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## UNIFORMITY OF ENVIRONMENTAL CONDITIONS AND PLANT GROWTH IN A HYDROPONIC CULTURE SYSTEM FOR USE IN A GROWTH ROOM WITH AERIAL CO<sub>2</sub> CONTROL\*

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VESSEY J. K., YORK E. K., HENRY L. T. and RAPER C. D., Jr. Uniformity of environmental conditions and plant growth in a hydroponic culture system for use in a growth room with aerial  $CO_2$  control. BIOTRONICS 17: 79–94, 1988. A portable system of hydroponic culture was developed that maintained temperature, pH, and nutrient concentrations of circulating nutrient solutions. The hydroponic system is used within a controlled-environment room (CER) for control of aerial environment. The CER was equipped with an autocalibrating system for atmospheric  $CO_2$  control. The control systems for the hydroponic chambers were able to maintain acidity within  $\pm 0.2$  pH units and the temperature with  $\pm 0.5^{\circ}$ C. Mixing time for the 200-liter volume of solution within a hydroponic chamber was less than 12 min. The  $CO_2$  control system was able to maintain aerial concentrations within  $\pm 10$  ppm CO<sub>2</sub> during the light period. The only gradient found to occur within the hydroponic chambers or CER was a slight gradient in aerial temperature along the length of hydroponic chambers. Growth of soybeans [Glycine max (L.) Merr.] was characterized during a 3-week period of vegetative development by leaf number and area, plant dry weight, total N content of plants, and N depletion from the nutrient solution. The growth characteristics among populations for three hydroponic chambers within the CER were not significantly different, and the percent standard errors of means of the measurements within populations from each chamber were nearly all less than 10%. Thus, the uniformity of plant growth reflected the uniformity of environmental conditions.

**Key words:** aerial temperature; CO<sub>2</sub> control; *Glycine max* (L.) Merr.; nutrient solution; pH control; root-zone temperature; soybean.

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#### **INTRODUCTION**

The uniformity of environmental conditions for growth of plant material in experiments is important in identifying treatment effects on physiological responses. Uniformity of growth conditions is even more important in experiments which require statistical analysis of characteristics between populations of plants. Hydroponic culture of plant material therefore is well suited for many physiological studies of plants, because the physical and nutritional conditions around root systems can be kept much more uniform and can be more precisely measured than in solid media.

A system has been built for the growth of populations of plants in hydroponic culture in the Southeastern Plant Environment Laboratories at N. C. State University. The system consists of four portable, hydroponic chambers, each with individual pH and temperature control of the root compartments. The hydroponic chambers provide a constant recirculation of nutrient solution between root compartments and a large reservoir, thereby providing relatively stable nutrient concentrations over several days. The system, which has been designed for low maintenance, is housed within a controlled-environment room (CER) equipped with an auto-calibrating system for atmospheric  $CO_2$  control.

In this paper the physical and design characteristics of the hydroponic chambers and control systems are described. The performance of the system, in terms of uniformity of environmental conditions and uniformity of growth of populations of vegetative soybeans within the system, also is presented.

#### MATERIALS AND METHODS

#### Design of hydroponic chambers

The hydroponic chambers (Fig. 1), which measure  $137 \times 46 \times 80$  cm, are located in a  $3.65 \times 2.44$  m CER (1). Each chamber consists of three root compartments which measure  $43 \times 43 \times 23$  cm (Figs. 1, 2). The three root compartments hold a combined volume of 100 1 of nutrient solution. Each root compartment is divided into four cells of equal size, with the cap for each cell constructed to hold up to four plants (Figs. 1, 3), for a total of 48 plants per hydroponic chamber. The root compartments are constructed of 64 mm polyvinyl chloride (PVC) and all joints have been heat welded with PVC rod. Each individual root compartment is insulated with a closed-cell foam insulation (R15 Armaflex<sup>®</sup>, Armstrong World Ind., Atlanta, GA).

The reservoir (Fig. 2), which measures  $91.5 \times 35.5 \times 33$  cm, is insulated with the same material as the root compartment and is situated below the root compartment. The reservoir holds 100 l of nutrient solution, which added to the 100-l volume of the root compartments results in a total volume of 200 l for the entire hydroponic chamber. All control sensors (temperature, pH, and solution level) are located in the reservoir.

