration than DPR. There was a direct, negative effect of increasing ethylene concentration reducing gas exchange as well as an indirect ethylene effect on leaf epinasty, which reduced light capture and C\textsubscript{A}. While the C\textsubscript{A} was comparable, there was a lower DPR in hypobaric than ambient pressure plants – independent of ethylene and under non-limiting CO\textsubscript{2} levels (100 Pa pCO\textsubscript{2}, nearly three-fold that in normal air). This research shows that lettuce can be grown under hypobaria (\textless{}25\% of normal earth ambient total pressure); however, hypobaria caused no significant reduction of endogenous ethylene production.

Introduction
Advanced Life Support Systems (ALSs) with the capacity to recycle resources and produce food are critical for the exploration of space (Wheeler et al. 2001). Important biological components include the use of higher plants for supplemental air and water purification, nutritional and psychological benefits. Plants grown in space environments will be subjected not only to reduced gravity but also to the conditions designed mainly to fulfill requirements for the human environment (Wheeler et al. 2001). The International Space Station has CO\textsubscript{2} concentrations in its atmosphere that are many times in excess of those on earth (Campbell et al. 2001). The potential range of environmental conditions in an ALS includes total gas pressures as low as 54 kPa (8 psi) and CO\textsubscript{2} concentrations as high as 700 Pa (7000 \textmu{}mol mol\textsuperscript{-1}), some 19 times greater than that on earth [National Aeronautics and Space Administration (NASA) 2004] and in great excess

Abbreviations – C\textsubscript{A}, CO\textsubscript{2} assimilation; DM, dry mass; DPR, dark period respiration; GC, gas chromatograph; g\textsubscript{s}, stomatal conductance; LPPG, low-pressure plant growth system; RGR, relative growth rate; RWC, relative water content; SLA, specific leaf area.
of the range that researchers are studying global change (Amthor 1991). Plants can tolerate wide variation in concentration of the essential gases, O\textsubscript{2} and CO\textsubscript{2}, RH and, possibly also, total gas pressure (Corey et al. 2002, He et al. 2003, 2006, 2007, Spanarkel and Drew 2002). Important environmental variables that have received limited research effort in relation to an ALS include hypobaria at elevated CO\textsubscript{2} partial pressure.

There are important engineering and efficiency advantages in growing plants at hypobaria in biomass production in a controlled Martian or lunar extraterrestrial base environments. The reduced pressure differential between the plant growth facility and the external environment would reduce structural requirements, permit lighter materials to be used in its construction and improve safety issues by reducing external and internal pressure differentials. This would further reduce the payload volume and mass required for deployment (He et al. 2003).

A hypobaric condition would reduce gas leakage from the controlled environment space to the external environment. The Martian ambient pressure varies from 0.2 to 0.9 kPa, whereas the ambient pressure is near 0 kPa for a lunar mission. Pressure gradients between the two environments drive leakage, with higher differences resulting in greater leakage. Furthermore, hypobaric conditions in the plant growth facility would require less buffer gas, typically N\textsubscript{2}, to be transported or obtained in situ to supplement the physiologically active gases (CO\textsubscript{2} and O\textsubscript{2}). Earlier studies have demonstrated that plant germination and seedling growth are possible at hypobaric conditions (Andre and Massimino 1992, Corey et al. 2002, He et al. 2003, Musgrave and Strain 1988, Schwartzkopf and Mancinelli 1991, Spanarkel and Drew 2002). Plants can be grown at high altitude, with pressures well below 70 kPa (Davies et al. 2005, Gale 1972), although the inevitable association between decreasing temperature and increasing altitude confounds the issue of the effect of pressure alone. The question here is whether the rates of vegetative growth and morphogenesis compare closely with those at ambient pressure. A major potential limitation to plant growth under hypobaria is if the partial pressure of O\textsubscript{2} (pO\textsubscript{2}) is reduced, oxidative phosphorylation can become limited (Drew 1997). Seedlings germinated and grew during a week-long study at 6 kPa total gas pressure, provided the atmosphere was comprised predominately of oxygen (pO\textsubscript{2} = 5 kPa; approximately 83\% O\textsubscript{2}); but at lower total pressures and therefore less O\textsubscript{2}, seeds failed to germinate (Schwartzkopf and Mancinelli 1991). Low total pressure (21–24 kPa) did not inhibit seed germination, and initial growth as long as pO\textsubscript{2} was 5 kPa or more (Musgrave and Strain 1988). Hypobarcic environments are typically associated with hypoxia (low O\textsubscript{2}) condition, particularly when total gas pressure is reduced below 50 kPa. Hence, there is a need to supply sufficient partial pressures of \( pO_2 \) to avoid hypoxia under hypobactic conditions.

