CO_2 RETENTION AND APPLICATION FREQUENCY OPTIMIZES YIELD PER GAS USED IN HYDROPONIC LETTUCE

RETENCIÓN DE CO_2 Y FRECUENCIA DE APLICACIÓN OPTIMIZA PRODUCCIÓN POR UNIDAD DE GAS USADO EN LECHUGA HIDROPÓNICA

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ABSTRACT

CO_2 injection can be used to enhance plant growth and it was used in hydroponic lettuce cultivation. Anti-aphid mesh covered lettuce plants to retain CO_2 and to prevent its dissipation into the surroundings. A spatial temporal study of CO_2 concentration between lettuce leaves was carried out, showing the effects of fan air circulation driven by a fan. One and four CO_2 injections per hour were applied to lettuce grown in PVC tubes inside tunnels. Gas concentration was 25 % higher with multiple injections when the mesh covered the lettuce plants. Multiple injections helped to optimize CO_2 waste, and lettuce weight using the mesh increased 87 % with respect to control lettuce weight produced without mesh. Single injection increased lettuce weight by 35.5 % without mesh.

Additional key words: CO_2 injection, lettuce growth, hydroponics, anti-aphid mesh.

RESUMEN

La inyección de CO_2 induce crecimiento en las plantas y fue usado para producir lechugas en hidroponia. Una malla anti-áfidos cubrió las lechugas para retener el CO_2 y evitar su disipación al medio ambiente. Un estudio espacial y temporal de la concentración de CO_2 entre las hojas de lechuga se realizó, mostrando los efectos de la circulación del aire causados por el extractor. Una y cuatro aplicaciones de CO_2 por hora fueron aplicados a la lechuga producida sobre tubos de PVC dentro de túneles. La concentración del gas fue 25 % mayor con aplicaciones múltiples cuando la malla cubría las lechugas. Aplicaciones múltiples optimizaron el consumo de CO_2, incrementando el peso de la lechuga 87 % con respecto al control que no usó malla. El aumento de peso para una sola aplicación de CO_2 sin malla fue del 35.5 %.

Palabras clave adicionales: inyección de CO_2, crecimiento de lechuga, hidroponia, malla anti-áfidos.

INTRODUCTION

Mexican lettuce exports to the US increased from 7 thousand metric tons in 2000 to 52 thousand metric tons in 2006 (Global Trade Atlas, 2007). One way of meeting market increase trends is by enhancing plant growth with injections of CO_2 into the ambient air or irrigation water. Doubling CO_2 concentration from 300 to 600 ppm increases growth rates of most non woody plants by about 33 % (Kimball, 1983). Kretchmann and Howlett (1970) recorded a 10 to 20 % increase in lettuce yields using atmospheric CO_2 injection. Lettuce with aerial CO_2 injection for one hour periods at concentrations ranging between 800 and 1000 ppm increased fresh mass by 23.63 % (Costa, 2001).

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Metabolic activity of plants enriched with CO_2 through irrigation increased flower and fruit production (Durao and Galvao, 1995). Monthly use of 155 kg·ha^{-1} CO_2 via irrigation water increased lettuce production by 20.5 % when compared with lettuce grown under normal conditions (Gomes *et al*., 2005). Furlan *et al.* (2001) used irrigation water enriched with CO_2 and reported an increase in lettuce head diameter, number of leaves and yield of heads of 15.9, 5.55 and 28.8 %, respectively, relative to plants without CO_2 application.

In places with high levels of radiation, CO_2 can be applied throughout all the day using intermittent CO_2 cycles within a closed loop in order to save gas. Zipori *et al*. (1986) applied gas until the greenhouse reached 28 ºC. Afterwards of an aeration period of 10-20 minutes and if the temperature was below 24 ºC, CO_2 was injected again reaching 1000±200 ppm. Tomato yield increased with intermittent carbon dioxide application. Mortensen (1984) applied intermittent one hour CO_2 cycles to *Chrysanthemum*, followed by one hour without carbon dioxide enrichment. Dry matter obtained with this technique was almost similar to the continuous carbon dioxide enrichment treatment. Alscher and Krug (1989) controlled CO_2 cut off when wind velocity was over 3 m·s^{-1} and/or irradiance was greater than 30 W·m^{-2}. Gas cut off resulted in an extended lettuce growth period of 6 days and did not present savings with respect to continuous gas application.

