Micro-bubbles – their contribution to cotton plant performance under a soil less culture medium

by

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Abstract

Cotton plants are extremely sensitive to water logging. Short term water logging associated with wetting front of drip and subsurface drip irrigation can also impede the cotton root functioning under the field conditions. This study examined the effect of aerated water (in the form of micro bubble) on cotton plants water uptake, leaf photosynthesis and growth in the soil less culture medium. The results suggested that exposing the cotton plant to deoxygenated water, similar to hypoxia in the field conditions damage the plants ability to recover upon transferring them to aerated conditions later on. Plant grown in the aerated water with micro bubbles maintained greater leaf photosynthetic rate, transpiration rate, slowed the wilting symptoms and accumulated more plant biomass for same duration of growth. Exposing cotton plants to deoxygenated water not only at the beginning, but also in between the aerated water treatments, exerted sever consequences on cotton plants growth.

Introduction

Cotton in Australia is grown in Queensland and New South Wales and is a valuable commodity in Australian agriculture. The Department of primary industries points out that the majority of cotton grown in Australia is exported to Asia, which plays a major role in the Australian economy. Cotton production in Australia is mostly carried out on cracking grey clay soils that have low drainage rates. This can be a major problem to farmers as cotton is known to be less tolerant to water logging than other farmed plants in Australia. Cotton seeds are usually sown in winter and grown using furrow irrigation, until summer when substantial rainfall from the wet season occurs. This increases the risk of water logging which leads to anaerobic soil conditions. Another contributor to water logging is poor field formation such as, excessive length, poor slope and poor bed formation (Bange, Milroy & Thongbai 2003). An immediate effect of water logging on cotton is the decrease in oxygen in soils, which is required for root respiration and growth and may contribute to substantial yield losses (Thongbai et al. 2001). Reductions in photosynthesis in the leaves and the whole plant and the reduction of nutrient uptake from the roots have also been found

as consequence of water logging on cotton by a number of researchers (Bange, Milroy & Thongbai 2003).

Pervious research in CQuniversity suggested that oxygation- use of aerated water can be useful for overcoming the temporal and spatial hypoxia associated with wetting fronts in drip irrigated crops. Microbubbles increase oxygen levels in water and may be delivered to cotton plants via Sub-surface Drip Irrigation (SDI) to increase oxygen levels in waterlogged soils (Bhattarai, Su & Midmore 2005).Use of pressure differential venturi such as Mazzei air injector was largely utilized on those early studies. The promise of this system is based on an availability of operating pressure in irrigation plot for the air injector. The benefit demonstrated in earlier research with aerated water irrigation by subsurface drip where aerated water is directly injected to the root mass in the wetting front. Majority of the cotton industry in Australia is not under pressurised irrigation rather it is irrigated by furrow. Therefore targeted air injection in the root zone becomes complicated in the case with flood/furrow irrigation system. Therefore, aerating the water sources such as dam, lagoon and use such aerated water with furrow irrigation could potentially open up new opportunities in cotton irrigation. Seair diffusion systems manufacture a type of aeration device that can be utilized to produce proportionately more micro bubbles in water reservoirs that can be utilized independent of irrigation methods. The aim of this experiment was to evaluating the effect of seair diffuser in stability of micro bubble in the water if the rate of cotton plants root respiration would be increased by supplying aerated water consisting of microbubbles introduced by Seair diffuser into the irrigation water sources.

Methods and Materials

Introduction of micro-bubbles estimating its fractions in water

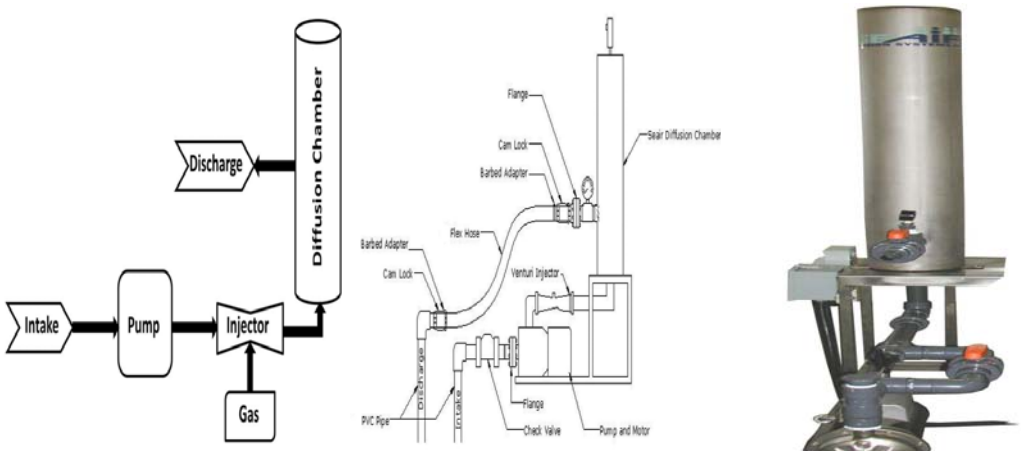

Figure1 : Seair SA-75 diffusion system for air injection into the irrigation water (flow direction, details of the make up and the commercial product for the use by the industry.

A 1478 L capacity water tank was connected with the sea air diffusion system for aerating the water source for irrigation purpose. Tape water available to CPWS compound was used for filling the tank. Tap water recorded an Electric conductivity of 225 µS / cm. The water was aerated for 2 hours before sampling. The pressure differential across the mazzei was maintained at 30-15 PSI and in the outlet end of the seair diffusion chamber it was 10 PSI.

The water samples were collected in 3 L glass bottles inside the water tank and from the tap that used to fill the water tank. All macro-bubble from the glass bottle was allowed to escape so that at the end of sampling period some bottles have only aerated water and others have the tape water only.

These bottles were completely full with the same volume of water (L), and the fraction of micro bubbles in the water was determined on the basis of gravimetric determination based on the weight difference between these two treatments.

The gravimetric determination suggested that the fraction of the micro-bubbles in the aerated water was 4% by volume of the water. This conclusion is based on the observation of the 20 each water bottle samples measured for the control and seair diffuser aerated water. The salinity level and water temperature was maintained as close as possible for the both treatments, hence, air fraction in the sample water is not affected by ions and temperature difference.

Growth cabinet conditions

The growth cabinet was programmed to have a fourteen hour day cycle and a ten hour night cycle. The temperature was set at twenty-eight degrees Celsius during the day and twenty-two degrees Celsius at night. The humidity was set to 80-90% and the light intensity during the day cycle was programmed to be at 400µmol m^2/sec. These were the conditions of the growth cabinet throughout the entire experiment.

Germination

A germination tray was placed into a hydroponic tray and filled with prepared saturated Fytocell. *Gossypium hirsutum* seeds were placed one centimetre deep into the prepared Fytocell. The hydroponic tray was then filled to approximately one centimetre deep with distilled water and placed into the growth cabinet. