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Effect of hypobaric conditions on ethylene evolution and growth of lettuce and wheat

Chuanjin He[1], Fred T. Davies Jr.[1] *, Ronald E. Lacey[2], Malcolm C. Drew[1], Denise L. Brown[2]

[1] Department of Horticultural Sciences, Texas A&M University, College Station, Texas 77843-2133, USA
[2] Department of Biological and Agricultural Engineering, Texas A&M University, College Station, Texas 77843-2117, USA

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Summary

Elevated levels of ethylene occur in enclosed crop production systems and in spaceflight environments, leading to adverse plant growth and sterility. There are engineering advantages in growing plants at hypobaric (reduced atmospheric pressure) conditions in biomass production for extraterrestrial base or spaceflight environments. Objectives of this research were to characterize the influence of hypobaria on growth and ethylene evolution of lettuce (*Lactuca sativa*) and wheat (*Triticum aestivum*). Plants were grown under variable total gas pressures [from 30 to 101 kPa (ambient)]. In one study, lettuce and wheat were direct seeded, germinated and grown in the same chambers for 28 d at 50 or 101 kPa. Hypobaria increased plant growth and did not alter germination rate. During a 10-day study, 28-day-old lettuce and 40-day-old wheat seedlings were transplanted together in the same low and ambient pressure chambers; ethylene accumulated in the chambers, but the rate of production by both lettuce and wheat was reduced more than 65 % under 30 kPa compared with ambient pressure (101 kPa). Low O_2 concentrations [partial pressure of O_2 (pO_2) = 6.2 kPa] inhibited ethylene production by lettuce under both low (30 kPa) and ambient pressure, whereas ethylene production by wheat was inhibited at low pressure but not low O_2 concentration. There was a negative linear correlation between increasing ethylene concentration and decreasing chlorophyll content of lettuce and wheat. Lettuce had higher production of ethylene and showed greater sensitivity to ethylene than wheat. The hypobaric effect on reduced ethylene production was greater than that of just hypoxia (low oxygen).

Key words: hypobaria – hypoxia – *Lactuca sativa* – low pressure – *Triticum aestivum*

Abbreviations: DM = dry mass. – g_s = stomatal conductance. – LAR = leaf area ratio. – Pn = net photosynthesis. – R = gas constant. – T = temperature

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* E-mail corresponding author: f-davies@tamu.edu

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Introduction

The exploration of space will require the development of Advanced Life Support Systems (ALS) that will have the capacity to recycle resources and produce food (Wheeler et al. 2001). Such life support systems will likely combine biotechnology and physicochemical processes for air and water recycling. The biological component will include the use of higher plants for air and water purification, as well as providing food and psychological benefits (NASA 1998). The National Aeronautics and Space Administration (NASA) has a research and development effort to build such systems as part of the NASA Advanced Life Support System Program (Corey et al. 1997, Schwartzkopf and Mancinelli 1991), and Lunar Base Agriculture (Ming and Henninger 1989).

Plants grown in space-flight environments will be subject not only to microgravity, but also to the conditions designed mainly to fulfill requirements for the human environment (Wheeler et al. 2001). The International Space Station is expected to have CO_2 concentrations in its atmosphere that are many times in excess of those on Earth. The potential range of environmental conditions in an Advanced Life Support System include total gas pressures as low as 60 kPa, and CO_2 as high as 700 Pa (7000 nmol mol^{-1}) some 20 times greater than on Earth (NASA 1998). Plants can tolerate wide variation in concentration of the essential gases O_2, and CO_2, relative humidity, and possibly also, total gas pressure (Spanarkel and Drew 2002).

Important environmental variables that have received little research effort in relation to an Advanced Life Support System are: (i) total atmospheric gas pressure, (ii) high CO_2 partial pressure, up to 70 Pa or higher, greatly in excess of the range currently studied by researchers interested in global change (Amthor 1991), (iii) wide differences in humidity and (iv) effects of trace gases – including ethylene under low pressure conditions.

There are several important advantages, from an engineering viewpoint, associated with growing plants at hypobaric conditions in biomass production in extraterrestrial base or space-flight environments:

- A reduced pressure differential between the plant growth facility and its external environment would permit less massive materials to be used in its construction. This would further reduce the payload volume and mass required for deployment.
- A hypobaric condition would reduce atmospheric leakage from the facility to its near 0 kPa external environment.
- Hypobaric conditions in the plant growth facility would require less N_2 gas to be transported or obtained *in situ* to supplement the physiologically active gases (CO_2 and O_2).

A number of earlier studies have demonstrated clearly that plant germination and seedling growth are possible at hypobaric conditions (Andre and Massimino 1992, Corey et al. 2002, Costes and Vartepetian 1978, Gale 1973, Goto et al. 2002, Musgrave and Strain 1988, Schwartzkopf and Mancinelli 1991, Spanarkel and Drew 2002). It is also a common observation that plants can be grown at high altitude, where pressures are well below 100 kPa (Davies et al. 2002), although the invariable association between increasing altitude and decreasing temperature confounds the issue of the effect of pressure alone. For instance, potatoes are grown worldwide under a wider range of altitude, latitude, and climatic conditions than any other major food crop-from sea level to over 4000 m elevation, with a total gas pressure of 61 kPa (Davies et al. 2002). The question here is whether the rate of germination, vegetative growth and morphogenesis, final yield and seed viability, compare closely with those at ambient pressure.