Another way to save gas could be through the use of meshes to act as an extra barrier on the air movement. For roll-up openings without screen and for a wind direction parallel to the greenhouse axis, both computed and simulated values show that air speed has relative high values near the openings and reduced values near the center of the greenhouse (Campen and Bot, 2003). The use of an antibemisia screen reduces the mean air velocity inside the greenhouse by 30 % and the use of an anti-aphid screen by 70 % compared to the values of air velocity for a greenhouse without screen (Bartzanas *et al*., 2004). Temperature and humidity distribution inside the greenhouse follows the air velocity profile and in regions with small air velocities the air was warmer and more humid compared with the air in regions with high air velocities (Linker *et al.*, 2002).

This paper analyzes the effect on lettuce weight, leaf area index and root size of single and multiple CO_2 injections through a drip tape running inside a PVC hydroponics recirculating system. CO_2 is a volatile gas and its contact time with lettuces has to be increased so a system was designed to place and remove a mesh to increase CO_2 retention and concentration between leaves. Multiple CO_2 injections per day were provided to increase lettuce yield per CO_2 used.

MATERIALS AND METHODS

Experimental setup

Two plastic tunnels 6 m long, 2 m wide and 1.7 m high were built at the Plant Science Experimental Field of the Universidad Autonoma Chapingo. Two 10 cm-diameter PVC tubes (6 m long) were fixed in each tunnel at a height of 1 m with a slope of 5 %. An "Aqua Traxx" drip tape (Del Toro, Inc.), was fixed inside each PVC tube just over the nutrient solution for CO_2 injection (Figure 1a). Drip tape specifications were 0.75 L·h^{-1} outflow at an operating pressure of 10 psi.

A cylinder stored CO_2 and used a solenoid valve distribute the gas. Between the solenoid valve and the cylinder a butterfly valve, a high pressure regulator, a flow meter, and a pressure manometer were installed in series. CO_2 was injected into the drip tape at a rate of 0.5 L/plant/day for one month. This injection technique was named IPT (internal PVC treatment).

Because CO_2 is a volatile gas, a mechanism was designed to cover the lettuce plants with an anti aphid mesh which worked as a diaphragm retaining the gas surrounding the plants (Figure 1b). Two pistons were fixed from the top of the tunnel to move the stretched mesh toward the PVC tube (approximately 40 cm). A compressor provided the air to activate the pistons through electro valves; the mesh covered the lettuce 5 seconds before gas injection stopped. Ten minutes later, a timer turned on the electro valve and the air left the pistons making the mesh rise to the top of the tunnel. The electro valves were protected against power outages with a 12 V motorcycle battery. Air compressed in the tank lasted 5 days of piston operation. Because this mechanism reduced radiation within the ITP tunnel, a mesh was installed at the top of the tunnel containing the control treatment (no CO_2 injections).

The system had to control the four operations: nutrient recycling, gas application, fan control and mesh placement and removal. The four operations had to be synchronized for proper operation. A more sophisticated timing routine was required to start and stop the fans to control temperature and prevent moisture from forming during multiple injections. Ten minute cycles were required as injection + retention take 2.5 + 7.5 minutes.

Plant management

Lettuce var. *Coolguard* seedlings were produced in trays supporting twenty plants each with peat as substrate. Lettuce

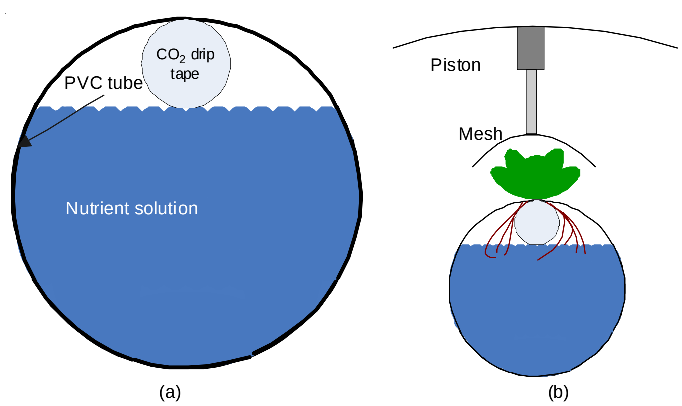

Figure 1. CO_2 (a) applied inside the PVC, and (b) piston-mesh mechanism.

seedlings were transplanted 30 days after sowing to small plastic funnels spaced 30 cm apart on the topside of the nutrient recirculating PVC tubes. For each experiment new plants were planted in both tunnels.

