Controlled environment chambers for investigating tree response to elevated CO_2 and temperature under boreal conditions

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Abstract

A closed CO_2 and temperature-controlled, long-term chamber system has been developed and set up in a typical boreal forest of Scots pine (*Pinus sylvestris* L.) near the Mekrijärvi Research Station (62°47'N, 30°58'E, 145 m above sea level) belonging to the University of Joensuu, Finland. The main objectives of the experiment were to provide a means of assessing the medium to long-term effects of elevated atmospheric CO_2 concentration (EC) and temperature (ET) on photosynthesis, respiration, growth, and biomass at the whole-tree level and to measure instantaneous whole-system CO_2 exchange. The system consists of 16 chambers with individual facilities for controlling CO_2 concentration, temperature, and the combination of the two. The chambers can provide a wide variety of climatic conditions that are similar to natural regimes. In this experiment the target CO_2 concentration in the EC chambers was set at a fixed constant of 700 μmol mol^{-1} and the target air temperature in the ET chambers to track the ambient temperature but with a specified addition. Chamber performance was assessed on the base of recordings covering three consecutive years. The CO_2 and temperature control in these closed chambers was in general accurate and reliable. CO_2 concentration in the EC chambers was within 600-725 μmol mol^{-1} for 90 % of the exposure time during the "growing-season" (15 April - 15 September) and 625-725 μmol mol^{-1} for 88 % of the time in the "off-season" (16 September - 14 April), while temperatures in the chambers were within ±2.0 °C of the ambient or target temperature in the "growing season" and within ±3.0 °C in the "off season". There were still some significant chamber effects. Solar radiation in the chambers was reduced by 50-60 % for 82 % of the time in the "growing season" and 55-65 % for 78 % of the time in the "off season", and the relative humidity of the air was increased by 5-10 % for 72 % of the time in the "growing season" and 2-12 % for 91 % of the time in the "off season". The crown architecture and main phenophase of the trees were not modified significantly by enclosure in the chambers, but some physiological parameters changed significantly, *e.g.*, the radiant energy-saturated photosynthesis rate, transpiration rate, maximum photochemical efficiency of photosystem 2, and chlorophyll content.

Additional key words: climate change; pine forest; *Pinus sylvestris* L.

Received 4 August 1999, *accepted* 30 December 1999.
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Abbreviations and symbols: Chl - chlorophyll; EC - elevated CO_2 concentration; ECOCRAFT - European Collaboration on CO_2 Responses Applied to Forests and Trees; ET - elevated temperature; FACE - Free Air Carbon Dioxide Enrichment; F/F'_m - the maximum photochemical efficiency of PS2; IPCC - Intergovernmental Panel on Climate Change; IR - infrared radiation; PMMA - polymethyl-methacrylate; PPFD - photosynthetic photon flux density; PS - photosystem; PVC - polyvinyl chloride.

Acknowledgements: This work was undertaken as part of the ECOCRAFT (European Collaboration on CO_2 Responses Applied to Forests and Trees) programme funded by the European Commission DG XII, and the programme on the Response of the Boreal Forest Ecosystem to Climatic Change and Its Silvicultural Implications funded by the Academy of Finland and the University of Joensuu. Logistic support from the staff of the Mekrijärvi Research Station, University of Joensuu, and assistance in data transfer from Mr. Alpo Hassinen are gratefully acknowledged. Numerous people have also assisted with the assembly and operation of the system at the field site.

Introduction

Environmental engineering has been applied for several decades to investigate the response of technical or biological material to physical and chemical effects. Most research into the response of plants to gaseous pollutants has been performed in open-top field chambers (Heagle *et al.* 1973, Drake *et al.* 1989, Lee *et al.* 1993, Wang *et al.* 1995), automated, null balance greenhouses (Oechel *et al.* 1992), chamberless Free Air Carbon Dioxide Enrichment (FACE) rings (Hendrey *et al.* 1993), or branch bags (Barton *et al.* 1993, Kellomäki and Wang 1997a, Wang and Kellomäki 1997). Each approach has contributed greatly to our understanding of the integrated responses of plants to the environment at a given location. However, there are particular strengths and weaknesses in the different approaches that ultimately determine which design is best for each research question.

When studying boreal forest ecosystems, two unique points regarding the choice of exposure system have to be taken into account. Firstly, the facility should be adequate for long-term and year-round field studies. This is because the dominant component of the forest ecosystem comprises trees, certain features of which, such as their size, longevity, complexity, and enormous adaptability and potential for acclimation require *in situ* experimental observations of a considerable duration, at least a complete phase in the tree's lifetime. Secondly, the facility should enable accurate, stable control of temperature and CO_2 and allow combinations of changes in these. This is because not only are the well known effects of EC on trees greatly dependent on temperature (Long and Drake 1991, Wang *et al.* 1996) but also it is the forests of the boreal zone that will experience the greatest warming in the future (IPCC 1996). These facts imply that temperature manipulation, alongside CO_2 concentration, is necessary in order to study the impact of climate change on boreal forest trees.

Over the period 1991-1997, we used a set of semi-closed chambers to investigate the ecophysiological responses of boreal trees to both EC and ET (Wang *et al.* 1995, 1996). The chamber had several obvious weaknesses, however, such as excessively high internal temperatures on some days in summer, a high vapour pressure deficit coinciding with the temperature elevation in early spring and late autumn, and pronounced gradients in CO_2 concentration, temperature, and wind velocity across the chamber. These unexpected moisture load and heat stress conditions and non-uniform patterns of exposure led to difficulties in interpreting the results (Kellomäki and Wang 1996, Wang 1996). In view of the above experiment, we set up a new series of closed chambers with computer-controlled systems which allow different combinations of environmental variations (see Fig. 1 in Kellomäki and Wang 1998a). Although the chamber system is designed for a European collaborative research programme to assess the likely effects of EC and ET on forest trees (ECOCRAFT; see Jarvis 1998), it could equally well be used with little or no modification to measure carbon fluxes under other sets of conditions, such as altered water or nutrient availabilities, or variations in trace gases other than CO_2. The system has been running *in situ* since 1996 in a typical boreal forest of Scots pine (*Pinus sylvestris* L.) near the Mekrijärvi Research Station (62°47'N, 30°58'E, 145 m a.s.l.) belonging to the University of Joensuu, Finland. The present paper describes the technical details of the closed chambers and the performance of whole chamber system, and presents a few results to demonstrate the efficiency of such controlled environments, with special emphasis on the effects of CO_2 and temperature on trees.