A major potential limitation to plant growth at a hypobaric condition is the partial pressure of O_2 (pO_2) which can become limiting to oxidative phosphorylation (Drew 1997). Seedlings germinated and grew during a seven-day test at 6 kPa total gas pressure provided the atmosphere was comprised predominately of oxygen (pO_2 = 5 kPa; ≅ 83 % O_2); but at lower pressures and therefore also less O_2, seeds failed to germinate (Schwartzkopf and Mancinelli 1991). Other studies also found that low total pressure (21–24 kPa) did not inhibit seed germination and initial growth as long as pO_2 was 5 kPa or more (Musgrave and Strain 1988).

Hypobaric environments are associated with hypoxia (low O_2) condition, particularly when total gas pressure is reduced below 50 kPa. Hence the need to supply sufficient partial pressures of O_2 to avoid hypoxia under hypobaric conditions. However, NASA also has safety concerns since fire can be more easily propagated in enclosed environments with oxygen enriched atmospheres above 23.5 % (U.S. Code of Federal Regulations 1999, Title 29, Part XVII, Section 1910.146). However, Goto et al. (2002) grew *Arabidopsis* at total pressure of 23 kPa with partial pressure O_2 of 21 kPa in a hypobaric chamber equipped with a heater and other electronic devices without problems. Safety may be a concern since oxygen levels exceeded 91 % (vs. 21 % in normal air) under these hypobaric conditions.

Plants can respond adversely to low ethylene levels (Klassen and Bugbee 2002). Elevated levels of ethylene have been reported in enclosed environments (Abeles et al. 1992) and implicated in microgravity – spaceflight experiments (Wheeler et al. 1996). Martin and Sinnaeve (1987) reported that wheat grew poorly in an ambient (101 kPa) chamber with ethylene at 100–200 nmol mol^{-1} (ppb). When the ethylene was removed by an activated alumina column, the plants grew normally. Chronic exposure to ethylene levels of 50 to 100 nmol mol^{-1} reduced growth of lettuce and Easter lilies (Wheeler et al. 1996). On the Russian space station, Mir, ethylene ranged from 1000 to 1700 nmol mol^{-1} [about 1000 × to 1700 × greater than ethylene in terrestrial agricultural, open field conditions] leading to abnormal growth and sterility (Campbell et al. 2001); ethylene levels are around 50 nmol mol^{-1} on the international space station (ISS). This has led to adverse changes in plant growth and sterility (Bugbee 1999, Campbell et al. 2001, Levinskikh et al. 2000, Stutte 1999, Wheeler et al. 1996). Under hypoxia (low oxygen) one would expect that higher (rather than lower) levels of ethylene occur (Drew 1997). It would be profoundly interesting to characterize ethylene levels and other volatile organic compounds (Stutte 1999), particularly with the expected differences in gas diffusion rates at low pressure.

Hence, the objectives of this research were to characterize the influence of hypobaric conditions on growth and ethylene evolution of lettuce (*Lactuca sativa*) and wheat (*Triticum aestivum*).

Materials and Methods

Plant growth condition and plant growth system

Lettuce (*Lactuca sativa* L. cv. Buttercrunch) or wheat (*Triticum aestivum* L. cv. USU-Apogee) were germinated in 2.5 L-volume plastic pots which were filled to within 1 cm of the top with fine-grade calcined clay (particle size less than 1 mm). The calcined clay was pre-washed with deionized water and allowed to drain before sowing. After the seeds were germinated, plants were supplied with a modified Hoagland's nutrient solution containing 2.0 mmol/L KNO_3, 3.0 mmol/L $Ca(NO_3)_2$, 1.0 mmol/L $NH_4H_2PO_4/(NH_4)_2HPO_4$, 0.5 mmol/L $MgSO_4$, 0.1 mmol/L Fe as Fe-EDTA, micronutrients and adjusted to a pH of 6.3. Nutrient solution (150 mL/day) was supplied to plants in pressure chambers by a peristaltic pump (Cole Parmer, Chicago, Il, USA, model No. 7016) located outside the chambers. This solution flowed through a valve at the top of each chamber into the middle of the pot about 2 cm deep below the surface of the calcined clay.

Seeds were germinated and seedlings grown in pots (a single lettuce plant or groups of three plants for wheat) of calcined clay at normal atmospheric pressure (101 kPa) in controlled growth chamber for 28 d (lettuce) or 40 d (wheat). Uniform plants were selected and then transferred with their pots to pressure chambers for low pressure experiments. The pressure was lowered by vacuum pump to 70, 50 or 30 kPa. For germination studies under low pressure, 22 lettuce seeds and 20 wheat seeds were direct seeded and grown under low (50 kPa) and ambient pressure (101 kPa) for 28 days.