The solution was prepared by mixing 180 L of water with the following fertilizers: 116 ppm potassium nitrate, 216 ppm calcium nitrate, 64 ppm magnesium sulfate, 1.94 ppm ferrous sulfate, 0.72 ppm copper sulfate, 0.5 ppm manganese sulfate, 0.22 ppm zinc sulfate, 0.67 ppm boric acid and 53 mL phosphoric acid. The solution kept in a tank was recirculated by a pump every 15 minutes during the day and for 15 minutes every two hours during the night. However, to prevent CO_2 displacement to the tank by air turbulence caused by nutrient solution recirculation, the cycle was modified to pump 10 minutes and rest 20 minutes. Two CO_2 injections were applied during pump turn off in the multiple injection treatment.

Experimental methodology

To study gas distribution during injection, 23 days after transplant, two lettuce plants were selected: one near the fan extractor and another near the opposite end of the tunnel. CO_2 concentration measurements were taken under different gas flows with and without mesh cover (Figures 2a and b). CO_2 concentration was first measured without air movement, beneath the first leaf, between first and second leaf and between the second and third leaf of the lettuce plant closer to the fan. The first leaf was the one closest to the PVC tube (Figure 2a). On other hand Figure 2b shows CO_2 retention measurements (with mesh) at the same site on the two selected lettuce plants samplinh the air were taken every second. Gas measurements were obtained three times with a CO_2 probe (model CO2-BTA, Vernier®) and stored in a data logger (model LabPro, Vernier®). The three measurements were averaged and plotted.

Position of tape drip holes relative to those made in the PVC tubes, where the lettuce plants grew, varied during installation. The effect on IPT lettuce growth, according to drip tape displacement with respect to the PVC hole, was analyzed. Also, temperature, pH and relative humidity (RH) were measured daily in each tunnel. Temperature and relative humidity were

Figure 2. Probe measuring CO_2 in the leaf (a) without mesh and (b) inside the mesh.

acquired with a sensor (model H7625B1006, Honeywell), while nutrient solution pH was measured with hand equipment (HI 98130, Hanna).

The experiment considered two types of carbon dioxide application: one hourly application and several applications per hour. The hourly application for single injection lasted 10 minutes, while hourly multiple injection cycles were twice for 5 min each and 4 times for 2.5 min each, from 9 AM to 3 PM. The retention curve of Figure 4 at 40 $L \cdot min^{-1}$ and covered with mesh was used for the analysis helping to simulate the gas concentration under each treatment.

All lettuce heads were weighed daily, their roots measured with a ruler and leaf area determined with a color camera. Leaf area was the 2-D area covered by the lettuce leaves. The image file was imported by the ImageJ software and the area was calculated. Measurements were obtained from lettuce grown in both tunnels with and without mesh and compared. Data were analyzed with correlations between transplant and harvest lettuce weights, root lengths, and leaf area indexes using SAS software (ver. 9, 2008). Maximum and minimum values of root size, leaf area index and lettuce weights were obtained for each treatment and the standard deviation calculated.

RESULTS AND DISCUSSION

Tunnel environment

Temperature and relative humidity (RH) were sampled above lettuce leaves. Atmospheric temperature inside the IPT tunnel was two degrees higher than inside the control tunnel where no CO_2 was injected. Figure 3a shows that during the first two days after transplanting when lettuce seedlings had four true leaves, temperature differences between control and uncovered IPT lettuce atmosphere reached 1.5 °C. After the 17th day, temperature was analogous for the three treatments.