The nutrient solution is pumped from the reservoir at a rate of 161 min<sup>-1</sup> by



Fig. 1. Floor plan of the CER containing the four hydroponic chambers. Individual root compartment, root cells, and the location of building-supplied coolant (ethylene glycol) lines and air inlet ducts for  $CO_2$  distribution are indicated.

a totally enclosed pump (Fig. 2). A check valve in the supply piping for the root compartments keeps the root compartments from draining if the pump should fail and enables the reservoir to be drained and refilled separately from the root compartments. Thus, half of the nutrient solution can be changed without exposing the roots to air.

Past the check valve, the nutrient solution is pumped into two 13 mm PVC supply pipes which run along the length of both sides of the root compartments (Figs. 2, 3). Supply lines feed solution into the bottom corner of each of the four



ROOT COMPARTMENTS

Fig. 2. Block diagram of hydroponic chamber showing root compartments, reservoir, and flow pattern of nutrient solution through supply and return lines.

cells within the individual root compartments. This configuration eliminates gradients of temperature and nutrients that can occur by delivery of solution along extended distances of uninterrupted lateral flow. After flowing through the root cells the nutrient solution drains through a common standpipe at the center of each root compartment (Fig. 3). The three standpipes within one chamber are connected to a common 51 mm drain pipe that returns to the top of the reservoir. The solution is aerated by the tumbling action as it returns through the standpipes and drain line into the reservoir. Periodic measurements with a Clark-type, dissolved-O<sub>2</sub> probe during the growth of soybeans in the chambers indicated that O<sub>2</sub> concentration of the solution in the root compartment cells remained near saturation.

The reservoir, root compartments, and pump are supported by a metal frame. Swivel casters are attached to the frame to make the unit portable. After disconnecting the supply and return lines, the root compartments can be removed from the frame to give access to the reservoir for cleaning or maintenance of components.

#### Control of temperature in root compartments

The temperature of the nutrient solutions within the hydroponic chambers is regulated by solid-state, Barber Colman Model 560 proportional temperature controllers (Barber Colman Co., Rockford, IL) (Fig. 4). The temperature of the nutrient solution for each chamber is sensed by a platinum (100  $\Omega$  at 0°C) resistance temperature detector (RTD) in the reservoir and displayed on the temperature controllers which are located on the exterior of the CER. The temperature at which the nutrient solution is to be maintained within a working range of 10 to 35°C is set on the controller. Proportional control of cooling is attained by an electric actuator driving a proportional control valve to regulate the flow of building-



Fig. 3. Axonometric drawing of a single root compartment showing the layout of the four root cells, covers of root cells capable of holding four plants each, and return and supply lines. Arrows indicate flow of nutrient solution from the supply port, through a root cell, and discharge through the central stand pipe.

supplied coolant (ethylene glycol) through a cooling coil in the reservoir (Fig. 4). In a prototype system (2), chilled ethylene glycol was supplied by a 1.99 kW constantflow, portable refrigerator unit. A building-supplied liquid heat source for proportional control of heating is not available; therefore, heating is facilitated by an accessory output on the temperature controller. When the temperature falls below the set-point, the controller closes a solid-state relay to activate a 500 W tubular heating element in the reservoir (Fig. 4).

The temperature controller has dual set-points which allow programming of two control temperatures during a 24-h cycle. Either of the two set-points may be selected on 15 min increments by a timing mechanism with a single-pull, doublethrow switch.

As a safety feature, a Magnetrol Series 81 liquid level controller (Magnetrol Inc., Downers Grove, IL) detects loss of solution in the reservoir and deactivates the heating element (Fig. 4).

#### Control of pH in root compartments

The pH level of the nutrient solution in a hydroponic chamber is sensed by Cole-Parmer #5993-35 pH electrodes (Cole-Parmer Inst. Co., Chicago, IL) located



Fig. 4. Schematic diagram of component parts of temperature control system of nutrient solution within a hydroponic chamber. SPS, 24 h setpoint selector; SSR, solid state relay; SOV 1–2, shut off valve; PCV, proportional control valve; EA, electric actuator; LLC, liquid level controller; LLP, liquid level probe; RTD, resistance temperature detector.

in the reservoirs. The pH of the solution is displayed on a Horizon Model 5650 pH monitor/controller (Horizon Ecology Co., Chicago, IL) which is located on the exterior of the CER.