The low pressure plant growth system consisted of two transparent, polyacrylic (Plexiglas) cylindrical chambers. Each chamber was 89 cm high, 29 cm in inner diameter and 6 mm in wall thickness, with a total volume of 59.5 L. The chambers were modified following the system described by Spanarkel and Drew (2002). The chambers were placed inside a controlled environment room (area 2 m × 3 m) which regulated light, day length, and temperature. Lighting was approximately 480 $\mu mol\ m^{-2}\ s^{-1}$ at canopy level inside the pressure chambers provided by fluorescent (Philips Lighting Co., Somerset, NJ, USA, Model No. CW1500) and incandescent lamps of the controlled environment room (EGC, Chagrin Falls, OH, USA). Environmental conditions also included a 13 h/11 h light/dark phase and a maximum/minimum temperature of 25/20 °C. All experiments were run in tandem with a control (normal air pressure) – so the gaseous atmosphere in one chamber was maintained at low pressure (30, 50 or 70 kPa) while the control chamber was maintained at 101 kPa.

Gas control and chamber volume change per unit time

Measurement of internal vessel pressure was accomplished using a strain-gauge-based pressure transducer (Ashcroft K-2, Dresser Instruments, Addison, TX) with an accuracy of 1 % F.S (Brown 2002). The pressure transducers output was a 4–20 mA signal that was directly proportional to the pressure inside each vessel. The current signal was converted to a voltage and amplified using a series of operational amplifier circuits. The converted signal was then recorded by a PC and an NI-PCI-605XE data acquisition card (National Instruments, Austin, TX). Total pressure in the two vessels was adjusted by either adding compressed air from a gas cylinder or using a vacuum pump to evacuate air from the vessels. The flow of compressed air into the vessels was controlled by a mass flow controller (DFC2600, Aalborg, Orangeburg, NY). LabVIEW software running on a Dell Precision Workstation (Austin, TX) controlled and recorded pressure in each vessel and recorded internal vessel temperature. Temperature was measured using a thermistor circuit. The circuit output was linear over the range of temperatures.

The average leak rate of the low pressure vessel for an initial pressure of 25 kPa was 1.5 % chamber volumes per day (Purswell 2002). The leak rate was very low compared to other low pressure systems. The Perfect Gas Law ($PV = nRT$) was used to determine the pressure vessel leak rate over a 24-h period. The mass of gas lost was converted to volume removed using the Perfect Gas Law and the known values for temperature and change in pressure. [See the materials and methods section on purge rate of low pressure vessels for greater detail].

Gas composition at reduced pressures

The first set of experiments were designed to characterize how lettuce and wheat responded to lower total gas pressure with the ratio of partial pressure of N_2 (pN_2) and O_2 (pO_2) remaining constant with the earth's atmosphere at 110 m above sea-level (ambient at College Station, TX, USA). The partial pressure of CO_2 (pCO_2) at our growth facility under ambient conditions was 81 Pa (810 $\mu mol\ mol^{-1}$ [ppm]) and was reduced with total pressure accordingly. Hence, all gas ratios were constant at reduced pressure, but not controlled independently with changes in total pressure. The partial pressures of N_2, O_2 and CO_2 at the various test pressures are given below. They were based on atmospheric composition, total pressure and the Perfect Gas Law.

Total gas pressure (kPa)	Partial pressure N_2 (kPa)	Partial pressure O_2 (kPa)	Partial pressure CO_2 (Pa)
101	80.02	20.9	81
70	55.44	14.5	56
50	39.66	10.3	40
30	23.78	6.2	24

Hypoxic (low O_2) effect on ethylene evolution

To evaluate hypoxic effect on ethylene production under ambient pressure, uniform 28-day-old lettuce (1 seedling per pot) and 40-day-old wheat (3 seedlings per pot) were selected and transferred with their pots into 4 sealed glass jars (15.5 L volume). Jars were lubricated with high vacuum silicon at the top edges and covered by glass plates. The glass plate had inlet and outlet lines for gas passage. Air {20.9 % O_2 [v/v] or pO_2 of 20.9 kPa} and pre-purified N_2 gas were mixed from pressurized cylinders and regulated by electronic mass flow controllers (Scott Specialty Gases, Model 5878) to give an O_2 concentration of 6.2 % (v/v), which was equivalent to 6.2 kPa pO_2 at 30 kPa total gas pressure. The mixed gases were then passed into the glass jars from the inlet line at the top leading to the bottom at a flow rate of 800 mL min^{-1} per jar. The mixed air was expelled from the jars with an outlet line at the top of each jar. For ambient O_2 level (pO_2 of 20.9 kPa), plants received air from the pressurized cylinder passed from the top of the jar to bottom at the same flow rate as the hypoxic treatment. Ethylene evolution was measured at 24 h, 48 h, or 72 h after treatment. Both inlet and outlet lines from the jar were closed for 1-h incubation, gas was drawn from each jar and injected into a GC for ethylene determination.

Ethylene determination of cylindrical chambers

Atmospheric samples for ethylene analysis were drawn manually from a valve at the top of each cylinder. There was no significant change in total atmospheric pressure in either chamber during gas sampling. Ethylene concentration was measured by withdrawing gas from chambers at 11:00 (3 h after the start of the light period) every other day beginning at the first day of each experiment. A 1-mL volume of gas was withdrawn using a syringe and injected into a digital gas chromatograph (Photovac 10 plus, PerkinElmer, Inc., Norwark, Conn) with a photo detector and compressed air (ultra zero grade, Praxair Inc.) as carrier gas. The ethylene concentration in the hypobaric chamber was calculated according to the Perfect Gas Law: $PV = nRT$; where P = pressure, T = temperature, n = moles, R = gas constant and T = temperature.