Relative humidity inside the IPT tunnel where CO_2 was injected was 15 % higher than inside the control tunnel. Relative humidity arrived to 45 % RH (Figure 3b), which caused lettuce to yellowing. For this reason, a fan was introduced to reduce moisture and presence of fungi. The fan was turned on for 20 minutes before CO_2 application and turned off when gas was injected. Once the moisture within the IPT tunnel was extracted, a relative humidity of 30 % was measured.

It is important to note that carbon dioxide was not inside the nutrient solution, and when applied it moved by convection towards the lettuces. Although Aqua Traxx drip tape was designed for use with water, CO_2 gas injection was uniform, and no pressure reduction was observed. However, CO_2 tank pressure had to be reduced to avoid tape damage. The tape can be used below mulches, and holes on the plastic should coincide with the drip tape apertures.

The pistons worked properly, moving the mesh to cover the lettuce plants without damaging them. Water drops appeared below the mesh due to plant evapotranspiration as air movement decreased. Water drops disappeared when the stretched mesh was spaced 10 cm from the lettuce plants. On the 13th day electricity failed so air within the compressor moved the pistons and the recirculating pump did not work, but lettuce continued to grow. Although pistons can work continuously for 5 days, dissolved oxygen in the nutrient solution decreases and lettuce growth can be retarded. A pneumatic pump could be the solution for places where power failures are continuous. Another option could be to design a more intelligent system that "remembers" when power fails and applies water and gas injections at another moment during the day.

Previous experiments showed that, when the mesh came into contact with lettuce heads, quality was lost due to friction, so any contact between the anti-aphid mesh and plant leaves was avoided. If no anti-aphid mesh is available, fiberglass mesh, common in house windows, can be used instead.

CO_2 concentration in lettuce plants

When the solenoid valve was closed, CO_2 gas concentration under the lettuce decreased to ambient CO_2 concentration in lettuce plants (300 ppm). Figure 4 shows that a flow of 30 L·min^{-1} during normal gas application resulted in a CO_2 concentration of 1000 ppm having a peak at 1050 ppm. No exceeding gas was present one minute after stopping the application. The valve was opened to provide 40 L·min^{-1} of CO_2 for a concentration of 5020 ppm under the first lettuce leaf, and

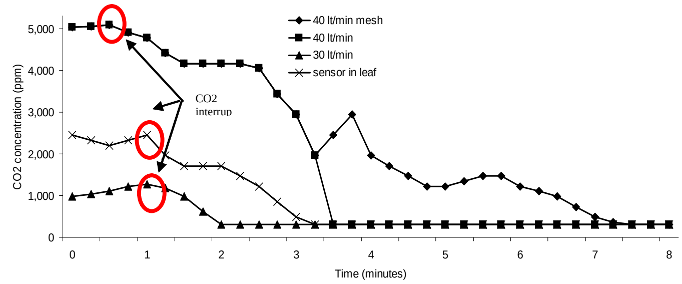

Figure 4. CO_2 concentration measured under different flows with mesh.

took 3.5 minutes to reach 300 ppm after stopping CO_2 application. The CO_2 retained by the mesh, shown by the line with the square ticks, took 7.5 minutes to achieve the ambient CO_2 concentration of 300 ppm.

The effect of the fan can be observed in Figure 5 when the sensor was placed beneath the first leaf during CO_2 injection and CO_2 interruption. The mesh retained 6 % more CO_2 during injection. However, gas retention had an unusual peak which varied according to plant location within the tunnel. The retention peak of the plant closer to the fan was 82.8 % higher than that of the plant located farther from the fan. Maximum CO_2 concentration of 12 % was found near the tunnel end where the fan was located and CO_2 from other plants accumulated. The carbon dioxide sensor measured only 5000 ppm explaining the flat responses of Figure 5.

During gas injection without mesh cover and without fan operation, the bottom leaf received the entire CO_2 dosage. The concentration at this point was 93 % higher than the gas measured between the first and second leaf (Figure 6). It was 356 % higher than that measured between the second and third leaf. Ambient carbon dioxide concentration (300 ppm) was detected five cm above the lettuce head.