The desired pH can be set on the controller within a range of +0.2 pH unit. When the pH of the nutrient solution moves outside the high or low limit of this range, a relay in the controller activates the following mechanisms. A Masterflex<sup>®</sup> Model PD 7520-00 peristaltic pump (Cole-Parmer Inst. Co., Chicago, IL) with dual 7014-20 pump heads pumps acid (0.01 N H<sub>2</sub>SO<sub>4</sub>) and base  $[0.01 \text{ N Ca}(OH)_2]$  from their respective 20 l reservoirs (Fig. 5) at 100 ml min<sup>-1</sup> through Tygon tubing into 3-way stainless steel solenoid valves (Fig. 5, SV9-SV10). Depending on whether the high or low limit relay on the controller has been activated, either the acid or the base solenoid valve is opened, which loads a series of four acid or four base 2-way solenoid valves (Fig. 5, SV1-SV8). The relay on the controller also opens the corresponding 2-way acid or base solenoid valve which results in the appropriate solution being pumped into the reservoir return line of the hydroponic chamber. Finally, the controller activates either the acid or base delivery totalizer which records the length of time the system is activated. When the pH controller senses that the pH has returned to the set-point, the system is deactivated.

To operate this system, customized circuitry was designed to provide a low voltage (24 V DC) signal and the communication circuits among the controller, pump, solenoids, and totalizers. The pump, solenoid valves, and acid and base reservoirs are housed on a metal frame "pumping station" which is located adjacent to the CER.

MONITOR/CONTROLLER



Fig. 5. Schematic diagram of control system of nutrient solution pH. A1–A4, acid delivery totalizers; B1–B4, base delivery totalizers; SV1–SV8, solenoid valves (2-way NC); SV9–SV10, solenoid valves (3-way NO); PH1–PH2, peristaltic pump

#### Control of $CO_2$ concentration in the CER

A computer automated system was developed to control the  $CO_2$  concentration of the air within the CER at preset levels from ambient to 1000 ppm. The system continuously monitors the  $CO_2$  concentration within the CER and makes additions of  $CO_2$  to the CER at the beginning of every minute if required. Key features of this system are a real-time graphics display and an ability to autocalibrate.

Air from the center of the CER is continuously sampled by a Dasibi Model A0218-B gas sampling pump (Dasibi Environmental Corp., Glendale, CA) at a rate of 1 1 min<sup>-1</sup> (Fig. 6). The sample passes through a solenoid valve and flowmeter (Fig. 6, SV1 & FM1, respectively). The CO<sub>2</sub> concentration of the sample is determined by an ANARAD Model AR-50 infrared gas analyzer (IRGA) (Anarad Inc., Santa Barbara, CA). The analog output from the sensor of the IRGA is continuously processed by a 12 bit A/D board, and the digital signal is sent to an Apple<sup>®</sup> IIE computer. The incoming digital signal to the computer is processed by a custom software program. The program can give a real-time display of the CO<sub>2</sub> concentration at 1 min increments for a period of 1 h on the computer monitor. At the end of each hour, the program writes to disk the CO<sub>2</sub> concentrations that were recorded in the 1 min increments.

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heads.



Fig. 6. Schematic diagram of control system of  $CO_2$  concentration in the CER. SV1–SV4, solenoid valves (NC); FM1–FM4, 0–1000 ml min<sup>-1</sup> flowmeters; PS1–PS2, gas cylinder pressure sensors; MSZ, manual step zero; MDZ, manual direction zero; MSS, manual step span; MDS, manual direction span; ZERO, front panel override; SPAN, front panel override; SAMP., front panel override; DELIV., front panel override.