Description of the chamber system

Overview: The main objective in the design of the chamber system was to provide means of assessing the medium to long-term effects of EC and air temperature on the growth and physiology of trees. The system therefore comprises sixteen individual chambers to allow for four treatments: (*1*) ambient temperature and CO_2 concentration (CON), (*2*) doubled ambient CO_2 (EC), (*3*) elevated ambient temperature (ET), and (*4*) EC and ET. Twenty trees of approximately the same crown size and height were chosen, and sixteen of these were enclosed individually in the closed chambers in 1996, while another four were grown under ambient conditions outside the chambers and were used as comparisons (OUT). To reduce shading from adjacent trees, all other trees within 2 m from a chamber were cut down one year prior to the experiment running. Development and test-running of the chamber system began in 1994 and normal operation started in the growing season of 1996.

Chamber structure: The chamber is of a cylindrical structure with eight walls (Fig. 1). The four walls facing south and west are constructed from 12 pieces of double-wall glass (*K-glass+AS Green, Eglas Oy,* Imatran, Finland) on a frame of aluminium angle brackets, and the four north and east-facing walls from double-wall acrylic sheets (standard 16 mm PMMA). The outer glass sheet is an anti-sun glass with low transparency to infra-red radiation (Table 1) and the inner one is a special heating glass. The inner ribs of the acrylic sheet form a series of closed cells which confer stiffness and mechanical strength. The acrylic sheets have high thermal and acoustic insulation properties and high transparency (*ca.* 85 % transparency within the range of 400-700 nm). Four small vents with baffles are provided at the bottom of the north and east-facing walls, and a lateral door of size 2.0±0.8 m on the north-facing wall provides access to the chamber. The walls of the chamber extend 50 cm below the mean ground-surface level in order to sever any root connections and to prevent the soil in the heated chambers from freezing. The chamber has a conical roof consisting of eight triangular acrylic plates to allow precipitation to run off. The precipitation collected from the roof is ducted into the chamber and then distributed uniformally by means of four swaying nylon belts hanging in the upper part. The seams between the aluminium brackets and the acrylic sheeting or heating glass are sealed with rubberized gaskets. A "*W*-type" duct (see Fig. 1) containing a blower (*K250M*, max. air flow 0.25 $m^3\ s^{-1}$) and a manual baffle is located to the north of each chamber, in order to control air exchange between the chamber and outside. A heat exchanger of size 1.0×0.6×0.2 m is installed in the top of the chamber. Each chamber covers a ground area of 5.9 m^2 and has an internal volume of 26.5 m^3.

Table 1. Transmission of the transparent materials used to cover the chambers.

Material	Transmission [%] 320-380 nm	380-780 nm	780-1000 nm	1000-1500 nm
Pilkington Anti-sun glass	34	76	33	32
Heating glass+Anti-sun glass	12	68	21	18
Acrylic (16 mm PMMA)	82	85	87	87

Fig. 1. Photograph of a closed chamber at Mekrijärvi Research Station (62°47'N, 30°58'E, 145 m a.s.l.), University of Joensuu, Finland.

CO_2 and temperature control: The CO_2 control system for each chamber consists of a CO_2 transmitter (*GMP 111*, Vaisala, Finland), a control module (*ISM 112, Gantner Electronic*, Austria), and a CO_2 proportional valve (Fig. 2). Unlike the usal infra-red CO_2 analyzer, the detector used in the CO_2 transmitter is a single-wavelength non-dispersive infra-red gas sensor that is not sensitive to other gases, *e.g.*, water vapour. At the same time, the transmitter is of sufficiently small size, low cost, and long-term stability that one can be placed directly in each chamber. On the other hand, the sensor is diffusion aspirated, *i.e.*, CO_2 enters it through a permeable membrane, and consequently the response time is affected by the ventilation rate in the chamber. The *ISM 112* module has the dual function of data acquisition and control, and can be employed for both the direct and multi-channelled recording and processing of analogue and digital sensor signals or measured values, as well as for regulating the actuators. Given a relatively constant rate of ventilation, the target concentration of CO_2 in each chamber is achieved by coordination between the transmitter, module, and CO_2 proportional valve. On receiving a signal from the transmitter, the module compares the measured concentration of CO_2 with the target value and decides whether to switch the proportional valve on or off. The required accuracy of CO_2 control can be attained by repeatly adjusting the related parameter values in the PID regulation system of the module, and the lag in the response time of the CO_2 transmitter can also be taken into account.

The temperature in each chamber is controlled by a heat exchanger linked to a refrigeration unit (*CAJ-4511YHR*, 3 kW, *L'Unite Hermetique*, France), a set of heating glasses, and adjustment of the air flow rate into the chamber. The evaporator coils of the heat exchanger, of size 1.0×0.6×0.2 m, were custom made for the chamber system. This large size allows a low coil-to-air temperature gradient and reduces condensation and icing on the coil, resultant desiccation of the chamber air, and loss of cooling efficiency. Air is pulled through the evaporator coils and cycled into the chamber by two 25-cm axial flow fans, each with a rated capacity (free air) of 0.22 m^3 s^{-1}, thus providing a nominal air turnover time in the chamber of approximately 50 s. We estimated that resistance to air flow through the evaporator coils increases the turnover time to no more that 2 min. We have also installed a 1.5 kW resistance heater on the evaporator coils in the current system for defrosting the evaporator. The refrigeration system consists of 3 kW air-cooled condensing units equipped with crankcase and head pressure controls, crankcase pressure regulating valves, and refrigerant accumulators. Properly adjusted, these accessories protect the compressor units from overloading and keep them operating efficiently under the highly variable conditions during field season. The refrigeration is switched on and off by a module-controlled magnetoelectric valve (see Fig. 2). The heating