After gas injection was stopped, CO_2 was measured to determine retention between leaves. The lowest CO_2 concentration was found beneath the first leaf. Gas concentration,

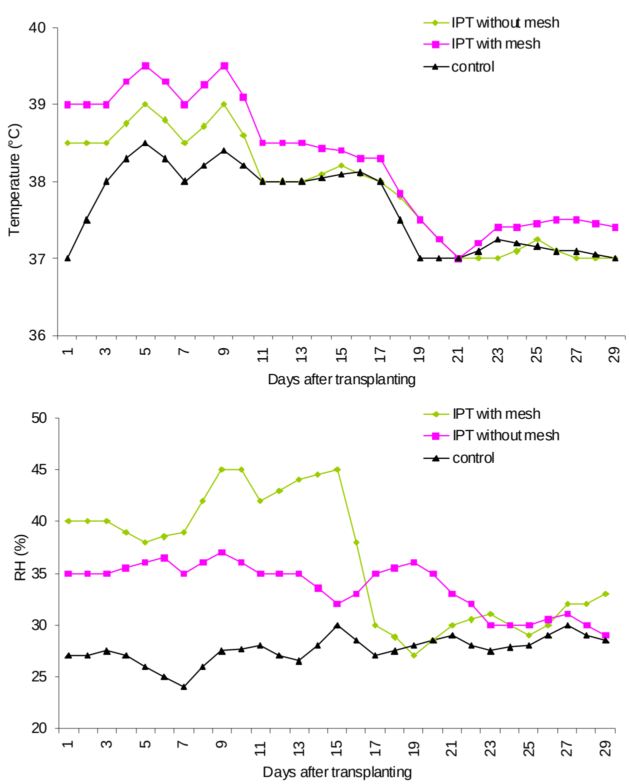

Figure 3. Daily (a) temperature and (b) relative humidity obtained for control and CO_2 injection treatments.

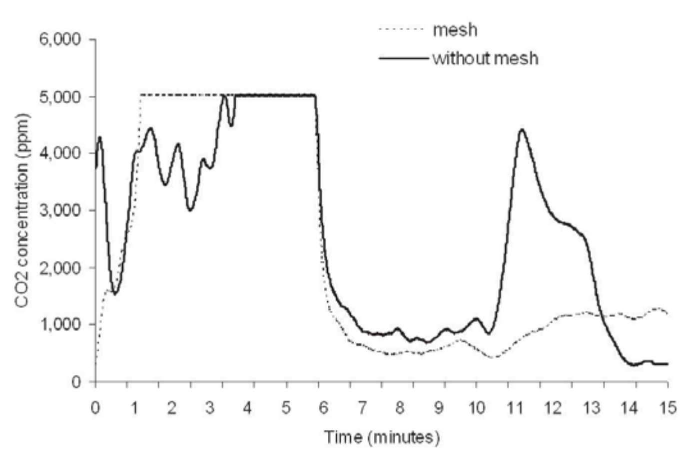

Figure 5. CO_2 concentration measured on the lettuce plant closer to the fan covered and uncovered with mesh after fan application.

determined as the area below the curve, was 203 % higher between the first and second leaf and 416 % higher between the second and third leaf. After integrating injection with retention periods, the gas below the first leaf was 63 and 90 % higher than the gas concentration between the first and second leaf and between the second and third leaf, respectively.

Gas distribution between lettuce leaves differed and was not uniform, as it is in lettuce plants which received CO_2 injected through irrigation water (Gomes *et al.*, 2005). CO_2 dosage increases nutrient uptake and water, so application changes might cause differences in leaf nutrients (Andriolo *et al.*, 2006; Buwalda and Warmenhoven, 1999). However, leaf edges did not burn out (turning brown) as reported by Costa (2001) when applications reached concentrations of 3600 ppm.

Lettuce yield with single CO_2 injection

Figure 7 shows that IPT lettuce without mesh weighed 35.55 % more than control. Two slopes, which show how growth under the three treatments becomes markedly differentiated, are clearly noted on the three curves: one lasting from the 9th to the 19th day, and another from the 20th to the 29th day. Growth from the 9th to 19th day is critical, affecting final harvest weight. The control lettuce slope was the smallest with a value of 0.142, while IPT with mesh had the steepest slope, 0.2. After the 20th day slopes were similar for each of the three treatments.