If the CO<sub>2</sub> concentration is determined to be lower than the preset level, a digital signal is sent from the computer to a custom-built D/A board (Fig. 6). An analog signal then is sent to a custom-built solenoid control board which opens a solenoid valve at the CO<sub>2</sub> supply (Fig. 6, SV4). This valve will remain open from 10 to 60 s in response to a preselected, input option in the computer program. The quantity of CO<sub>2</sub> that is supplied to the CER also can be fine-tuned by manual adjustments to a 0 to 1000 ml m<sup>-1</sup> flowmeter (Fig. 6, FM 4). The CO<sub>2</sub> addition then is injected to the CER along the entire length of inlet air ducts on one side of the CER (Fig. 1) to provide an even distribution of CO<sub>2</sub> in the CER. Although in the present configuration CO<sub>2</sub> concentration is being controlled in only one CER, the system is designed for simultaneous control of up to four CER's. Because of the time lag of sampling the air from multiple CER's, precision of control would be expected to decline with each additional CER under the control of the system.

#### Automatic and manual calibration of the $CO_2$ control system

A feature designed into the  $CO_2$  control system is its ability for automatic cali-

bration. This is done once or twice during every 24 h period in response to an input option in the computer program. During autocalibration, the computer temporarily shuts down the control function and opens, in sequence, solenoid valves which supply either zero gas(N<sub>2</sub>) or span gas (950 ppm CO<sub>2</sub>) to the IRGA (Fig. 6, SV2 and SV3). If the calibration gases are determined to be within 5 ppm of their true concentrations, the program switches back to its control function. If the concentration of the calibration gases are determined to deviate more than 5 ppm from their true value, a signal is sent to a stepper control board via the D/A output board (Fig. 6). The stepper control initiates an increase or decrease in the calibration motor drives on the IRGA. This process is then repeated until the determinations of the concentrations for the calibration gases are within 5 ppm of their true values.

The system also can be calibrated manually by using keyboard entries to put the control function on hold and by activating a manual enable override. The solenoid values of the calibrating gases then can be operated with switches which manually override the solenoid control board. Likewise, the stepper control board is overridden by manual switches which allows the zero and span concentrations of  $CO_2$  to be adjusted on the IRGA.

#### Plant culture

Soybean [*Glycine max* (L.) Merr. cv Ransom] seedlings were transferred into the hydroponic chambers after four days of germination on germination paper at 25°C. Each seedling was held in place in the top of the root cells (Figs. 1, 3) by a foam disc. Four seedlings were placed in each root cell, for a total of 48 plants per chamber. The nutrient solution on which the plants grew initially contained 1.0 mM NO<sub>3</sub><sup>-</sup>, 0.25 mM H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 1.25 mM K<sup>+</sup>, 0.5 mM SO<sub>4</sub><sup>-</sup>, 0.25 mM Ca<sup>+</sup>, 0.25 mM Mg<sup>+</sup>, 19  $\mu$ M B, 3.7  $\mu$ M Mn, 7.2  $\mu$ M Cl, 0.3  $\mu$ M Zn, 0.13  $\mu$ M Cu, 0.05  $\mu$ M Mo and 10.0  $\mu$ M Fe (II) as 300 Fe-Sequestrene (Ciba-Geigy Ltd.). Temperature of the nutrient solution was controlled at a set-point of 24°C. The plants were grown at day/night aerial temperatures of 26/22°C and light/dark periods of 9/15 h. The photosynthetic photon flux density in the CER during a 9 h light period was 700 $\pm$ 50  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> from a combination of cool white fluorescent and incandescent lamps (1). The 15-h dark period was interrupted after 6 h by the incandescent lamps for a 3 h period to suppress floral initiation (3). The aerial CO<sub>2</sub> concentration was controlled at a set-point of 400 ppm.

During a pretreatment period of 9 days until the plants had attained the third trifoliate stage, the nutrient solution in the reservoir was replaced once. On day 0 of the treatment period, the nutrient solution from both the root compartments and the reservoir was drained and replaced with fresh solution.

On day 0 of the treatment period, 4 plants were harvested randomly from each chamber, and the plants were thinned to 3 plants per cell (36 plants per chamber). Thereafter, 4 plants from each chamber were harvested at 3- to 4- day intervals over a 3-week period. Each plant was divided into leaves, stems plus petioles, and roots. Number of leaflets were counted and leaf area was determined with a LI-COR LI-3000 area meter (LI-COR, Lincoln, NE). The plants were frozen at  $-20^{\circ}$ C until they were freeze dried. The plant parts then were weighed and ground in a

Wiley mill (40 Mesh). The ground plant material was analyzed for total nitrogen by a micro-Kjeldahl technique which included a predigestion with salicylic acid for more efficient recovery of  $NO_3^-$ -N.