Ethylene is produced by plants throughout their life cycle. Terrestrial atmospheric ethylene levels rarely exceed 10 nmol mol\(^{-1}\) (ppb) (Abeles et al. 1992, Klassen and Bugbee 2004). However, elevated ethylene levels are of particular concern in tightly sealed bioregenerative life support systems developed by NASA (Bugbee 1999, Wheeler et al. 1996). Complete sterility has been reported with wheat associated with ethylene in growth chambers on the Russian MIR and NASA-International Space Station (Campbell et al. 2001). Several studies have reported the effects of ethylene on gas exchange in a number of species (Gunderson and Taylor 1988, 1991, Khan 2004a, 2004b, Woodrow et al. 1989) under ambient pressure. Ethylene at 10 nmol mol\(^{-1}\) (ppb) was reported to directly inhibit foliar gas exchange in Glycine max (Gunderson and Taylor 1988, 1991). Other researchers have reported that ethylene has no direct effect in gas exchange (Pallaghy and Raschke 1972, Woodrow et al. 1989), but rather an indirect effect via leaf epinasty, leading to reduced light capture and photosynthesis. We are not aware of any longer term study that clearly implicated ethylene effects on carbon dioxide (CO\textsubscript{2}) assimilation (\( C_A \)), dark period respiration (DPR) and plant growth. In addition, hypobaria has been reported to decrease respiration and increase ethylene removal from tissues of stored vegetables and fruits (Burg and Bug 1966). We hypothesized that ethylene would decrease plant gas exchange and growth but that hypobaria would reduce endogenous ethylene and mitigate its effect. Hence, the objectives of this research were to determine the influence of ethylene on \( C_A \), DPR and subsequent growth of lettuce (Lactuca sativa L. cv. Buttercrunch) under ambient and hypobactic conditions.

**Materials and methods**

**Low-pressure plant growth system**

The low-pressure plant growth system (LPPG) system is a fully automated system, capable of controlling pressure and gas concentrations in ambient or reduced pressure growth chambers (He et al. 2006, 2007). The LPPG system consisted of six growth chambers designed to operate at pressures as low as 5 kPa (Fig. 1). The six chambers were housed in an environmentally controlled growth room. Total pressures, partial pressures of oxygen (pO\textsubscript{2}) and carbon dioxide (pCO\textsubscript{2}) were controlled and monitored during experiments. The LPPG was
a semi-closed system because O$_2$, CO$_2$ and N$_2$ were added and controlled. Temperature was recorded, although not controlled directly by the LPPG system. Temperature control and lighting were provided by placing the LPPG system in an independent plant growth room. The pressure and control systems of each chamber were independent so that conditions could be set to satisfy any statistical experimental design. The LPPG operating system and parameters measured are explained in greater detail (He et al. 2007).

**Plant growth conditions**

Lettuce (*L. sativa* L. cv. Buttercrunch) was germinated in 20 × 14 cm plastic pots that were filled to within 1 cm of the top with fine-grade calcined clay [Profile Greens; Profile Products LLC, Buffalo Grove, IL (particle size <1 mm, 74% porosity, 0.56 g ml$^{-1}$ bulk density and 2.5 g ml$^{-1}$ particle density)]. The inert, calcined clay was prewashed with deionized water and allowed to drain thoroughly before sowing. After the seeds were germinated, plants were supplied with a modified Hoagland’s nutrient solution (pH 6.3) containing 4.0 mM Ca(NO$_3$)$_2$, 1.0 mM KH$_2$PO$_4$/K$_2$HPO$_4$, 2.0 mM KNO$_3$, 1.0 mM MgSO$_4$, 50 µM Fe as Fe-EDTA, 1 mM NaCl and micro-nutrients: 50 µMB, 10.0 µMMn, 1.0 µMCu, 2.0 µMZn and 0.3 µMMo.

Ten days after imbibition, three seedlings were transplanted to 20-cm self-watering pots (S-series pots; Apollo Plastics Ltd, Mississauga, Ontario, Canada) with a volume of (4 l) filled with prewashed calcined clay. The reservoir of the self-watering pots can hold 1 l nutrient solution. The lettuce seedlings were allowed to grow in normal atmospheric pressure in a controlled growth chamber for another 14 days. Only containers with uniform, 24-day-old plants were selected and transferred to the LPPG chambers for the treatments, so plants were 34-day-old at the termination of 10-day studies. Each chamber contained one pot with three seedlings (*n* = 1). For the 10-day study, 1 l of nutrient solution was in the reservoir at the beginning of the experiment, and an additional 1 l was added during the study through an air-lock system.

Lighting was approximately 600 µmol m$^{-2}$ s$^{-1}$ at canopy level inside the pressure chambers provided by Sylvania 400 watt metal halide (M400U) lamps with a 12 h/12 h light/dark phase, maximum/minimum temperature of 26.1 ± 0.6°C/20.0 ± 0.1°C and maximum/minimum RH of 91.2 ± 3.7% (dark period)/83.7 ± 2.7% (light period). Supplementary CO$_2$ was added during the light cycle to maintain a minimum set point level of
100 Pa pCO$_2$. Without supplementary CO$_2$ during the light period, CO$_2$ levels would fall within several hours to a CO$_2$ compensation point of around 2–4 Pa CO$_2$ (20–40 $\mu$L l$^{-1}$ equivalent at 101 kPa) at 21 kPa pO$_2$ (data not reported).

Gas exchange of lettuce plants as affected by ethylene

Two long-term (10-day) and one short-term (60-h) study were designed to characterize the effects of ethylene on C$_A$ and DPR of lettuce under light conditions of 600 $\mu$mol m$^{-2}$ s$^{-1}$.