Purge Rate in low pressure vessels

In order to limit ethylene buildup in the vessels, the system was set to purge a small amount of gas from one or both vessels in 30 minute intervals during some tests. Gas was pulled out of the low pressure vessel, and pressure and temperature before and after gas removal were recorded. If both vessels were flushed for a particular experiment, these values were used to determine the mass percent of vessel atmosphere removed from the low pressure vessel. Then the same percent of gas based on mass was removed from the ambient pressure vessel. Pressure and temperature before and after gas removal were recorded for the ambient pressure vessel as well.

The amount of gas removed from each chamber was determined using the Perfect Gas Law (PV = nRT). Mass of gas removed equaled moles of gas removed over molecular weight of the gas (in this case, air, which has an MW of approximately 29 g/mol). Therefore, the moles of gas removed from each chamber equaled $P_iV/(RT_i) - P_fV/(RT_f)$ and the mass of gas removed equaled $n_{removed}/MW_{air}$. The percentage of gas removed is $n_{removed}/(P_iV/(RT_i))$. If $T_i = T_f$, the percentage of gas removed by mass simplifies to $(P_i - P_o)/P_i \times 100\,\%$.

The mass of gas removed was converted to volume removed using the Perfect Gas Law and the known values for temperature and change in pressure. The average volume purged from the vessels was 2.4 % vessel volume every 30 minutes.

Net photosynthesis, stomatal conductance, chlorophyll, leaf area and plant dry mass

Net photosynthesis (*Pn*) and stomatal conductance (g_s) were measured by LiCOR LI-6400 (Lincoln, Nebraska) immediately before and after the treatment of plants in chambers. The Li-COR 6400 was programmed with constant leaf chamber block temperature at 25 °C; a fixed 360 $\mu L\,L^{-1}$ CO_2 was provided from a 12-gram cartridge; and the light source was an LED 6400 R/B at 300 $\mu mol\,m^{-2}\,s^{-1}$.

Chlorophyll was measured with a Spad 501 (Minolta Camera Co. LTD, Japan) immediately before and after plants were incubated in chambers. The content of total chlorophyll was determined by extinction coefficients with N,N-dimethylformamide extraction (Inskeep and Bloom 1985, Davies et al. 2001). Leaf area was measured by LI-3000 portable area meter (LiCOR, Lincoln, Neb.). Plant dry mass was determined by the method described by Davies et al. (2000).

Because of the size of the low pressure cylinders, only two could be accommodated within the controlled environment room. Replication was achieved by repeating experiments under the same conditions over time, with one chamber at low and the other at ambient pressure as a control. All reported data were pooled from repeated, independent experiments. It was not possible to have direct access to plants during the experiments, so measurements of net photosynthesis (*Pn*) and stomatal conductance (g_s) were completed within 10 min after plants were removed from the chambers under ambient total air pressure and oxygen concentration; during this brief period a steady state of *Pn* and g_s was maintained. Chlorophyll, *Pn* and g_s were measured only at the very beginning and immediately at termination of an experiment.

Statistical tests of the significance of differences between treatments were based on ANOVA and mean separation by Duncan's multiple range test ($P < 0.05$) or standard error (±SE).

Results

Long term direct seeding under hypobaric, purged environments

Lettuce and wheat seeds were direct seeded, germinated and grown in the same chambers for 28 days at 50 kPa (purged) or 101 kPa (non-purged). Treatments were evaluated for germination rates, plant growth, and ethylene evolution. Increased growth occurred with both direct-seeded species at 50 kPa (Fig. 1). Dry mass and leaf area of lettuce increased 32 % and 124 %, respectively, at 50 kPa compared to ambient pressure (Table 1). There were no differences in germination rate (90–91 %) between treatments for either species (Table 1). Low pressure increased dry mass and leaf area of wheat, respectively, 8 % and 3 % compared to plants at ambient pressure.

Low pressure enhancement of lettuce growth was more dramatic than with wheat at 50 kPa (Fig. 1). Concentrations of ethylene were 109.8 vs. 6.2 nmol mol^{-1}, respectively, under ambient and hypobaric conditions (Table 1). Essentially a 94 % reduction of ethylene occurred under purged, hypobaric conditions (Table 1). Further experiments were therefore designed to distinguish the effects of purging under low and

Table 1. The effect of hypobaric conditions and purging on dry mass, leaf area, germination rate (GR) and ethylene evolution of wheat and lettuce.

Pressure kPa	Ethylene (nmol mol^{-1})	Lettuce[1] Dry Mass (g)	Lettuce[1] Leaf Area (cm^2)	Lettuce[1] GR (%)	Wheat[1] Dry Mass (g)	Wheat[1] Leaf Area (cm^2)	Wheat[1] GR (%)
101[2]	109.8	0.151	58.2	91	3.01	229.6	90
50[3]	6.2	0.20	130.5	91	3.26	236.3	90
HE[4]	−94 %	+32 %	+124 %	0 %	+8 %	+3 %	0 %

[1] Plants were 28-days after their direct seeding. Ethylene concentration in chambers was measured at the end of the experiment; lettuce and wheat were germinated and grown in the same chambers.

[2] Ambient chamber was non-purged; n = 1.

[3] Hypobaric chamber was purged at 2.4% of vessel volume every 30 min to lower ethylene concentration and other volatiles; n = 1.