On harvest days, half the nutrient solutions were replaced in each chamber by draining and refilling the reservoirs with fresh solution. Every day during the experimental period, a sample of nutrient solution from each chamber was analyzed for the concentrations of  $NO_3^-$  and  $H_2PO_4^-$  with a Dionex Model 10 ion chromatograph (Dionex Corp., Sunnyvale, CA) using an AS4A anion separator column. After determining the concentrations of these ions, addition of stock solutions of 1.0 M  $Ca(NO_3)_2$ , 1.0 M  $Mg(NO_3)_2$ , and 1.0 M  $KH_2PO_4$  were made to bring the concentrations were again sampled and analyzed to verify the concentrations of  $NO_3^-$  and  $H_2PO_4^-$ .

#### **RESULTS AND DISCUSSION**

To test the performance of the control systems, the spatial and temporal variations were measured for temperature and ionic concentrations within and between hydroponic chambers. Variations in  $CO_2$  concentration and temperature were also measured within the CER. Measurements were made both before and during the growth of vegetative soybeans in the chambers. Additionally, for an indicator of the uniformity of growth conditions among chambers, leaf number and area, plant dry weight, total N content of plants, and  $NO_3^-$  depletion from nutrient solutions were determined for populations of soybeans which were grown in the hydroponic chambers.

#### Mixing time of the nutrient solutions within chambers

Before soybean seedlings were transferred into the chambers, the time required for complete mixing of the nutrient solution in the chambers was evaluated. A short mixing time is important to prevent gradients of nutrient concentrations, pH, or temperature within the root compartments which could result in non-uniform root environments.

Since the flow rate of the solution pumps for the chambers is 161 min<sup>-1</sup>, the nominal turn-over time of the entire 2001 of solution is 12.5 min. However, due to the multiport design of the supply lines to the root compartment which results in individual supply lines feeding each of the 12 root cells within the root compartments, the mixing time apparently is faster than would be indicated by the turnover time.

Mixing time was evaluated by first filling the hydroponic chambers with the nutrient solution described in the previous section. Stock solution of  $NO_3^-$  and  $H_2PO_4^-$  then were made to the reservoir in quantities that would increase the concentration of these ions in the total 2001 of solution by 25% to 1.25 mM  $NO_3^-$  and 0.313 mM  $H_2PO_4^-$ . Samples were taken from a root cell in each of the hydroponic chambers at 2 min intervals after the addition of the stock solutions to determine when the solutions equilibrated at the higher ionic concentrations. In all chambers, the steady-state concentrations of  $NO_3^-$  and  $H_2PO_4^-$ , and thus complete mixing,

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Fig. 7. Air temperature of the CER and solution temperature in the four hydroponic chambers over a 24-h period. Increase in the air temperature during the night period is due apparently to absorption of radiation by the thermocouple from the incandescent lamps which interrupt the dark period.

occurred within 12 min after the additions. The time is sufficiently short to suggest that sustained gradients of  $H^+$  (pH), nutrient concentrations, and temperature which could effect whole-plant growth do not exist in the chambers.

#### Temperature gradients within hydroponic chambers

Temperatures of the nutrient solutions were measured at 18 sites to determine if a temperature gradient existed within the root cells of the root compartment. Temperatures were measured in a root cell at 9 points on a  $30 \times 39$  cm grid system located on a plane 2 cm below the surface of the nutrient solution and again on the same grid system located 2 cm above the bottom of a root cell. The temperatures were measured in series by a single thermocouple connected to a computer system which recorded the temperature 12 times over a min at each point and gave the average and range of the readings. No temperature gradients were observed in the root cell either on the vertical or horizontal planes. The temperature range around the control setting of  $24^{\circ}$ C was  $\pm 0.5^{\circ}$ C.

Temperatures were also recorded in the same manner at single points in root cells at opposite ends of a hydroponic chamber to determine if a temperature gradient occurred along the length of the chambers. The averages of these two readings were within  $0.2^{\circ}$ C, indicating that a gradient among the cells did not exist.