Experiment 1 consisted of 101 (ambient total pressure)/21 kPa pO$_2$ (normal oxygen at normal pressure) or 25 (low total pressure)/12 kPa pO$_2$; in previous studies, C$_A$ and plant growth and development were comparable between these two treatments (He et al. 2006, 2007). Plants were then subjected to naturally occurring endogenous ethylene or maintained in an ethylene-scrubbed environment for the 10-day growth cycle. Plant growth measurements were also taken. There were a total of 4 treatments × 3 replications (n = 3) = 12 chambers required for experimental runs, with 6 chambers in the growth room. In experiment 2, plants were in the chambers for a 10-day growth cycle and ethylene scrubbed for 5, 10, 15, 20 or 1440 min day$^{-1}$ to create a gradient of endogenous ethylene levels in different chambers; plants were grown under 101/25 kPa pO$_2$ or 25/12 kPa. Ethylene effects on gas exchange and plant growth were determined. There was a total of 10 treatments × 2 replications (n = 2) = 20 chambers required for experimental runs. Experiment 3 was a short-term, dose study to assess effects of exogenous sources of ethylene on plant gas exchange. Exogenous ethylene was applied during the last 60 h (2.5 days) of the 10-day plant growth cycle from 0 to 1000 nmol mol$^{-1}$ (ppb), which is explained in greater detail in the following section. Plants were at 101/25 or 25/12 kPa total pressure/pO$_2$. Only plant gas exchange was assessed. There was a total of 2 ± low and ambient total pressure × 4 exogenous ethylene levels = 8 treatments × 2 replications (n = 2) = 16 chambers required for experimental runs.

CO$_2$ assimilation

In experiments 1, 2 and 3, the C$_A$ was measured by rate of CO$_2$ uptake (draw down) during the light period cycle (He et al. 2007, Wheeler 1992). The CO$_2$ accumulated in the chambers during the dark period cycle (without supplemental CO$_2$). To determine C$_A$, slopes of the regression lines of CO$_2$ concentration over time (min) in each chamber were determined during the light cycle. The pCO$_2$ were measured by CO$_2$ sensors within each chamber independently at 1-min intervals during the light cycle. The change of pCO$_2$ in each chamber was related to C$_A$ during the light period cycle. The minimum CO$_2$ concentration set point was 100 Pa (pCO$_2$) for all the treatments in ambient and low-pressure chambers. When CO$_2$ levels were drawn down (assimilated by plants in the chamber) to 100 Pa, which was equivalent to 1000 $\mu$mol mol$^{-1}$ at 101 kPa total pressure, the system added supplementary gas to maintain the set point level CO$_2$ in each chamber through the remaining duration of the light period cycle. The rates of C$_A$ were obtained during the first 100 min (10 min after lights were turned on during the light cycle). Hence, at 1-min intervals, there were 100 measurements taken in determining the daily C$_A$ in each chamber (one container per chamber with three seedling plants per container) per treatment. For experiment 1, each point on the figure regression lines was based on the daily slopes of C$_A$ of lettuce plants from three replicated chambers (n = 3) per treatment, while two replicated chambers per treatment were used in experiments 2 and 3 (n = 2).

Dark period respiration

In experiments 1, 2 and 3, the DPR was measured by CO$_2$ accumulation in chambers during the dark period (He et al. 2007, Richards et al. 2006, Wheeler 1992). The pCO$_2$ were measured by CO$_2$ sensors within each chamber independently at 1-min intervals during the dark period. During the dark period cycle, no supplementary CO$_2$ was added to chambers, and CO$_2$ was allowed to rise without maximum set points. To avoid any residual effects of the light cycle, data during the first 10 min of the dark cycle were not used for calculation. Hence, there were seven hundred and ten 1-min interval measurements taken nightly for DPR in each chamber per treatment. DPR was determined by the slopes of the regression equations of accumulated CO$_2$ concentration in each chamber during the dark period cycle. Each point of the regression lines was based on the slopes of the DPR of lettuce plants. For experiment 1, there were three replicated chambers, while two chambers per treatment were used in experiments 2 and 3.

Ethylene determination and treatment in cylindrical chambers

For the three experiments, ethylene concentration was measured by withdrawing gas from chambers daily at 09:00 h, except the first day of a given experiment. A 1 ml volume of gas was withdrawn using a syringe and injected into a digital gas chromatograph (GC) (Photovac 10 plus; PerkinElmer, Inc., Norwalk, CT) with a photoionization...
detector and compressed air (ultra zero grade; Praxair Inc., Bryan, TX) as carrier gas. The sensitivity of the GC was 150 fmol. There was no significant change in total atmospheric pressure in chambers during gas sampling. The ethylene concentration in the hypobaric chamber was calculated according to the Perfect Gas Law (PV = nRT, where n, moles; P, pressure; R, gas constant and T, temperature) (He et al. 2003).

For experiment 3, an exogenous ethylene concentration of 2% ethylene was made by mixing pure ethylene with nitrogen gas in a 2-L glass jar. The resulting concentration of ethylene were calculated according to the Perfect Gas Law and were checked with the GC after injection to LPPG chambers. Air in the growth room was tested for background ethylene contamination with the GC; no detectable ethylene contamination occurred in the growth room during ethylene treatment experiments. For experiment 3, lettuce was grown in ethylene-free chambers (continuously scrubbed, with no detectable ethylene for 7.5 days). Then, exogenous ethylene was applied to individual chambers to achieve concentrations of ethylene at 0, 200, 500 and 1000 nmol mol$^{-1}$ (ppb), and lettuce plants were exposed for 60 h (2.5 days). Concentrations of ethylene were adjusted to designed concentration by adding or scrubbing ethylene prior to the light period.

The scrubbing system to remove endogenous ethylene generated by lettuce plants consisted of a stainless steel column filled with potassium permanganate to strip ethylene from the air, as it is circulated over the cooling coils and returned to a given chamber. The scrubbing system was very effective in removing ethylene, as shown by ethylene effects on lettuce plant growth (Fig. 1) and detectable ethylene levels in the chambers (Fig. 2A, B). In experiment 2, to achieve different gradients of endogenous ethylene levels, chambers were scrubbed daily of ethylene for 5, 10, 15, 20 or 1440 min, and ethylene concentrations checked daily with the GC.