[4] HE (Hypobaric Effect) = [(Hypobaric – Ambient)/Ambient] × 100%.

ambient pressure, and of hypoxia in the regulation of ethylene production.

Ethylene accumulation in chambers decreased under hypobaric and hypoxic conditions

During a 10-day study containing transplanted 28-day-old lettuce or 40-day-old wheat seedlings in sealed chambers, ethylene gradually accumulated (Fig. 2). However, there was a 70 % reduction in final ethylene concentration in the atmosphere in non-purged chambers under 30 kPa total pressure (hypobaria) with partial pressure pO_2 of 6.2 kPa (hypoxic) compared to non-purged, ambient plant chambers (Fig. 2).

Table 2. The effect of hypoxia (low O_2) on ethylene evolution of lettuce and wheat under ambient pressure (101 kPa) without purging.

Oxygen (% v/v)	Ethylene Evolution (nmol mol^{-1} g^{-1} DM h^{-1}) Lettuce[1]	Ethylene Evolution (nmol mol^{-1} g^{-1} DM h^{-1}) Wheat[1]
6.2	16.5 b[3]	9.8 a
20.9	19.7 a	10.7 a
Hypoxic Effect[2]	−16.2 %	−8.4 %
Pr > F	0.0004	NS[4]

[1] Lettuce and wheat seedlings were 28- and 40 d-old, respectively at time of treatment initiation; treatments were terminated after 72 h and dry mass was determined.

[2] Hypoxic effect = {[ethylene (6.2 kPa O_2) – ethylene (21 kPa O_2)]/ ethylene (21 kPa O_2)} × 100%.

[3] Mean separation by Duncan's multiple range tests ($p \leq 0.05$); Means with the same letter in the same column are not significantly different.

[4] NS = nonsignificant; n = 3.

Table 3. Hypobaric effect on dry mass, chlorophyll content (Chl), and ethylene evolution of lettuce and wheat.

	Lettuce[1] Dry Mass	Lettuce[1] Chl	Lettuce[1] Ethylene	Wheat[1] Dry Mass	Wheat[1] Chl	Wheat[1] Ethylene
30 kPa[2]	8.74	30.9	31.5	19.79	46.5	29.7
30 kPa[3]	10.8	34.7	10.1	20.72	47.4	10.9
101 kPa[2]	8.29	25.4	76.8	18.23	45.4	67.0
101 kPa[3]	10.1	32.9	19.2	20.42	47.2	22.1
HE (%)[4]	+6.2	+12.5	-56.6	+4.8	+1.4	−54.4
PE (%)[5]	+22.7	+20.1	-72.9	+8.2	+2.9	−65.9

[1] Lettuce (28-day old) and wheat (40-day old) seedling transplants were studied for 10 days under 30 kPa (hypobaric) and 101 kPa (ambient). Treatments were evaluated by dry mass (g), chlorophyll content (μg cm^2) and ethylene evolution (nmol mol^{-1}).

[2] Chambers were non-purged.

[3] Chambers were purged at a rate 2.4% of total volume every 30 min; n = 1.

[4] HE (hypobaric effect) = {[30 kPa (purge + non-purge) – 101 kPa (purge + non-purge)]/101 kPa (purge + non-purge)} × 100 %.

[5] PE (Purge Effect) = {[purged (30 kPa + 101 kPa) – non-purge (30 kPa + 101 kPa)]/non-purge (30 kPa + 101 kPa)} × 100%.

Purging the chambers greatly reduced ethylene accumulation with lettuce or wheat grown under hypobaric or ambient conditions (Fig. 2). However, greatest reduction occurred in purged, low-pressure chambers.

Another experiment was conducted at ambient total pressure to test the effect of low oxygen (pO2 of 6.2 kPa) and ambient (pO2 of 20.9 kPa) pressure on ethylene evolution. At ambient pressure, there was a significant 16.2 % reduction in ethylene production of lettuce seedlings under hypoxia, and an 8.4 % reduction in wheat, which was not significant (Table 2).