When lettuce head diameter reached 6.5 cm (9th day) temperature differences decreased to 0.5 °C due to evapotranspiration. After the 17th day, lettuce head diameter was over 13 cm and evapotranspiration decreased the temperature in both tunnels; relative humidity was maintained at 30 % RH with fan operation. It is unknown whether all the CO_2 entered the stomata or if leaves bounced the gas back to the tube. CO_2 measured in the nutrient tank was 450 ppm which is insignificant, compared with measurements of 6000 ppm during nutrient recycling.

Figure 8 shows a correlation plot between transplant weight and final lettuce weight. The slope of IPT lettuces (with mesh) is 7.67, while the control lettuce slope is 6.0. For example, a lettuce plant weighing 6 g at transplant produced a final weight of 64 g under the control treatment, while a 6 g plant transplanted to the IPT treatment was harvested weighing 110 g. Under both treatments a 2 g of difference at transplant ended in a final weight gain of approximately 15 g.

Homogeneous transplant size is required to obtain harvest lettuce uniformity for proper packing and marketing. Thus, when averaging lettuce weights, transplant lettuce weight must be considered in order to reduce standard deviations. The statistical transplant size variance of 15 affected final harvest weight (variance = 67), and although an initial difference of one gram among plants may not seem important, it causes harvest differences of 12 to 15 g. The buffer effect that CO_2 has in water

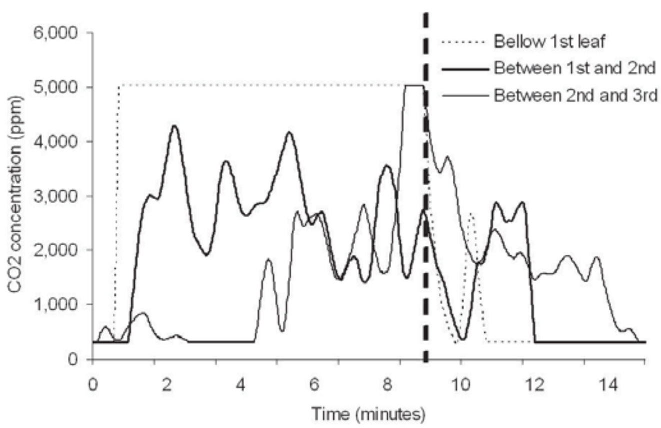

Figure 6. CO_2 concentration measured between leaves during (0-9 min) and after (9-15 min) injection.

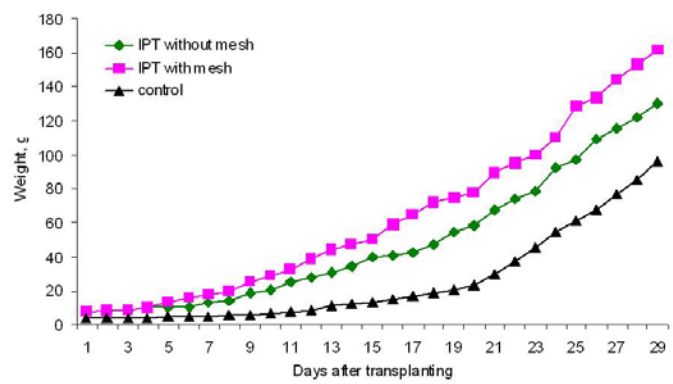

Figure 7. Daily lettuce weight obtained for control and IPT injections.

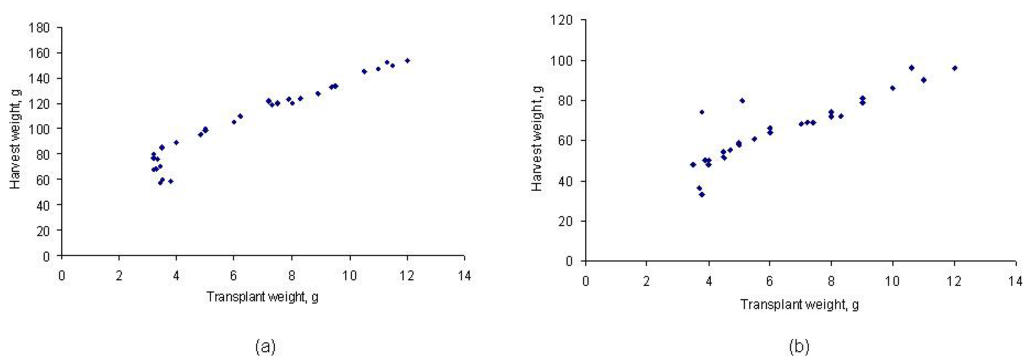

Figure 8. Relationship between transplant and harvest weight for (a) IPT and (b) control lettuce plants.