**Plant growth determination**

In experiments 1 and 2, at the termination of the 10-day studies, plants were removed from chambers and leaf area, specific leaf area (SLA) (cm$^2$ g$^{-1}$), leaf and root dry mass (DM) leaf/root ratio (g g$^{-1}$) were determined. Relative growth rate (RGR) [(ln total plant DM$_2$ – ln total plant DM$_1$) (time$_2$ – time$_1$)] was also determined. Leaf area was measured with a LI-COR LI-3000A leaf area meter (LI-COR, Lincoln, NE). Relative water content (RWC) [(fresh mass – DM) (saturated mass – DM)] was also determined. For digital imagery of foliar area, a digital camera (Sony Cyber-shot DSC-P200, Sony Corporation, Physiol. Plant. 135, 2009).
Tokyo, Japan) was set on the top of the LPPG. Photos were taken daily at 09:00 h. The distance and focal length between the camera and the plant was fixed from the top of chambers. Relative foliar area was determined by pixels of digital images as described by Klassen et al. (2003).

Statistical analysis

Three experiments were designed to characterize the ethylene effects on CA, DPR and plant growth. Experiment 1 was a 10-day, 2 × 2 factorial experiment of ambient (101/21) and low-pressure (25/12 kPa pO2) plants, which were either scrubbed of ethylene or allowed to accumulate endogenously produced ethylene (n = 3). Experiment 2 was also a 10-day, factorial experiment of two pressure × five ethylene concentrations endogenously generated (n = 2). The coefficient of linear regression (r²) was also determined for slopes of CA, DPR and selected plant growth parameters (n = 2). Experiment 3 was a short-term, dose study in a factorial design of two pressure × four levels of exogenous ethylene during the final 60 h of the plant growth cycle (n = 2). Plants were grown in chambers for 7.5 days in chambers that were continuously scrubbed of ethylene prior to exposure to exogenous ethylene.

Low and ambient total pressure chambers were run concurrently. Chambers were alternated during treatments between low and ambient total pressure to avoid any chamber effects. There were no chamber effects. All reported data were pooled from repeated independent treatments. There were six chambers in the growth room, so replications of treatments were run with combinations of total pressure and partial pressure of pO2 and repeated as needed under the same environmental conditions, as previously explained. There were three plants per container and one container per chamber, with each container as a single replicate. ANOVA was also conducted to determine main effects and interactions, with mean separation by Duncan’s multiple comparison or ±SE for both gas exchange and growth analysis. Coefficient of linear regression (r²) was also determined for CA and DPR with growth parameters of lettuce under 101/21 kPa or 25/12 kPa (total pressure/pO2).

Results

Experiment 1: endogenous ethylene accumulation under ambient and low total pressure

Endogenous ethylene accumulated by lettuce plants in the non-scrubbed, LPPG chambers increased during the 10-day study. Within 3–4 days, ethylene was around 200 nmol mol⁻¹ (ppb) for both ambient (101/21 kPa pO2) and low-pressure (25/12 kPa pO2) chambers (Fig. 2A, B). At the termination of the 10-day study, ethylene reached 1119 and 936 nmol mol⁻¹, respectively, for plants under ambient and low total pressure; while there was a trend of 16% lower ethylene in hypobaric than ambient pressure chambers, it was not statistically different (Fig. 2A, B, Tables 1 and 2). Ethylene levels in the ethylene-scrubbed chambers were NS, regardless of total pressure. Hence, the scrubbing system very efficiently removed ethylene from the chambers (Fig. 2A, B, Table 1).

Experiment 1: effect of endogenous ethylene on CA, DPR and plant growth

By day 3, endogenously produced ethylene reduced the CA of lettuce plants, regardless of total atmospheric pressure (Fig. 2C, D). Ethylene also decreased the DPR to statistically significant levels by day 7 for ambient and day 9 for low-pressure plants (Fig. 2E, F). The

<table>
<thead>
<tr>
<th>Total pressure (kPa)</th>
<th>C2H4 treatments</th>
<th>C2H4 level in chamber (nmol mol⁻¹)</th>
<th>CA (Pa CO2 min⁻¹)</th>
<th>DPR (Pa CO2 min⁻¹)</th>
<th>CA/DPR ratio</th>
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<tbody>
<tr>
<td>101</td>
<td>Accumulated</td>
<td>1119a†</td>
<td>2.45b</td>
<td>0.50bc</td>
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<td></td>
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<td>11b</td>
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<tr>
<td>25</td>
<td>Accumulated</td>
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<td>2.30b</td>
<td>0.46c</td>
<td>5.03ab</td>
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<td>Significance</td>
<td>Pressure (Pres)</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
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<tr>
<td></td>
<td>C2H4</td>
<td>NS</td>
<td>**</td>
<td>***</td>
<td>NS</td>
</tr>
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<td></td>
<td>Pres × C2H4</td>
<td>NS</td>
<td>NS</td>
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Table 1. Experiment 1: effect of total atmospheric pressure and ethylene ([C2H4], either scrubbed or endogenously produced) on CA, DPR and the CA/DPR ratio of lettuce (Lactuca sativa L cv. Buttercunch) in chambers of one pot/chamber and three seedling plants per container. Measurements taken during the last day of a 10-day study; plants were 34 days old at termination. †Values are means, with mean separation within columns by Duncan’s multiple comparison. Letter(s) within columns are not significantly different (p = 0.05). n = 3. NS, non-significant; *P < 0.05; **P < 0.01; ***P < 0.001.
There was a negative linear correlation between increasing endogenous ethylene on \( \text{CA} \) and DPR during a 10-day study (Fig. 4). As ethylene increased, there was a reduction of \( \text{CA} \) at 1.2427 and 1.2435 Pa CO\(_2\) min\(^{-1}\) per chamber, respectively, for both ambient and low-pressure plants (Fig. 4). Ethylene reduced DPR by 0.143 and 0.139 Pa CO\(_2\) min\(^{-1}\) respired by lettuce, respectively, for both ambient and hypobaric plants (Fig. 4). There was a significant ethylene effect on plant gas exchange, while total pressure and the interaction of ethylene \( \times \) pressure were non-significant.