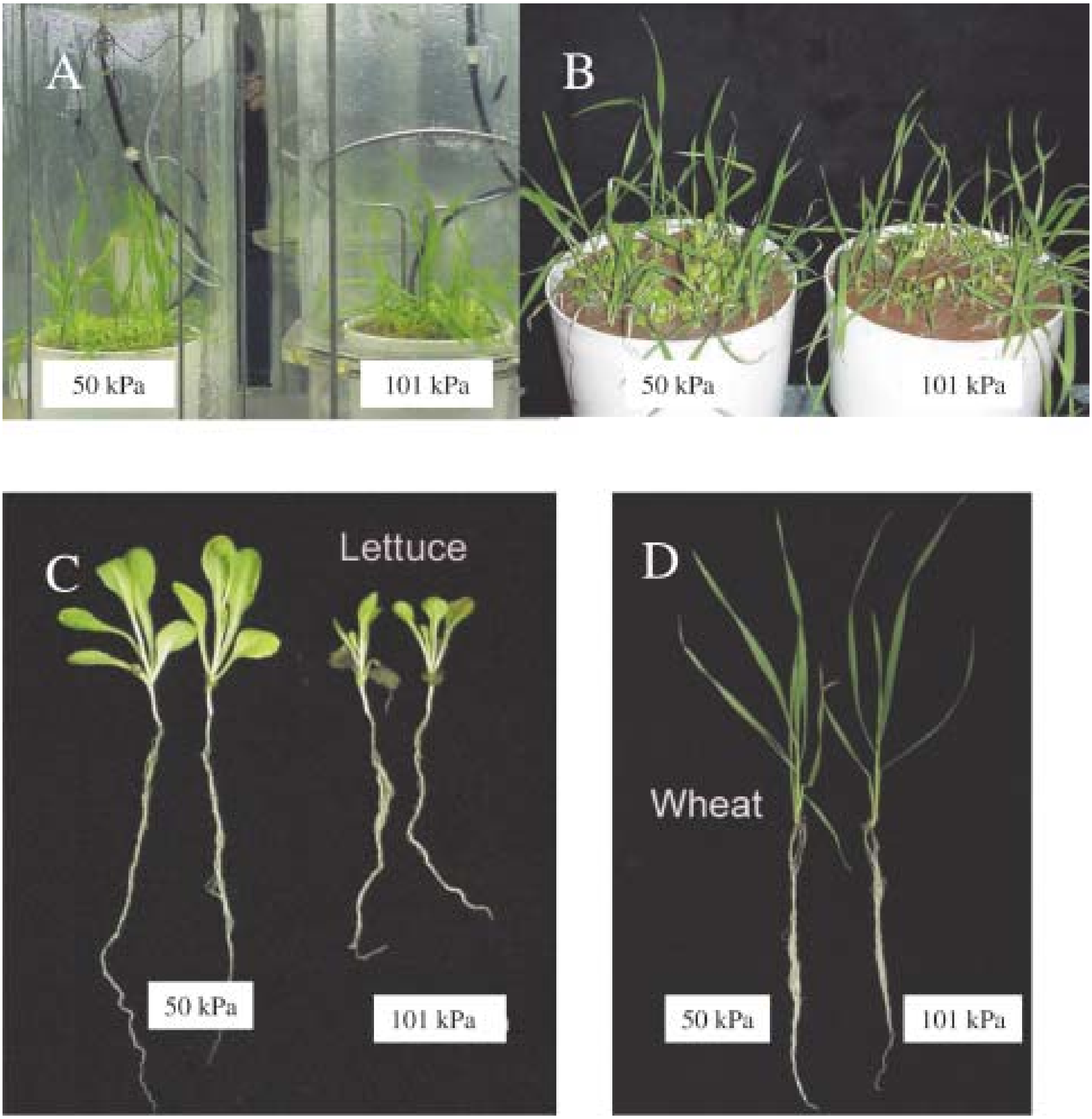

Figure 1. (A) Growth of lettuce and wheat plants 28 days after their direct seeding into non-purged, ambient (101 kPa) and purged, low pressure (50 kPa) chambers. (B) Growth was more optimal at 50 kPa than ambient. (C) Lettuce was more sensitive than (D) wheat to ethylene and had greater growth in 50 kPa than ambient chambers; n = 1.

Hypobaric and/or purging effects on chlorophyll, ethylene and dry mass of lettuce and wheat

Dry mass, chlorophyll and final concentration of ethylene were measured under hypobaric (30 kPa) and ambient (101 kPa) conditions with or without purging chambers for lettuce (28-day-old at transplanting) and wheat (40-day-old at transplanting) during a 10-day study. Values for dry mass accumulation and chlorophyll tended to be highest at 30 kPa with purging compared to 101 kPa without purging chambers for both lettuce and wheat (Table 3). Under non-purged conditions, dry mass and chlorophyll content of lettuce were higher under hypobaric than ambient pressure (Table 3). Hypobaric effects enhanced dry mass (+6.2 %), chlorophyll

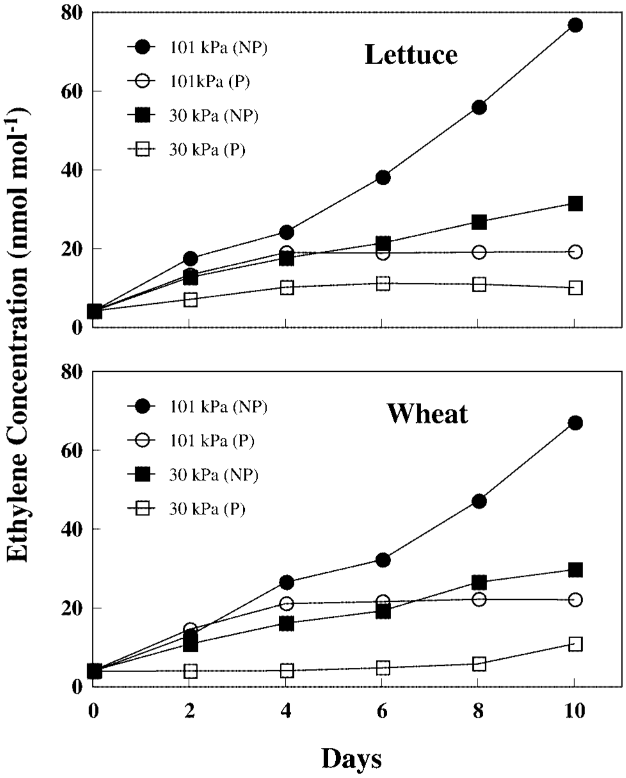

Figure 2. Ethylene concentration of lettuce and wheat during a 10-day study under 30 kPa (low) and 101 kPa (ambient) total gas pressure with purged (P) and non-purged (NP) chambers; seedling transplants of lettuce and wheat were, respectively, 28- and 40-day-old at time of transplanting; n = 1.