Table 1. Root length, fresh weight and leaf area (FA) after 27 days of growth with and without mesh.

Variable	Treatment								
	CO_2 without mesh			Control			CO_2 with mesh		
	max	min	Std dev	max	min	Std dev	max	min	Std dev
Root (cm)	21	11	1.95	29	22	1.19	20	11	1.98
Weight (g)	138	126	1.96	107	89	1.41	170	155	1.22
LA (pix^2)	115	97	2.55	117	97	2.21	117	95	2.35

solutions was not considered in this analysis.

Even though root and weight variables varied greatly among the treatments, leaf area calculated on harvested lettuce was similar in the three treatments (Table 1). During CO_2 injection, stomata close and transpiration decreases (Kimball, 1983; Cure and Acock, 1986). Morison and Gifford (1984) found that increasing ambient CO_2 increases leaf area. In our study, however, leaf area was not proportional to weight. An R^2 of 0.43 was obtained between harvest weight and harvest leaf area, showing that instead of leaf area, leaf thickness increased. Leaf area index showed the highest differences between lettuces even in the control treatment and can be correlated to radiation variations through the tunnels as well as non-homogeneity in the tunnel HR. It is interesting to note that the effect was similar in the three treatments having similar maxima and minimum values. Thus, leaf area cannot be used as a marketing parameter.

Standard deviation was obtained from each treatment and lettuces covered with the mesh presented a more uniform weight, resulting in a standard deviation of 1.22. It is interesting to note that the mesh keeps a continuity of gas present through the plant stomata. The highest standard deviation was obtained with gas application without the mesh. The variability was caused by differences in CO_2 application due to air movement and mismatch between dripper and PVC apertures. When lettuces grown under these circumstances were removed, standard deviation decreased to 1.32.

IPT lettuce roots exhibited a similar growth pattern with and without mesh and were shorter than control lettuce roots. Average control lettuce root length was 26.3 cm at harvest, while IPT lettuce roots were 16 cm long. It was noted that IPT root tips turned dark brown and were in general darker in color than control lettuce roots. Root growth depends on the solution and CO_2 affects it, showing a higher variability than in the control as can be noted on the standard deviation value encountered in Table 1. The R^2 regression value between root length and lettuce weight was 0.23.

Lettuce grown under the IPT technique was crispier than control lettuce and the bottom leaf tended to roll over the central leaves. For this reason, IPT lettuce bottom leaves were not removed as on control lettuce, thus, reducing labor.

Gas was removed after the 29th day for four days, and IPT lettuce heads dehydrated loosing approximately 28 % of their weight. Once the gas was reapplied, weight was recovered in 15 hours. Kaufman (2000) mentioned that lettuce bagged for salads wilt easily, but a CO_2 atmosphere could preserve the vegetables after packaging.

Table 2. Variation in average lettuce weight with relative coincidence between dripper and PVC apertures.

Ventilation	Mesh	Avg. lettuce weight (g) by dripper mismatch distance: 0 cm	5 cm	10 cm	15 cm
without	no	130	125	117	109
without	yes	162	139	127	114
with	no	147	119	113	103
with	yes	149	121	114	102

Coincidence between drip tape CO_2 emission holes and PVC holes affected yield. In lettuce plants with mesh without ventilation, average weight was 162 g when the drip tape CO_2 emission hole matched the PVC hole (Table 2). When the drip tape CO_2 emission hole was displaced 15 cm from the PVC hole, average lettuce weight was smaller, 114 g, but was still 15 g heavier than the average control lettuce weight. Smaller weight differences were found with the use of the mesh when ventilation was activated.