There was a negative linear correlation between increasing ethylene concentrations that reduced plant growth of both ambient and low-pressure plants – including the digital crop image (megapixels), leaf area, total plant DM and RGR during the 10-day study (Figs 5 and 6). There was a linear reduction of total plant DM; with increasing endogenously produced ethylene, there was a reduced growth rate of 2.0086 and 1.9119 g total plant DM (Fig. 6). A similar pattern occurred with RGR, with a reduction of 0.0339 and 0.0323 mg g\(^{-1}\) day\(^{-1}\) as ethylene accumulated, respectively, for

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**Table 2.** Experiment 1: effect of endogenous ethylene on \( \text{CA} \), DPR of lettuce \( (\text{Lactuca sativa L cv. Buttercrunch}) \) during a 10-day study. NS, non-significant; significant at * \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.001 \) (\( n = 3 \)).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>( \text{CA} )</th>
<th>DPR</th>
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<tbody>
<tr>
<td>Pressure (P)</td>
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<td>*</td>
</tr>
<tr>
<td>Ethylene (E)</td>
<td>***</td>
<td>***</td>
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<tr>
<td>Duration (D)</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>( P \times E )</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>( P \times D )</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>( E \times D )</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>( P \times E \times D )</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

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**Fig. 3.** Experiment 1: day 10, effect of endogenously produced ethylene or scrubbed at 101/21 and 25/12 kPa pO\(_2\) on (A) \( \text{CA} \) (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \)), (B) DPR (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \)) and (C) \( \text{CA}/\text{DPR} \) ratio of lettuce. For all parameters tested: total pressure (Pres), \( P = \text{NS} \); ethylene, \( P = \text{NS} \); Pres \( \times \) ethylene, \( P = \text{NS} \). Error bars represent the SE (\( n = 3 \)).
both ambient and hypobaric plants (Fig. 6). While there was a significant ethylene effect, total pressure and the interaction of ethylene × pressure were non-significant.

**Experiment 3: effect of differential concentrations of exogenous ethylene during a short-term, dose exposure of 12–60 h on CA and DPR of lettuce**

There was a negative linear correlation between increasing exogenous ethylene concentrations (injected into chambers) and CA and DPR with plant exposure to ethylene at 12, 36 or 60 h (Figs 7 and 8). An ethylene effect occurred after 12 h of exposure and increased through the termination of the treatment (60 h). There was an ethylene reduction of CA at 0.2557, 0.438 and 0.6048 Pa min$^{-1}$ per chamber for ambient pressure and at 0.2369, 0.4475 and 0.6364 Pa min$^{-1}$ per chamber for low-pressure plants, respectively at 12, 36 and 60 h (Fig. 7). The effect of exogenous ethylene on DPR had a similar pattern, with a reduction of 0.0271, 0.0642 and 0.0971 Pa min$^{-1}$ for ambient pressure and at 0.0235, 0.0591 and 0.0787 Pa min$^{-1}$ for low-pressure plants at 12, 36 and 60 h, respectively (Fig. 8).

**Table 3.** Experiment 1: effect of total atmospheric pressure and ethylene [(C$_2$H$_4$), either scrubbed or endogenously produced] on leaf area, digital crop image, SLA, leaf, root and total plant DM, RGR [(ln total plant DM$^{-2}$ / ln total plant DM$^{-2}$) (time$^{-2}$ - time$^{-1}$)] and RWC [(fresh mass − DM) (saturated mass − DM) $^{-1}$] of lettuce (Lactuca sativa L cv. Buttercrunch). Values are means, with mean separation within columns by Duncan’s multiple comparison. Letter(s) within columns are not significantly different (p = 0.05). n = 3. NS, non-significant; significant at **P < 0.01; ***P < 0.001.

<table>
<thead>
<tr>
<th>Pressure (kPa)</th>
<th>C$_2$H$_4$ treatment</th>
<th>Leaf area (cm$^2$)</th>
<th>Digital crop image (megapixels)</th>
<th>SLA (cm$^2$ g$^{-1}$)</th>
<th>Leaf DM (g)</th>
<th>Root DM (g)</th>
<th>Total plant DM (g)</th>
<th>RGR (mg g$^{-1}$ day$^{-1}$)</th>
<th>RWC (%)</th>
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<td>56.4$^{a}$</td>
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<td>1.22$^{a}$</td>
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<td>56.6$^{a}$</td>
<td>7.42$^{a}$</td>
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<tr>
<td>C$_2$H$_4$</td>
<td>***</td>
<td>**</td>
<td>**</td>
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<td>***</td>
<td>NS</td>
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<tr>
<td>Pres × C$_2$H$_4$</td>
<td>NS</td>
<td>NS</td>
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**Fig. 4.** Experiment 2: effect of endogenously produced ethylene at 101/21 and 25/12 kPa pO$_2$ on (A) CA and (B) DPR of lettuce plants during a 10-day study. For both parameters tested: total pressure (Pres), P = NS; ethylene, P < 0.001; Pres × ethylene, P = NS (n = 2).