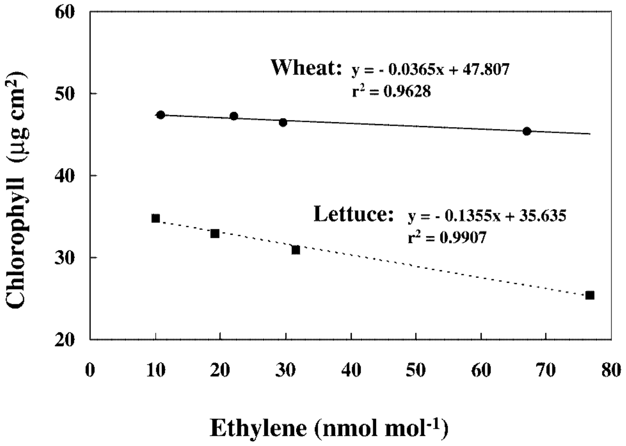

Figure 3. Correlation between chamber ethylene concentration and leaf chlorophyll in lettuce and wheat during a 10-day study; seedling transplants of lettuce and wheat were, respectively, 28- and 40-day-old at time of transplanting; n = 4.

content (+12.5 %), and reduced ethylene (56.6 %) in lettuce, regardless if the system was purged or not (Table 3). With lettuce the purging effect on the system was greater than the hypobaric effect. Purging led to increased dry mass (+22.7 %), chlorophyll content (+20.1 %) and a 72.9 % reduction in ethylene regardless of total gas pressure (Table 3). In wheat, the purging effect was also greater than the hypobaric effect, however percent differences in dry mass, chlorophyll and ethylene production were lower (Table 3).

There was a negative linear correlation between increasing final ethylene concentration in chambers vs. decreasing chlorophyll content (Fig. 3). The slope for chlorophyll vs. ethylene was more negative for lettuce (−0.1355) than wheat (−0.0365). Hence, lettuce had greater sensitivity to ethylene than that of wheat (Fig. 3).

Discussion

To our knowledge, this is the first report that hypobaric environments per se reduce ethylene evolution of lettuce and wheat (Fig. 2), and that lettuce is more sensitive to ethylene than wheat in sealed microenvironments (Figs. 1 and 3). Hypobaria increased plant growth and did not alter germination rate (Table 1). This research shows that plants can be successfully grown at hypobaric (as low as 30 kPa total gas pressure) and hypoxic (pO_2 = 6.2 kPa O_2) environments. Hypobaric conditions subsequently reduced the adverse effect of ethylene on plant growth. We found that the hypobaric (low pressure) effect on ethylene production was greater than that of just hypoxia (low oxygen).

Hypobaric conditions had no adverse effects on germination rate, net photosynthesis and stomatal conductance on lettuce and wheat

Wheat and lettuce are two plant species that have high potential of being included in the Advanced Life Support System for NASA (Bugbee and Salisbury 1989 a, b, Campbell et al. 2001, Wheeler et al. 2001). The hypobaric growth system originally designed in 1994, was initially limited to 70 kPa for the hypobaric chamber (Spanarkel and Drew 2002). The chambers in our research were subsequently modified to operate at variable hypobaric conditions less than 30 kPa with very low volume change per unit time, i.e. the average leak rate of the low pressure vessel for an initial pressure of 25 kPa was 1.5 % of the chamber volume per day, which is very low compared to other low pressure systems (Purswell 2002).

Iwabuchi et al. (1996) reported that there were no differences in net CO_2 assimilation and transpiration rate of spinach under three different total gas pressures and O_2 partial pressure conditions [total gas pressure/partial O_2 pressures of 101 kPa/21 kPa, 25 kPa/21 kPa and 25 kPa/10 kPa] during a 31-day study. However, Corey et al. (1997) observed that in lettuce the rate of *Pn* at reduced pressure was 14.6 % higher than at ambient pressure, and that decreased pO_2 resulted in enhanced rate of *Pn*, regardless of pressure.

We observed no differences in net photosynthesis or stomatal conductance of lettuce and wheat plants at 30, 50 or 70 kPa compared with ambient pressure (101 kPa) [data not reported]. However, our Li-COR 6400 measurements were taken outside the chambers under ambient conditions on individual leaves (not whole plant canopy). Photosynthesis and stomatal conductance are major components in plant growth. The increased yield under hypobaria was more likely due to reduction of ethylene production than to differences in plant gas exchange. Our results agree with Spanarkel and Drew (2002) who reported that lettuce under 70 kPa was normal in appearance, turgidity and quality, dry mass, and that photosynthesis was unaffected compared with ambient pressure. They maintained the same partial pressures of O_2 (21 kPa) and CO_2 (66.5–73.5 Pa) during both ambient and hypobaric conditions. However, in our study the ambient CO_2 in the growth room and chambers was 81 Pa (twice more than normal air); the total gas pressures of N_2, O_2 and CO_2 were then reduced proportionally under hyperbaria following the Perfect Gas Law.