Temperatures also were recorded to determine if the diurnal change in the aerial temperature of the CER ( $26^{\circ}C day/22^{\circ}C$  night) affects the capacity of the control system to maintain the temperature of the nutrient solution at a constant  $24^{\circ}C$ . A thermocouple was placed in each hydroponic unit and the computer scanned each thermocouple 12 times per min. The computer recorded the average temperature for 5 min intervals over a 24-h period. The control systems maintained constant temperatures of the nutrient solutions throughout the diurnal period (Fig. 7).

#### $CO_2$ concentration gradients in the CER

To determine whether  $CO_2$  concentration gradients occurred in the CER, 24 sites above the hydroponic chambers were monitored before and during the growth of plants. Samples were analyzed 5 and 65 cm above both ends and middle of each

of the four hydroponic chambers before seedlings were transferred into the chambers and at weekly intervals during the growth of the plants. During the growth period, sampling was begun at 3 h after the beginning of the light period. The air was sampled by a pump at a flow rate of  $1 \ 1 \ min^{-1}$  and analyzed for CO<sub>2</sub> concentration on an Anarad IRGA in the differential mode. The reference gas for the IRGA was an air-stream drawn from the same site in the middle of the CER utilized for the CO<sub>2</sub> control system. The preset level of CO<sub>2</sub> by the control system was 400 ppm.

In the empty chamber, no concentration gradients of  $CO_2$  were observed either in the vertical or horizontal plane. In fact, the air above the chambers was found to be within 1 ppm  $CO_2$  (detection limit of the IRGA) of the reference concentration (similar results were found when CO<sub>2</sub> concentration in the empty CER was controlled at 900 ppm). As well, concentration gradients of  $CO_2$  were not evident in the CER during the growth period of the plants. As would be expected, the variability for the  $CO_2$  concentrations increased when plants were present. No doubt this was due primarily to the proximity of the sampling tube to a leaf. Still, the maximum variability observed was within a range from -11.0 to +5.0 ppm of the reference gas. This indicates that the  $CO_2$  distribution line which runs along the length of the air inlet ducts and the air circulating system in the CER was adequate for uniform mixing of the  $CO_2$  injection into the CER. In contrast, when  $CO_2$  was injected through a single point source near the front of the CER, the CO<sub>2</sub> concentration decreased by as much as 100 ppm from the front to the back of the CER when actively photosynthesizing plants were present. The method of CO<sub>2</sub> distribution must be considered by researchers attempting to maintain uniform levels of  $CO_2$ within CER's.

#### Range of $CO_2$ concentrations in the CER

The CO<sub>2</sub> concentrations maintained in the CER were recorded in 1 min increments by the  $CO_2$  control system during the period of plant growth. These data showed that, during the light period when the CER was undisturbed, the  $CO_2$  concentration was maintained within a range of  $\pm 10$  ppm of the set-point of 400 ppm. Because a CO<sub>2</sub> scrubbing system was not included in the CO<sub>2</sub> control unit, CO<sub>2</sub> concentrations increased when someone entered the CER due to the person's res-These increases, which were never greater than 70 ppm  $CO_2$  above the piration. set-point, were transitory and the concentration returned to preset level within 30 min. Also, in the absence of a  $CO_2$  scrubbing system, the  $CO_2$  concentration slowly increased during the dark periods due to the dark respiration of the plants. The degree of this increase was dependent on the biomass of the plants. The maximum  $CO_2$  concentration recorded for a single sample at night was 450 ppm. The maximum mean CO<sub>2</sub> concentration for a dark period, was 426 ppm. These increases in  $CO_2$  in the CER during the dark period, and when a person entered the CER, occurred primarily because the CER has been modified to essentially a closedcirculation system. While this closed system exacerbates the increases in  $CO_2$ described above, it allows more accurate control of the CO<sub>2</sub> concentration during most of the light period when plants are photosynthesizing.

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Fig. 8. Number of leaflets (A) and leaf area (B) of soybeans grown in three hydroponic chambers over a 3-week period. Bars equal S.E. of means with n=4.