**Fig. 5.** Experiment 2: effect of endogenously produced ethylene at 101/21 and 25/12 kPa pO$_2$ on (A) digital crop image (megapixels) and (B) total leaf area of lettuce plants during a 10-day study. For both parameters tested: total pressure (Pres), P = NS; ethylene, P < 0.001; Pres × ethylene, P = NS (n = 2).
Discussion

To our knowledge, this is the first report to show the adverse effect of ethylene on CA and DPR, leading to reduced plant growth and development, under both hypobaric and ambient total pressure. There was a direct, negative effect of increasing ethylene concentration reducing gas exchange as well as an indirect ethylene effect on leaf epinasty, which reduced light capture and CA. The CA was more sensitive to increasing ethylene concentration than DPR. Hypobaria had no effect on endogenous ethylene accumulation, even though there was a non-significant trend of 16% lower ethylene with hypobaric than ambient pressure lettuce plants. This study further documents that lettuce can be grown under subambient pressure (≈25% of normal earth ambient total pressure) without adverse effect on plant growth and gas exchange. Regardless of ethylene levels, hypobaric plants had a lower DPR, but similar CA and plant growth and development, compared with ambient total pressure plants.

There was a negative linear correlation between increasing ethylene concentration and decreasing CA and DPR at relatively low concentrations of endogenous or exogenous ethylene during the 10-day studies. The CA of ambient and hypobaric plants was reduced at 200 nmol mol$^{-1}$ of ethylene [days 3–4], whereas DPR was reduced at 650–700 nmol mol$^{-1}$ of endogenously accumulating ethylene [days 7–9] (Fig. 2). Because of the very low volume change per unit time (low leak rates of the chambers), endogenous ethylene produced by plants rapidly accumulated to reach biologically significant levels (Abeles et al. 1992). The longer the lettuce plants were in the sealed chambers and the larger biomass

![Graphs and equations](https://example.com/graphs.png)

**Fig. 6.** Experiment 2: effect of endogenously produced ethylene at 101/21 and 25/12 kPa pO$_2$ on (A) total plant DM and (B) RGR (mg g$^{-1}$ day$^{-1}$) of lettuce during a 10-day study. For both parameters tested: total pressure (Pres), $P = NS$; ethylene, $P < 0.001$; Pres $\times$ ethylene, $P = NS$ ($n = 2$).

**Fig. 7.** Experiment 3: effect of exogenous ethylene on CA of lettuce plants at (A) 101/21 kPa pO$_2$ and (B) 25/12 kPa pO$_2$ at 12, 36 or 60 h exposure to an exogenous source of ethylene from 0 to 1 nmol mol$^{-1}$ $\times 10^3$ (ppb); total pressure (Pres), $P = NS$; duration, $P < 0.01$; Pres $\times$ duration, $P = NS$ ($n = 2$). See Table 4.

**Fig. 8.** Experiment 3: effect of exogenous ethylene on DPR of lettuce plants at (A) 101/21 kPa pO$_2$ and (B) 25/12 kPa pO$_2$ at 12, 36 or 60 h exposure to an exogenous source of ethylene from 0 to 1 nmol mol$^{-1}$ $\times 10^3$ (ppb); total pressure (Pres), $P = NS$; duration, $P < 0.01$; Pres $\times$ duration, $P = NS$ ($n = 2$). See Table 4.
produced, the greater ethylene biosynthesis and accumulation. Ethylene is produced by plants during normal growth and development and is usually released into the atmosphere without accumulating (Pierik et al. 2006). Martin and Sinnerwee (1987) grew wheat in a sealed soil-plant atmosphere system (Experimental Soil Plants Atmosphere System (ESPAS) chambers, Wageningen, the Netherlands). They reported that the ethylene concentration during the first 11 days accumulated to 100–200 nmol mol⁻¹, which was sufficient to affect plant growth. Ethylene levels of lettuce peaked with rapid plant growth at the time of harvest at NASA Biomass Production Chamber at 1.6–2.5 nmol m⁻² s⁻¹ (Wheeler et al. 2004). Spanarkel and Drew (2002) reported that ethylene concentration increased with time in both ambient and low-pressure (70 kPa) produced lettuce but was not affected by differences in total pressure, which agrees with our observations at ambient and low pressure (25 kPa).

Plants experiencing environmental stress frequently show enhanced production of ethylene (Morgan and Drew 1997). Increasing total gas pressure above ambient (hyperbaria) enhanced the ethylene biosynthetic pathway of maize seedlings (He et al. 1996). Elevated levels of ethylene have been implicated in spaceflight experiments at microgravity leading to adverse changes in plant growth and sterility (Bugbee 1999, Campbell et al. 2001, Levinskyk et al. 2000, Stutte 1999). Klassen and Bugbee (2004) reported that ethylene levels during the life cycle of plants was 100-fold greater in closed chambers than in outside environments. They studied the sensitivity of wheat and rice to low levels of atmospheric ethylene and found that significant variability in ethylene sensitivity between species and cultivars. We observed that lettuce cv. Buttercrunch was more sensitive to ethylene than wheat (He et al. 2003). When plants are grown in semi-closed chambers, accumulation of ethylene resulted in a smaller leaf area of ethylene-sensitive Arabidopsis compared with ethylene-insensitive, mutant plants; however, no difference in total leaf area was found when the plants were grown in well-ventilated conditions (Tholen et al. 2004). They concluded that there was little impact of endogenous levels of ethylene on the growth of non-stressed plants.

While hypobaric (25 kPa) plants had a non-significant, 16% lower ethylene than ambient lettuce plants – in an earlier study, He et al. (2003) reported 65% less ethylene with 30 kPa than 101 kPa lettuce. While low levels of oxygen (6 kPa pO2) (hypoxia) at ambient pressure inhibited ethylene, it was not as dramatic as hypobaria in reducing ethylene (He et al. 2003). Both the previous and the current studies were performed with ‘Buttercrunch’ lettuce – so why the difference in hypobaric effects on ethylene in the two studies? Some possible reasons were that the previous study was carried out in an earlier prototype LPPG system, with lower light (480 compared with the current 600 mol m⁻² s⁻¹), lower pO2 (6 vs 12 pO2) and four-fold lower pCO2 (24 vs 100 pCO2), leading to 12-fold lower ethylene (80 vs 1000 nmol mol⁻¹). The 2.4 pCO2 (24 ppm) was also approaching the compensation point of lettuce at lower light levels (Frantz et al. 2004). The decreased ethylene in the earlier study may have also been attributed to periodic purging inherent to the system in lowering the chamber pressure to maintain hypobaric conditions.

Burg and Burg (1966) reported that hypobaric storage reduced ethylene, which may be attributed to greater diffusion of ethylene out of leaf mesophyll cells and hypobaria maintaining cells more fully oxygenated, thus offsetting effects of low O₂. They hypothesized that hypobaria influence enzymes in ethylene biosynthesis by decreasing ethylene biosynthesis and binding. In hypobaric studies of Arabidopsis, there were no differential effects on gene expression of ACC synthase or ACC oxidase, which are critical for ethylene biosynthesis (Paul et al. 2004, Rao et al. 2006, Richards et al. 2006). It is also possible that the hypobaric storage effect is caused by low pO2 and not by reduced ethylene levels (Abeles et al. 1992). Hypobaria reduces endogenous ethylene levels within tissues via: (1) an immediate drop in partial pressures as gaseous phase materials vent from leaves in the process of establishing a new gas equilibrium, (2) reduction in ethylene biosynthesis because of reduced pO2 (which was not a factor with our research) and (3) increased diffusive evacuation from internal tissue air spaces to the external surrounding atmosphere (Abeles et al. 1992). Why was there a reduction of gas exchange by ethylene? Ethylene is generally associated with the breakdown of the photosynthetic machinery in the process of

<table>
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<th>C_A</th>
<th>DPR</th>
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<td>101</td>
<td>12</td>
<td>-0.256*</td>
<td>-0.027*</td>
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<td>36</td>
<td>0.438&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>Pres x duration</td>
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**Table 4.** Experiment 3: the effect of exogenous ethylene on the regression slopes of C_A rate, DPR after 12, 36 or 60 h of ethylene exposure. *Mean separation within columns by Duncan’s multiple comparison. Letter(s) within columns are not significantly different (p = 0.05). NS, non-significant; significant at **P < 0.01 (n = 2).**
senescence (Abeles et al. 1992). While most researchers have concluded that ethylene does not have a direct effect on photosynthesis (Abeles et al. 1992), there are reports that changes in photosynthesis are in response to altered stomatal and mesophyll effects (Khan 2004a, 2004b). Woodrow et al. (1988) reported that ethylene effects on photosynthetic carbon metabolism of tomato are indirect and not because of direct effects on photosynthetic processes per se. Woodrow et al. (1989) observed that ethylene-induced epinasty of Xanthium strumarium reduced light interception by the leaves of up to 60%. The speed of translocation of recently fixed carbon assimilate movement was not seriously impaired following ethephon treatment; however, within 24 h of ethephon treatment, the whole plant net carbon exchange rate expressed on a per plant basis or a leaf area basis had dropped by 35%. The apparent inhibition of net carbon exchange rate was reversed by physically repositioning the leaves with respect to the light source. Ethylene exposure also inhibited expansion of young leaves, which was partially reversed when the leaves were repositioned. Thus, ethylene indirectly affected net carbon gain and plant growth through modification of light interception (leaf epinasty) and altered sink demand without directly inhibiting leaf photosynthesis.

There is also evidence for a direct effect of ethylene on photosynthesis. Taylor and Gunderson (1988) reported that the high levels of ethylene (410 μl l−1 for 2 h) caused pronounced decline in C_A of Glycine max due more to a loss in the mesophyll tissue’s intrinsic capacity to assimilate CO_2 than to a reduction in stomatal conductance (g_s), per se. To overcome any ethylene-induced epinastic movement of leaves and change in orientation to light and subsequent reduction in C_A, leaves of G. max were positioned in a cuvette to prohibit any epinastic movement (Gunderson and Taylor 1991); ethylene-induced decline in C_A and g_s were evident within 2 h and led to declines of 80 and 62%, respectively, for C_A and g_s. While gas exchange in some species is insensitive to ethylene, their study suggests a biochemical basis for the ethylene response of gas exchange. The response mechanism has not been determined.