Vegetative growth of lettuce and wheat was affected by accumulated ethylene in sealed plant growth chambers

The role of the gaseous plant hormone, ethylene, in regulating aspects of plant development and plant responses to the environment is well established (Abeles et al. 1992, Campbell et al. 2001, Morgan & Drew 1997). Ethylene, in coordination with other plant hormones, controls such important physiological processes as leaf and flower senescence, leaf and flower abscission and fruit ripening. Ethylene is produced by plants during their growth and development, and is usually released into the atmosphere without accumulating (Klassen and Bugbee 2002). Because of the very low volume change per unit time (low leak rates) of the chambers, ethylene produced by plants slowly accumulated to reach biologically significant levels (Abeles et al. 1992). The longer the plants were in the sealed chambers, the more ethylene accumulated (Fig. 2). Martin and Sinnaeve (1987) grew wheat in a sealed soil-plant-atmosphere system (ESPAS chambers). They reported that the ethylene concentration during the first 11 d accumulated to 100–200 nmol mol^{-1}, which was sufficient to affect plant growth (Abeles et al. 1992). Spanarkel and Drew (2002) reported that ethylene concentration increased with time in both ambient and low pressure chambers, but was not affected by differences in total pressure. However, the volume change per unit time (leakage) was greater in their chambers and low pressure was limited to 70 kPa.

Plants experiencing environmental stress frequently show enhanced production of ethylene (Morgan and Drew 1997). Additional total gas pressure above ambient (hyperbaric) to maize seedlings enhanced ethylene production and the ethylene biosynthetic pathway (He et al. 1996, Sarquis et al. 1991). 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, Stutte 1999, Levinskikh et al. 2000). However, in our study there was no indication that hypobaric environments placed any particular stress on plants, as the rate of ethylene production decreased with reduced total gas pressure (Fig. 2). Hypoxia occurred at lower pressure [i.e. 6.2 kPa pO_2 at 30 kPa total pressure compared to 20.9 kPa pO_2 oxygen at ambient (101 kPa)].

Seed development was completely inhibited in soybean in an atmosphere of 5 % oxygen [5 kPa pO_2 at 101 kPa total pressure] (Quebedeaux and Haley 1973). We could not ascertain ethylene or hypobaric effects on reproductive development – since lettuce seedlings were harvested before maturity and the 40-day-old wheat seedlings started to flower, but did not have a long enough maturation period to set seed. While plants can tolerate low O_2, seed germination is more sensitive to hypoxia than vegetative growth. Under total pressure of 50 kPa [pO_2 was 10.3 kPa] there were no significant effects on germination rate for both lettuce and wheat (Table 3).

Plant root systems show dramatic increases in ethylene production rates under hypoxic conditions (Drew et al. 1989, He et al. 1994, Jackson et al. 1985). However, in these studies plants were frequently grown at ambient pressure in open systems, in which roots were under low O_2, but shoots were exposed to normal O_2 levels (20.9 kPa pO_2 at 101 kPa total pressure). Subsequently O_2 can diffuse from leaves to roots through gas channels (aerenchyma) and other plant structures. With our airtight, closed system – roots and shoots were under the same partial pressure of 6.2 kPa pO_2 under a total gas pressure of 30 kPa. The final concentrations of ethylene were reduced by 56.6 % and 54.4 % under 30 kPa [which corresponded with 6.2 kPa pO_2, compared to ambient pressure with 21 kPa O_2] for lettuce and wheat respectively (Table 3).

It can be argued that decreased ethylene may be due to the hypoxic effect in the chambers at decreased total pressure. We ran an experiment at ambient pressure, incubating lettuce and wheat in four separate air-tight glass containers flushed with ambient O_2 (20.9 kPa pO_2) and 6.2 kPa pO_2 (equal to pO_2 at 30 kPa total pressure) by mixing various amount of compressed air with pre-purified N_2 gas in a closed system (roots and shoots in the same atmospheric conditions). Hypoxia (6.2 kPa pO_2) inhibited ethylene production of lettuce by −16.2 %, which was statistically significant at $P \leq 0.05$, whereas wheat had an −8.4 % reduction of ethylene, which, although reduced, was not significantly different at $P \leq 0.05$ level (Table 2). Hence, the hypobaric effect on reduced ethylene was greater than just hypoxia. One possibility may be that increased diffusivity of O_2 under hypobaric conditions could have maintained cells under fully-oxygenated conditions, thereby inhibiting ethylene production and offsetting the usual effect of low O_2. Hypobaria may also be influencing enzymes in ethylene biosynthesis.

Another trend was that lettuce and wheat grew better under low pressure compared with ambient pressure in our experiments (Tables 1 and 3). This may be attributed in part to lower ethylene concentrations in hypobaric chambers than ambient ones.

Lettuce and wheat showed different sensitivity to ethylene in a closed system

There was a negative linear correlation between chlorophyll content and final ethylene concentration in seedling transplants exposed to total pressures of 30 and 101 kPa (Fig. 3). The slope of both chlorophyll and ethylene concentrations were more negative in lettuce than wheat (Fig. 3). When lettuce and wheat were direct seeded and grown for 28 d in the same chambers, we found that plant development in wheat was better than lettuce, especially when ethylene concentration in the chamber was higher. In summary, lettuce was more sensitive to ethylene than wheat.

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