#### Testing for aerial temperature gradients

The aerial temperatures above the hydroponic chambers were measured at the same 24 sites used for sampling the  $CO_2$  concentrations (see above). The temperatures were measured by a thermocouple within a shielded, aspirated box and a computer system which scanned the thermocouple 12 times per min and recorded the average over a 5-min period. The temperatures were measured 3 h after the beginning of the light period in the CER before the plants had been transplanted into the hydroponic chambers. The average of all the temperatures recorded at 5 cm above the chambers was within 0.1°C of the average of all the temperatures recorded at 65 cm. This indicates that no vertical gradient occurred for temperature within these heights above the hydroponic chambers. The averages of the temperatures from the six positions over each chamber were within 0.3°C of each other. This indicates that no temperature gradient occurred from the front to the back of the CER.

A small temperature gradient, however, occurred along the length of each hydroponic chamber. An average temperature difference of 0.6 and  $1.0^{\circ}$ C at heights of 5 and 65 cm, respectively, above the hydroponic chambers was observed between opposite ends of each chamber. The warmer temperature occurred at the ends of the chambers which were closer to a wall of the CER (Fig. 1). The placement of the hydroponic chambers off the center of the CER is necessary to enable access around the chambers.

The possible consequences of the slight temperature gradient along the length of the hydroponic units are taken into consideration in the experimental design of our studies. Since each hydroponic chamber is assigned to a single treatment, treatment effects are defined between chambers. Plants for harvest are selected at random from a population within each hydroponic chamber. The temperature



Fig. 9. Dry weight of leaves, stems plus petioles, roots, and whole plants of soybean plants grown in three hydroponic chambers over a 3-week period. Bars equal S.E. of means with n=4.

gradient along the length of the hydroponic chambers thus may increase the variability within populations, but since there is no temperature gradient between chambers, the variability among chambers is not affected. The temperature gradient along the length of the hydroponic chambers, however, does impose a limit on the differences on aerial temperatures that can be investigated.

#### Uniformity of growth and N uptake in the hydroponic chambers

Growth characteristics of the population of vegetative soybeans from each hydroponic chamber were measured over the 3-week treatment period. The results from only three of the chambers are given since a pH monitor failed in hydroponic chamber #3 during the treatment period.



Fig. 10. Nitrogen accumulation of plants as estimated by  $NO_3^--N$  depletion from nutrient solution (A) and total N content of plants (B) for soybeans grown in three hydroponic chambers over a 3-week period. In (B), bars equal S.E. of means with n=4.

As indicated by leaflet number (Fig. 8A), leaf area (Fig. 8B), and plant dry weight (Fig. 9), the growth of the plants among chambers was very uniform. Even with a sample size of only four plants per chamber per harvest, the percent standard error for the means of almost all data points was less than 10%. Also, by the end of the 3-week treatment period, no significant differences at a 0.95 probability level occurred for any of the measured characteristics among the populations.

Nitrogen uptake by plants, as measured by both cumulative depletion of NO<sub>3</sub><sup>-</sup> from the replenished solutions (Fig. 10A) and tissue analysis of harvested plants (Fig. 10B), also was similar among the hydroponic chambers. The data for cumulative NO<sub>3</sub><sup>-</sup> depletion can be converted to total N content by addition of the 636 mg N that had accumulated in the plants sampled at Day 0 (Fig. 10B). The converted data for NO<sub>3</sub><sup>-</sup> depletion and the data from tissue analysis can be used to calculate the relative accumulation rate of nitrogen (RARN). The RARN is equivalent to the slope (b) of the regression equation  $\ln N = a + b$  (day), where N is mg N plant<sup>-1</sup>. The RARN for plants which were grown in chambers 1, 2, and 4, respectively, were 0.125, 0.125, and 0.124 mg N · mg<sup>-1</sup> N · day for the converted depletion data and 0.125, 0.123 and 0.123 mg N · mg<sup>-1</sup> N · day for the tissue analysis data. The closeness of agreement between these methods show that the daily changes detected in depletion of ions from replenished solutions are valid indicators of changes in net uptake activity by roots.

The uniformity of the growth and N uptake characteristics of the vegetative

soybeans grown in the hydroponic chambers is a practical indicator of the uniformity of growth conditions in our culture system. With this uniformity, we can be confident that differences seen between treatments in experiments run in this facility (4, 5, 6) are true treatment effects.

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