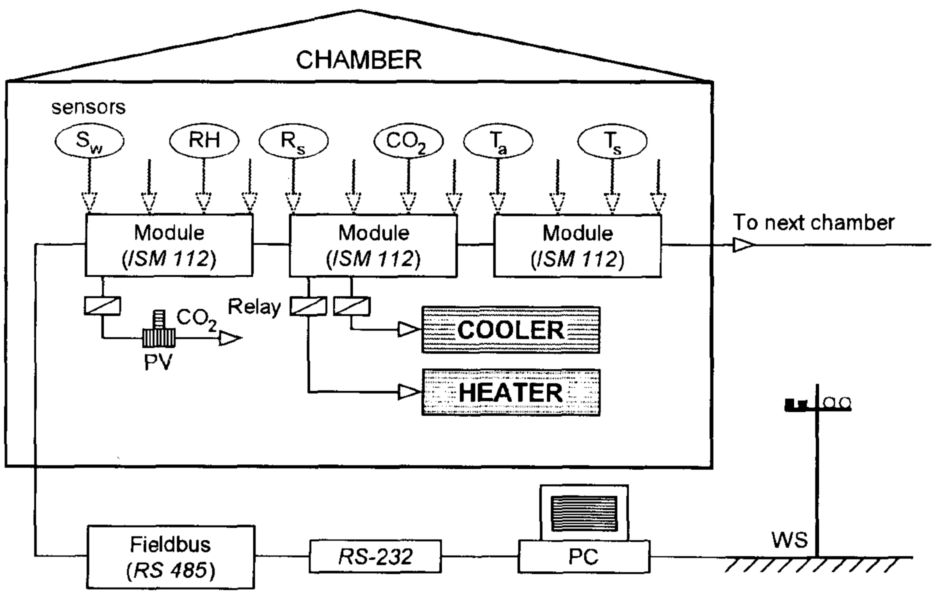

Fig. 2. Schematic representation of the closed-top chamber exposure facility shown in Fig. 1, including the dispensing and monitoring systems. PV: CO_2 proportional valve, PC: personal computer, WS: weather station, S_w, RH, R_s, CO_2, T_a and T_s: sensors for measuring volumetric soil water, relative humidity of the air, solar radiation, CO_2 concentration, and air and soil temperature, respectively.

system of each chamber consists mainly of 12 pieces of heating glass. Total heating power is 5.64 kW. For easy control of heating speed, the glass walls of each chamber are constructed by combining two types of heating glass, with nominal powers of 289 and 651 W.

Microclimate and soil: Measurements inside each chamber include air temperature, CO_2 concentration, relative humidity, solar and photosynthetically active radiation (in some chambers), soil temperature, and soil volumetric water content. The same measurements are also made at two locations outside the chambers. In addition, there is a weather station at a height of 9 m at the site in order to record solar radiation, net radiation, diffuse radiation, wind profiles, temperature, humidity, and precipitation. Details of all the instruments used in above measurements are summarized in Table 2.

Table 2. Details of the instructions for measuring microclimate variations in the experiment site at the Mekrijärvi Research Station, University of Joensuu, Finland.

Measurement	Sensor No.	location	mode & company
Inside chamber			
Solar radiation	2	1.5&3 m above the ground	*Ansa100, Itumic Oy*, Kuopion, Finland
PPFD	1	3 m above the ground	*LI-190SR, LiCor*, Lincoln., NE, U.S.A.
air temperature	1	middle of the crown	*HMP 141Y, Vaisala*, Helsinki, Finland
air humidity	1	middle of the crown	*HMP 141Y, Vaisala*, Helsinki, Finland
CO_2 concentration	1	middle of the crown	*GMP 111, Vaisala*, Helsinki, Finland
soil water	4	soil depths of 5&15 cm	*Theta Probe ML 1, Delta-T Devices*, Cambridge, U.K.
soil temperature	4	soil depths of 5&15 cm	*Pt-100, Muurlan Elektroniikka Ky*, Helsinki, Finland
Weather station			
solar radiation	1	9 m above the ground	*Ansa100, Itumic Oy*, Kuopion, Finland
net radiation	1	9 m above the ground	*Ansa100, Itumic Oy*, Kuopion, Finland
diffuse radiation	1	5 m above the ground	*CM21+CM121, Kipp and Zonen*, Delft, the Netherlands
PPFD	1	9 m above the ground	*LI-190SR, LiCor*, Lincoln, NE, U.S.A.
air temperature	1	9 m above the ground	*HMP45D & DTR13, Vaisala*, Helsinki, Finland
relative humidity	1	9 m above the ground	*HMP45D, Vaisala*, Helsinki, Finland
wind speed	1	9 m above the ground	*WAA15A, Vaisala*, Helsinki, Finland
wind direction	1	9 m above the ground	*WAV15A, Vaisala*, Helsinki, Finland
precipitation	2	5 m above the ground	*RG 13, Vaisala*, Helsinki, Finland

Data acquisition and storage: The core of the monitoring and control system consists of a computer and a set of control modules (*ISM 112, Gantner Electronic*, Austria). The computer runs the control programs (*ISM 100* configuration software, version *4.30*) and provides data storage facilities. The *ISM 100* configuration software can be combined flexibly with *FIX32 6.0* (*Intellution*, USA) and *EXCEL 5.0* (*Microsoft Corporation*, USA) to display graphs and process the data. Since each module has only four analogue inputs and two digital actuator outputs, three modules were used in each chamber to allow for all the various measurements and sensor types. A fieldbus *RS-485* was then used for simultaneous connection of all modules in the chamber at one line (see Fig. 2), with 16-bit A/D conversion for the input and 14 bit D/A conversion for the output. Digital output boards switch the 24 V DC supply to the various relays which regulate control functions.

Chamber performance

Effects of the chamber on the microenvironment: The construction of a chamber can in itself alter various components of the microenvironment, including temperature, vapour pressure deficit, iradiance and radiation quality, air mixing, and soil water (Fuhrer 1993, Körner *et al.* 1993, Payer *et al.* 1993, Wang 1996). This chamber effect was analyzed here by comparing the main environmental variables inside and outside the controlled chambers (CON-OUT). As our system has now been operating at the site since 1996, we have

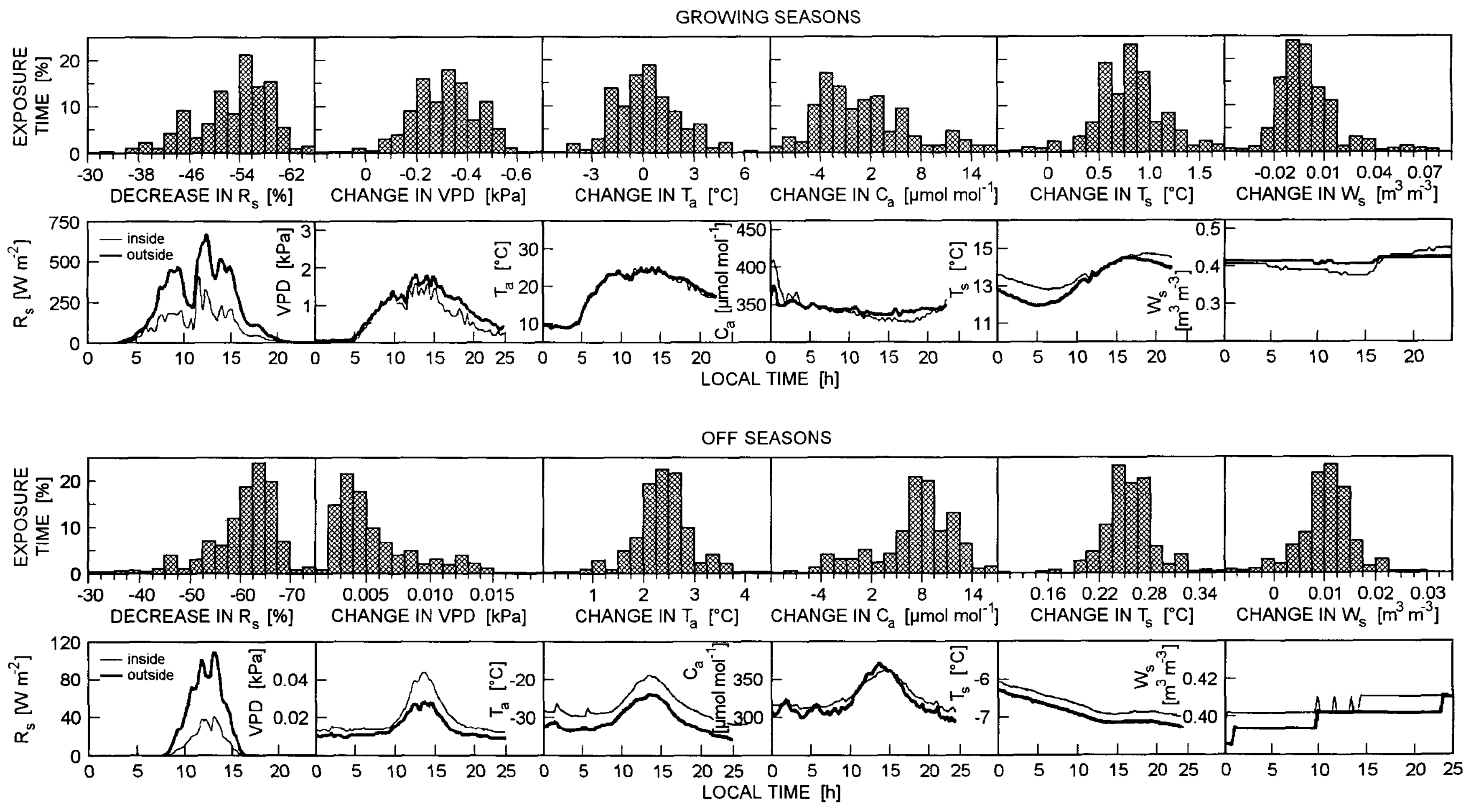

Fig. 3. Frequency distributions (*bar chart*) of changes in environmental variables *inside* control chamber compared with those *outside* the chamber, and mean daily patterns (*line chart*) of the environmental variables. The environmental variables include solar radiation (R_s) above the crown, vapour pressure deficit (VPD), air temperature (T_a), and CO_2 concentration (C_a) at the middle of the crown, and soil temperature (T_s) and soil volumetric water content (W_s) at 5 cm depth. The plots are based on 15-min means of 15-s readings taken from four replicates in each treatment. The data cover two "growing-seasons" (15 April-15 September 1997 and 1998) and two "off-seasons" (16 September 1997-14 April 1998 and 16 September 1998-14 April 1999).

plenty of recorded values for use in such an analysis. In view of the seasonal differences in the sensitivity of plants to environmental conditions and in chamber performance, the statistical analysis is made separately for the "growing season" and the "off season" (Fig. 3). The boundary dates are based on the annual rhythm of photosynthesis in Scots pine (Kellomäki and Wang 1998b).

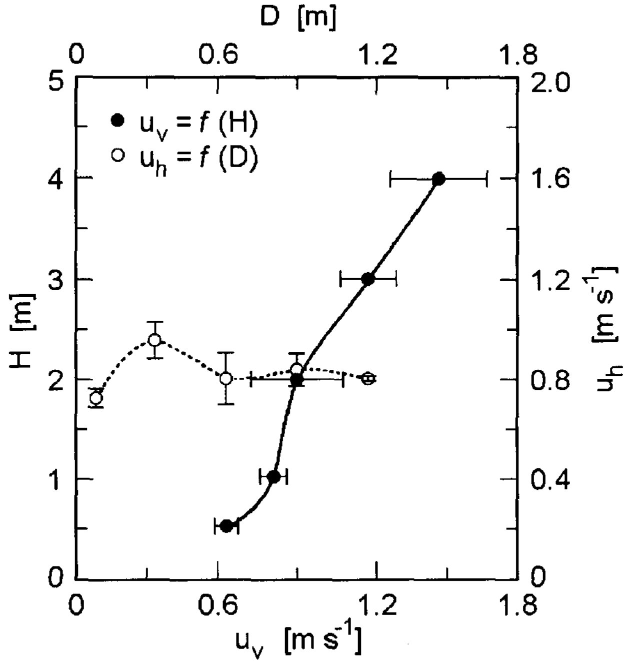

Fig. 4. Distribution of wind velocity within a closed chamber. $u_v = f(H)$ denotes the wind velocity measured near the central axis of the chambers (u_v) as a function of height above the ground (H), and $u_h = f(D)$ the wind velocity as a function of the distance from the wall of chamber (D). u_h was the mean of the wind velocities measured along four horizontal directions at a height of 2 m above the ground (about the middle of crown). The measurement was made at an air supply rate of 0.41 m^3 s^{-1} with two axial-flow fans on the heat exchanger running. Wind speed was measured by means of a thermal anemometer (*470-1, C&G Industrical Supply*, Houston, U.S.A.) using the manufacturers calibration.

The frequency distributions of the environmental variations indicate that:

(*1*) solar radiation (R_s in Fig. 3) in the chambers was reduced by 50-60 % for 82 % of the time in the "growing season" and by 55-65 % for 78 % of the time in the "off season". The variation in the reduction in solar radiation is related mainly to the position of sun and momentary weather conditions;

(*2*) changes in the relative humidity (RH) of the air in the chambers varied from -15 to +30 % in the "growing season", although the negative change accounted for only 4 % of the exposure time and originated mainly from interference from the cooling system, whereas the positive changes were in the range 5-10 % for 72 % of the time and were mainly the result of increased transpiration from the plants and soil surfaces in the chambers at high irradiance. In addition, a high irradiance also led sometimes to dew fall from the exchanger surface, even though the large surface area of the heat exchanger had been designed in order to reduce the temperature difference between the air and the exchanger surface. The "off season" change in RH varied from -8 to +20 %, with increases of 1.8-12.0 % for 91 % of the time. The slight increase in RH was related to the higher chamber temperature (T_a in Fig. 3);

(*3*) air temperature in the chambers was maintained within the ambient range ±2.0 °C for 86 % of the time in the "growing season" (T_a in Fig. 3). The extreme increase of 5-6 °C shown in Fig. 3 resulted from a short-term fault in the cooling system. The 2-3 °C increase for 83 % of the time in the "off season" can be attributed to a low air exchange rate;

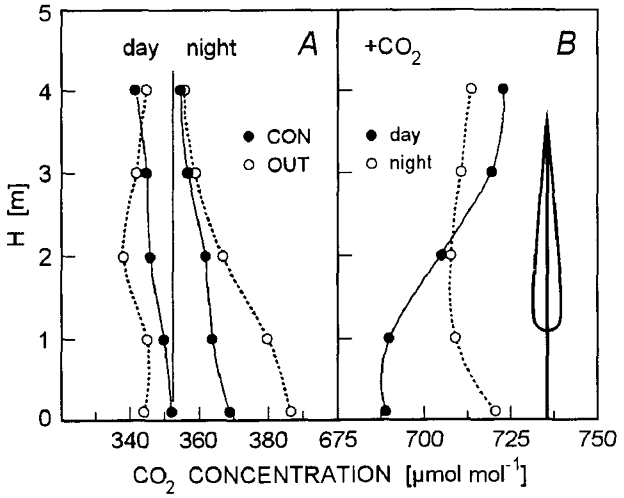

Fig. 5. Vertical distribution of CO_2 concentration within a closed chamber on a typical summer sunny day [12:00-15:00 on 17 June 1999 for day time (day) and 02:00-05:00 on 18 June 1999 for night time (night)]. Measurements were made separately in the centre of a control (CON) and a CO_2-enrichment chamber, and in the crown of a sample tree outside the chamber (OUT) at 5 heights (H) using two portable CO_2 gas analyzers (*LCA-4, Analytical Development Company*, UK).

(*4*) as a result of changes in RH and T_a, the calculated vapour pressure deficit (VPD in Fig. 3) in the chambers showed decreases of 0.2-0.4 kPa for 88 % of the time in the "growing season" and increases of 0.001-0.005 kPa for 90 % of the time in the "off season";

(*5*) the change in CO_2 concentration in the chambers during the normal operation period varied from -10 to +25 µmol mol^{-1} (C_a in Fig. 3), occasionally approaching

1500 μmol mol[-1] when the chamber access door was opened in order to make plant measurements and then closed abruptly. The increases (+) in C_a may be associated with the accumulation of CO_2 from soil and plant respiration processes during the night-time, and the decreases (-) with CO_2 uptake by plant photosynthesis during the daytime as shown by a typical daily course of CO_2 concentration in the chamber. In the "off season", a 4-10 μmol mol^{-1} increase in C_a was evident for 72 % of the exposure time, indicating that the air exchange rate was not enough to remove the CO_2 that had accumulated in the chamber;

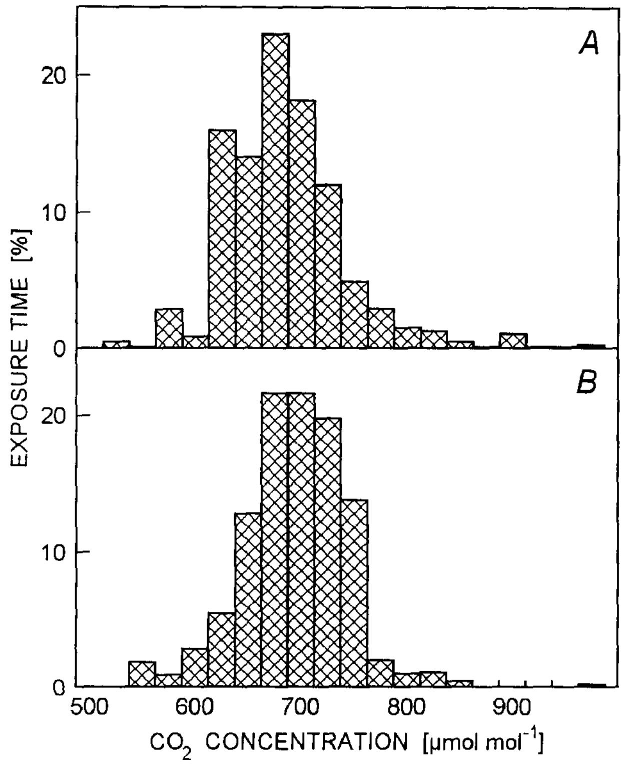

Fig. 6. Frequency distributions of CO_2 concentration in the CO_2-enriched chambers in (*A*) two "growing-seasons" (15 April-15 September 1997 and 1998) and (*B*) two "off-seasons" (16 September 1997-14 April 1998 and 16 September 1998-14 April 1999). 15-min means of 15 s readings taken from four replicated chambers with CO_2 elevation.

(*6*) corresponding to the increase in air temperature, a 0.5-1.0 °C increase in soil temperature for 81 % of the time occurred in the "growing season" and a 0.22-0.28 °C increase for 84 % of the time in the "off season" (T_s in Fig. 3);

(*7*) as soil water was supplied to the chambers promptly on the basis of the ambient soil water content, the patterns of soil water changes inside and outside the chambers were similar in all seasons (W_s in Fig. 3).

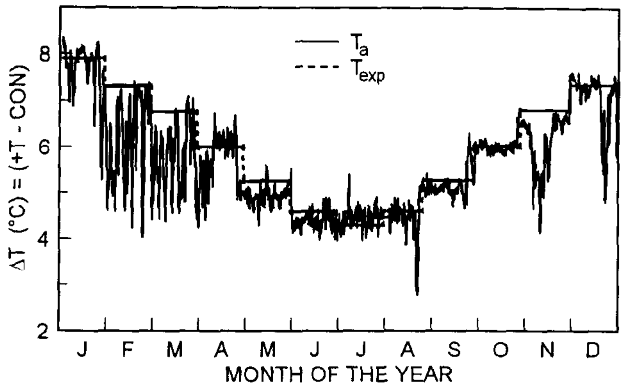

Fig. 7. Comparison between the expected increase in air temperature (T_{exp}) in the chambers and the measured increase (T_a). The calculations for ΔT_a are based on hourly means of 15 s readings taken from four replicates in each treatment (ET and CON). The readings cover a period from 15 April 1997 to 15 April 1999.

The air in the chambers is replenished at a measured rate of about 0.4 m^3 s^{-1} during the growing season, and this, when adding the air flow from two axil-flow fans on the heat exchanger, implies that the air circulation in the chambers is about 0.8 m^3 s^{-1}. Because of this constant circulation, the wind profile within the chamber is different from that outside. Distributions of wind velocity in a vertical (in the centre) and a horizontal (at the middle of the canopy) direction are shown in Fig. 4. Wind velocity is highest directly above the top of the canopy and declines with increasing distance downwards. In addition to this vertical gradient, a horizontal gradient in wind velocity is also evident from the centre of the chamber towards the wall. Even so, the wind speed in the middle of the crown is maintained at about 0.7 m s^{-1}. The CO_2 concentration on a typical sunny day in summer (Fig. 5) is generally slightly lower inside the chambers than outside during the daytime (about 10 μmol mol^{-1}), with no noticeable gradient within a control chamber or outside (CON and OUT in Fig. 5*A*). This differs somewhat from the situation with in a CO_2-enriched chamber (Fig. 5*B*). There are higher CO_2 concentrations near the ground in both the control and the EC chambers at night.

CO_2 enrichment and temperature elevation: The aim was to maintain the CO_2 concentration in the EC chambers at 700 μmol mol^{-1} throughout the year, and the statistical results indicate that this target was in general achieved very well (Fig. 6), concentrations being in the range 600-725 μmol mol^{-1} for 90 % of the time in the "growing season" (Fig. 6*A*) and 625-725 μmol mol^{-1} for 88 % of the time in the "off season" (Fig. 6*B*). The markedly low values mainly resulted from the opening of the chamber door and the markedly high ones partly from respiration by a person working in the chambers or partly from temporary faults in the CO_2 control system, especially during the growing season.

The warming treatments were designed to correspond to the climate scenario predicted for the site after doubling of the atmospheric concentration of CO_2 (Kellomäki and Väisänen 1997). This implies an increase in air temperature of 0.43-0.79 °C above the current ambient level, depending on the month of the year (Fig. 7). Actual measurements based on hourly means of 15 s recordings show that the air temperatures in the temperature-elevated chambers were close to the target temperatures in the "growing season", but below the target for most of the time in the "off season". Furthermore, there were larger relative fluctuations in the control temperature during the "off season". The standard deviations of the monthly measurements varied from ±0.4 to ±0.8 °C during the "growing season" and from ±0.5 to ±1.4 °C during the "off season".

Since there are no particular means of controling humidity or soil temperature in the chamber system, the increase in T_a causes considerable disturbance to these variables. Statistical results based on the two years of recordings indicate that the increase in T_a leads to a 5-21 % reduction in relative humidity for 87 % of the time (ΔRH, Fig. 8*A*), a 0.05-0.35 kPa increase in vapour pressure deficit for 65 % of the time (ΔVPD, Fig. 8*C*), and a 0.2-1.4 °C increase in the soil temperature for 90 % of the time (ΔT_s, Fig. 8*E*). Meanwhile, the changes in RH, VPD, and T_s (Fig. 8*A,C,E*) show typical annual patterns. The mean relative humidity decreases by only 5 % during the three main months of the growing season (June, July, August), but by 18 % in general and maximum of 33 % during the early spring (15 March-15 May) and late autumn (15 September-15 December). However, the increase in VPD during the "off season" is evident. A mean increase of 1.45 °C in soil temperature occurs during the winter months (1 November-31 March), but only of 0.45 °C during the other months of the year.

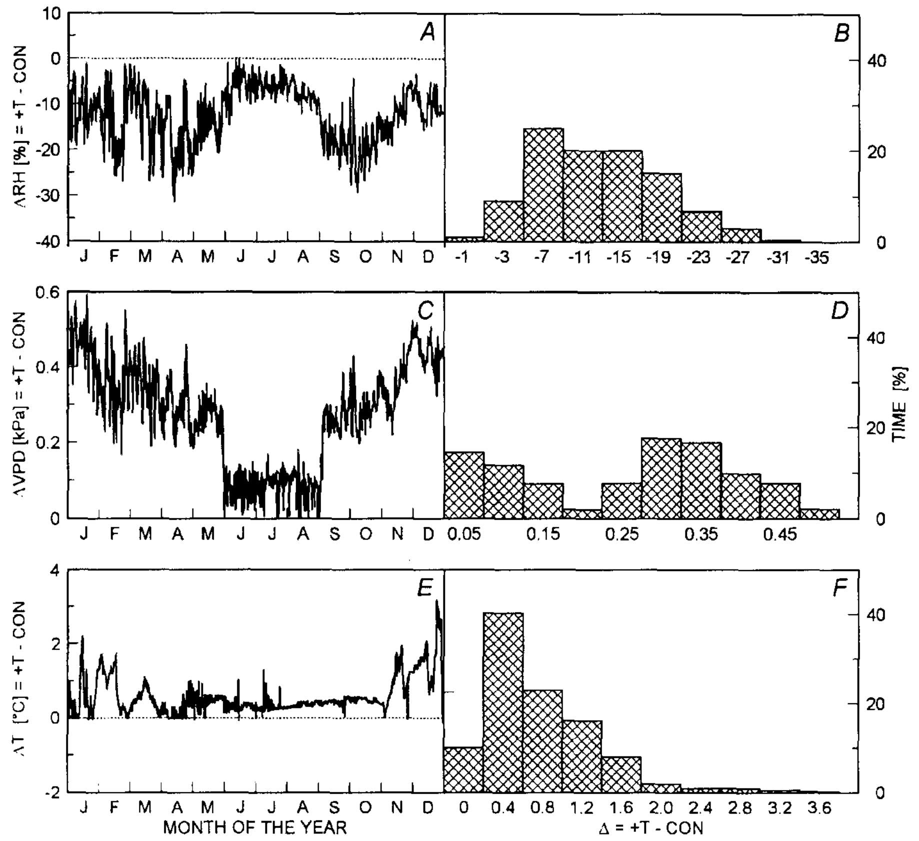

Fig. 8. Changes in relative humidity (RH), vapour pressure deficit (VPD), and soil temperature (T_s) at 5-cm depth caused by the increase in air temperature in the chambers. (*A*), (*C*), and (*E*) are annual courses separately for RH, VPD, and T_s, and (*B*), (*D*), and (*F*) frequency distributions separately for RH, VPD, and T_s. Plots are based on hourly means of 15 s readings taken from four replicates in each treatment (ET and CON). The readings cover a period from 15 April 1997 to 15 April 1999.

Effects of the chamber on physiological processes: It may be fairly difficult to determine the effects of the chamber on the growth and physiology of the trees because these involve numerous aspects of the physiological processes and vary according to the spatial and temporal scales on which the observations are made. Some of the parameters used to examine the chamber effects are listed in Table 3. The crown architecture and main phenophase were not modified significantly by the chamber but some physiological processes were greatly affected; *e.g.*, the radiant energy-saturated photosynthetic rate (P_{max}) and the mean daily sap flow (F_s) were reduced by 11 and 37 %, respectively, whereas the maximum photochemical efficiency of photosystem 2 ($\Delta F/F'_m$) and the chlorophyll (Chl) (a+b) content increased by 7 and 17 %, respectively. Significant modifications in Chl (a+b) and F_s were observed during the earlier period of the experiment but those in P_{max} and $\Delta F/F'_m$ only in the third year.

Table 3. Comparison of parameters related to growth and physiological processes of Scots pine between trees grown in the control chamber (CON) and outside the chambers (OUT). All parameters related to physiology and growth were means of measurements from four replicated chambers in late August each year. The statistical significance of the difference between CON and OUT p was determined by the Student's t-test, in which ns = no effect; *significant at p<0.05; **at p<0.01. P_{max} [μmol m^{-2} s^{-1}] denotes the radiant energy-saturated photosynthetic rate of two-year-old needles (Wang *et al.* 1995), $\Delta F/F'_m$ the maximum photochemical efficiency of photosystem 2 (Wang and Kellomäki 1997), R_D [μmol m^{-2} s^{-1}] dark respiration rate at 20 °C, g_s [mmol m^{-2} s^{-1}] the radiant energy-saturated stomatal conductance to CO_2 (Kellomäki and Wang 1997a), Chl (a+b) [g m^{-2}] the content of chlorophyll, F_s [kg tree^{-1}] the mean daily sap flow based on monthly measurements (Kellomäki and Wang 1998a), H_l [cm] length of living crown, A_c [m^2] crown projected area, A_l [m^2 tree^{-1}] total projected area of needles per tree, and ΔH_g [cm] and ΔD_g [mm] height and diameter growth of tree in previous year, respectively. Means of dates on which more than 80 % terminal buds of branches of a tree were bursting [d number].

	1996 OUT	CON	p	1997 OUT	CON	p	1998 OUT	CON	P
Physiology									
P_{max}	17.20±1.30	18.1±0.66	ns	16.60±0.92	17.0±1.11	ns	19.70±0.86	17.60±1.23	*
$\Delta F/F'_m$	0.72±0.04	0.74±0.02	ns	0.69±0.03	0.73±0.06	ns	0.72±0.02	0.77±0.03	*
R_D	1.26±0.08	1.32±0.05	Ns	1.07±0.10	1.14±0.07	ns	1.33±0.11	1.42±0.19	ns
g_s	145±13	152±10	ns	128±14	117±18	ns	157±12	142±21	Ns
Chl (a+b)	-	-	-	0.72±0.03	0.78±0.07	*	0.70±0.04	0.82±0.06	**
F_s	-	-	-	0.97±0.14	0.66±0.15	*	1.12±0.32	0.71±0.18	*
Growth									
H_l	285 ±11	281±9	ns	302±13	289±17	ns	311±12	292±21	ns
A_c	0.54 ±0.06	0.52±0.04	ns	0.76±0.05	0.72±0.07	ns	1.22±0.09	1.13±0.13	Ns
ΔH_g	-	-	-	26.8±1.8	28.2±1.03	ns	22.9±1.2	24.3±1.4	ns
ΔD_g	-	-	-	13.4±1.1	12.2±1.4	ns	17.1±1.2	16.0 ±1.4	Ns
A_l	1.84±0.03	1.79±0.06	ns	2.04±0.04	2.13±0.07	ns	2.48±0.05	2.65±0.11	Ns
Phenology									
Bud burst	-	-	-	149±8	147±7	ns	144±8	140±6	ns

Discussion

The closed, controlled-environment chamber system described here has proved to be a relative efficient means of studying the long-term responses of trees to specific CO_2 and temperature conditions at a boreal forest site. This system has several advantages over other current exposure systems such as open-top chamber (Drake *et al.* 1989, Wang *et al.* 1995) and free-air CO_2 enrichment (Hendrey *et al.* 1993), including continuous measurement of whole-system CO_2 flux, precise temperature control, and a more uniform CO_2 concentration throughout the chamber. In particular, the use of heating glass and the large chamber volume enables system to reproduce future annual temperature scenarios for whole trees of a large size *in situ*. In this respect the system also differs from other closed, mini-greenhouse systems, which have mainly been directed towards studying entire microcosms and only applied to vegetation of small stature in short-term experiments, ranging from beech saplings (Overdieck *et al.* 1998) to tundra vegetation (Oechel *et al.* 1992). The investment required for such a large-scale facility is large, however: approximately 0.1 million $ for development and testing, 0.3 million $ for construction materials and monitoring devices, 10 000 $ per year in CO_2 consumption, and 15 000 $ per year in power consumption. In addition, two engineers, one technician, and six scientists, trained in ecological physiology, soil science, plant nutrition, analytical chemistry, pest control, and computer science, respectively, were responsible for the technical servicing and scientific management of the system, and two trained persons were required to provide a 24-h emergency service at the site. All this is necessary to do precision, long-term experiments, lasting several years, with a minimum of loss.

As noted under "chamber performance", CO_2 and temperature control is in general accurate and reliable with these closed chambers. The differences between the CO_2 concentrations inside and outside the chamber varied from 0 to 35 µmol mol^{-1}, and the temperature differences from 0 to 2 °C. These variations are of similar magnitude to those reported in other well-controlled chamber experiments (*e.g.*, Jäger and Weigel 1993, Vourlitis and Oechel 1993, Norris *et al.* 1996). The variations in CO_2 concentration in the EC chambers are approximately symmetrical, giving a mean value of 684 µmol mol^{-1}, close to the target of 700 µmol mol^{-1}, with a standard deviation of 49 µmol mol^{-1}. Even so, some chamber effects still exist. First, the chamber system using a double layer of heating glass as the cover material reduces solar radiation by 50-60 %, which is larger than the 20-35 % reduction obtained with most open-top chambers covered with PVC (Weinstock *et al.* 1982, Ashenden *et al.* 1992, Wang 1996), but because this glass has a relatively low transmission in the IR spectral band of solar radiation and a high transmission for PPFD (Table 1), our measurements showed the hourly mean PPFD in the chambers to be reduced by only 34-40 % in the "growing season" and 38-45 % in the "off season" relative to the outside (Kellomäki and Wang 1998a). The reduction in PPFD entering the chambers may be the main reason for the significant modifications in certain parameters of photosynthesis such as P_{max}, $\Delta F/F'_m$, and Chl (*a*+*b*) (Table 3). Secondly, humidity in the chamber system is controlled only by adjusting the rate of air flow into the chamber. This method is subject to many limitations, such as the capacity of the blower, the adjustment interval, and the influence of CO_2 control. Consequently, as irradiance is high on most summer days, relative humidity is higher inside the chambers than outside in the afternoon, because the moisture arising from transpiration in the system can not be removed sufficiently rapidly. One direct result of the increase in humidity within the chambers may be the reduction in sap flow (F_s in Table 3). Thirdly, the wind speed in the middle of crown can be kept within 0.7-0.8 m s^{-1} (see Fig. 4) by means of the blower and the two axial-flow fans on the exchanger. Although this is certainly different from the actual windspeed profile outside the chambers, it is enough to maintain low needle boundary resistance for a pine tree with small needles (Kellomäki and Wang 1999), and to minimise gradients of CO_2 concentrations across the chamber (Fig. 5).

Two deficiencies in the accuracy of controlling the ET chambers are the considerable deviations between the actual control temperatures and the expected target temperatures in the winter months (Fig. 7) and the significantly lower humidity inside the chambers than outside in the early spring and late autumn (see Fig. 8*A*). The first deficiency arises because the heating system of the chamber does not react fast enough to the rapid changes in ambient temperatures. Most of these deviations were associated with exceptional air temperatures, often below –20 °C, and may therefore not be very significant for the physiological processes of the trees, which will be in a deep dormancy stage. The second deficiency occurs in the physiological recovery and the hardening periods of the trees (Kellomäki *et al.* 1993), and may have an important effect on their responses to the other treatment variables, in this case CO_2 concentration and temperature (Wang 1996, Kellomäki and Wang 1997*b*).

In conclusion, the following points are worth pointing out. (*a*) Compared with existing field exposure facilities, the closed chamber system described here provides relatively reliable and efficient means of studying long-term responses of trees to EC and ET at a boreal forest site. (*b*) Cooling and heating technologies are available to follow the ambient temperature and to provide a specified temperature addition over prolonged periods as required. This allows the long-term (year-round) interactive effects of EC and temperatures to be investigated, an approach that is hardly possible with open-top chamber or FACE rings. (*c*) The use of a CO_2 transmitter of small size allows CO_2 concentrations in a multi-chamber system to be monitored simultaneously. (*d*) The chamber system is suitable for growing a wide range of tree sizes from the seedling stage to mature trees *in situ*, and could equally well be used to measure CO_2 fluxes in other treatments involving water or nutrient availability, for example, with few or no modifications. Finally (*e*), in view of the significant reduction in solar radiation and relative humidity of the air, an available irradiation system and humidity control unit should be installed in any future implementation.

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