CO_2 remaining within the tube due to hole and dripper mismatch was transferred to the tank during nutrient solution recycling. The nutrient solution movement causes air turbulence and new air is sucked through the PVC holes taking with it the CO_2 in the tube. CO_2 measured in the nutrient solution tank was 5,000 ppm, corresponding to the maximum value measured by the probe.

CO_2 in the nutrient solution can affect pH. In our study IPT nutrient solution pH decreased from 6 on the first day to 5.6 twenty days later, but CO_2 inside the tank over the nutrient solution did not further affect pH. D'Andria *et al.* (1990) applied CO_2 in water and found that pH values decreased from 6.4 to 4.5.

Lettuce yield under multiple CO_2 injection

As CO_2 retention is considerable with mesh, multiple injection cycles were tested in order to obtain optimum applied CO_2 optimization. The gas applied on the three experiments was

Table 3. Average lettuce weight achieved under different CO_2 injection frequencies.

Injection frequency cycles (per h)	CO_2 Simulated concentration with mesh, ppm	Average lettuce weight (g) and standard deviation			
		With Mesh weight	With mesh Std dev	Without mesh weight	Without mesh Std dev
1 for 10 min	98,434	162.1	1.22	135.8	1.96
2 for 5 min	106,667	171.2	1.15	136.2	1.98
4 for 2.5 min	123,125	186.3	1.07	136.4	1.93

the same, but the concentration applied to the lettuces was different. The concentration value was not measured, and its value was obtained from simulation increasing when the mesh was used. However, CO_2 concentration in the treatment having 4 cycles per hour increased by 25 % with respect to a single injection of 10 min per hour and lettuce weight increased by 15 % with mesh.

It is important to mention that the control plants did not receive any gas application and that they appear on Table 3 only as a comparison tool. Average IPT lettuce weight at harvest with mesh in the 4 x 2.5 min treatment was 87 % higher than average lettuce weight of the control, and 15 % higher than harvest weight with a single CO_2 injection. Lettuce weight with the 2 x 5 min treatment was 70 % higher than control lettuce weight. Previous studies (Zipori *et al.*, 1986; Alscher and Krug, 1989; Mortensen, 1984) present closed loops for controlling intermittent CO2 enrichment. In all of these studies the application periods were longer, and only Zipori *et al*. (1986) reported higher yields in a tomato crop. No moisture problems with pathogens (Brandl and Amundson, 2008) were encountered with the mesh as the timing periods of lettuce cover were short. As the air movement with the mesh decreases (Bartzanas *et al*., 2004) the 4 x 2.5 min treatment lets better transpiration of moisture, reducing probable pathogen diseases.

Similar average weights were obtained with the treatment without mesh under different injection frequencies than in single gas injections. Weight standard deviation under all the treatments was close to 2 as air movement varied along the tunnel (Table 3). The lowest final weight standard deviation of 1.07 was obtained with the 4 x 2.5 treatment using mesh control. It can be understandable as a more uniform gas application takes place and growth is similar after 29 days. However, considerable differences in the treatment with mesh under different injection frequencies were found. This confirms the positive effect of retaining CO_2 around the plant during its growth. Roots with the 4 x 2.5 min multiple injection treatment were 7 % longer than control roots, while with the 2 x 5 min treatment the difference was only 4.8 %.

CONCLUSIONS

It can be concluded that CO_2 is retained within a mesh working as a diaphragm and that multiple CO_2 injections optimize its usage by the plant. Lettuce weight increased 87 and 62 % with multiple and single gas injections, respectively, relative to lettuce with null CO_2 application. Neither root length nor leaf area was a good indicator of harvest weight. Final lettuce weight was proportional to transplant lettuce weight and to the CO_2 injected between the 9th day and the 19th day. During injection, concentration was higher below the first leaf and decreased toward the third leaf. Once interrupted, CO_2 traveled through plants and the fan was responsible for higher concentration between the 2nd and third leaf. The stretched mesh spaced 10 cm over the lettuce heads avoided water drops from forming. Although tunnel temperature reached 39 °C, it did not limit lettuce growth. Maximum relative humidity of 45 % was removed by the fans. Mesh cover prevented CO_2 movement from plant to plant and although standard deviation on final lettuce weight was high in most treatments it can be reduced by using intermittent (4 x 2.5) CO_2 enrichment.

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