With our long-term, 10-day, study of lettuce, there was both an indirect and direct ethylene response to reduce plant gas exchange. Taylor and Gunderson (1991) exposed G. max to 10 nmol mol−1 of ethylene for a 5 h study and had dramatic reductions in gas exchange. We did not observe significant differences in C_A and DPR of lettuce plants until endogenously produced ethylene reached levels of around 200 and 700 nmol mol−1, respectively, for C_A and DPR (Fig. 2). Species differences, the very low-leakage rates of the LPPG system compared with conventional plant growth chambers could have accounted for some of these differences. Lettuce leaves in our study had an indirect response to ethylene with more upright and epinastic leaves, which potentially affected light capture and subsequent C_A. However, the LPPG system at 600 μmol m−2 s−1, with clear, acrylic chambers and considerable reflected side light would have minimized loss of light capture. With increasing ethylene concentrations, the epinastic orientation of leaves remained the same, but there was a dramatic decrease in gas exchange, suggesting a direct biochemical response to ethylene.

Why was DPR less affected by ethylene than C_A for both ambient and hypobaric plants? DPR is coupled to C_A and CO_2 levels were quite high during the dark period (up to 400 Pa – about 11.1-fold greater than normal air), while the 100 Pa pCO_2 set point during the light period was 2.8-fold higher than normal air (He et al. 2007); the higher CO_2 could lead to a suppression of ethylene, as occurs in controlled atmosphere storage (high pCO_2, low pO_2). High CO_2 is known to be a competitive inhibitor of ethylene action. The reduced sensitivity of DPR than C_A to ethylene may be because of the higher pCO_2 found during the dark period. Corey et al. (1996) reported that when total pressure of lettuce plants was reduced from 101 to 51 kPa, C_A increased 25%, while DPR decreased 40%; they suggested that there was a greater inhibition of photorespiration in reduced pO_2 at low pCO_2 – however, in our study, hypobaria had no effect on C_A but did reduce DPR. Richards et al. (2006) reported that hypobaria had no significant effect on differential expression of five key photorespiration enzymes, including RuBisCO; there was less than two-fold changes in regulation of targeted genes, suggesting no altered regulation within the photorespiratory pathway to hypobaria.

It has been reported that declines in both C_A and g_s shifted from first order (linear decline) to a zero order (saturation response) with increasing ethylene (Gunderson and Taylor 1988, 1991). They concluded that the response curves were similar to those documented for ethylene binding and for hormonally mediated ethylene actions. Our data indicated that declines in both C_A and DPR had a first order (linear decline) when ethylene concentrations in chambers were less than 1300 nmol mol−1 (Figs 4, 7 and 8). By differentially scrubbing endogenous ethylene (experiment 2) or applying various exogenous levels of ethylene (experiment 3), we were able to create an ethylene gradient. We also conducted a separate experiment (data not reported) with high concentrations of exogenous ethylene from 0 to 10 000 nmol mol−1 in a 3-day study and observed that C_A and DPR had a similar decline from 2000 to 10 000 nmol mol−1. This
indicated that the effects of ethylene on \( C_A \) and DPR were saturated at less than 2000 nmol mol\(^{-1}\).

Under ambient total pressure, Reuveni and Bugbee (1997) reported that high pCO\(_2\) of 260 Pa reduced \( C_A \) – 8% and DPR – 25% compared with 36 Pa CO\(_2\). In a study of *Arabidopsis* gene expression at low atmospheric pressure, less than one-half of the 200 genes dramatically upregulated or downregulated by hypobaria were similarly affected by hypoxia, suggesting that the response to hypobaria is unique and more complex than an adaptation to reduced pO\(_2\) (hypoxia) inherent to hypobaric environments, i.e., hypobaria does not equal hypoxia (Paul et al. 2004). Corey et al. (1996) also reported that 51 kPa, lettuce plants had 25% greater \( C_A \) and 40% greater DPR than 101 kPa plants; there was a greater effect at 41 CO\(_2\) than 81 Pa pCO\(_2\) – which was consistent with reports showing greater inhibition of photorespiration in reduced pO\(_2\) at low pCO\(_2\). In our research, all plants were grown under elevated (non-limiting) CO\(_2\) levels of 100 Pa pCO\(_2\) (1000 \( \mu \)l l\(^{-1}\)CO\(_2\) at ambient total pressure) – and while DPR was reduced at 25 kPa (hypobaria), the \( C_A \) was the same with the ambient.

Ethylene can cause Chl degradation, which may be the result of ethylene-induced aging rather than a direct ethylene effect (Abeles et al. 1992). Ethylene can also cause leaf abscission without the loss of Chl (Woltering et al. 1993). While we were unable to take measurements of Chl during this study, we did not observe any symptoms of chlorosis among treatments. In two previous studies, increasing ethylene (high of 75 nmol mol\(^{-1}\)) reduced leaf Chl of lettuce (He et al. 2003), and 25 kPa plants had higher Chl levels than 101 kPa lettuce plants from 6 to 21 kPa pO\(_2\) (He et al. 2007). Hypobaria is reported to enhance Chl retention (Burg 2004).

Ethylene reduced all growth parameters, including RGR (mg g\(^{-1}\) day\(^{-1}\)). The SLA was also reduced, indicating ethylene-induced thicker leaves, with reduced surface area. Lower SLA is a common response to abiotic and biotic stress (He et al. 2007), which can lead to reduced overall crop \( C_A \). The RGR is also correlated with SLA. There was an ethylene epinastic response, with leaves having a more upright, vertical orientation. Clearly, the ethylene-induced reduction in gas exchange had a negative impact on lettuce yield. We observed comparable vegetative growth and healthy roots between ambient and low-pressure plants when ethylene was scrubbed. While this research demonstrates that lettuce can be successfully grown under low pressure, hypobaria had no prophylactic effect on ethylene production.

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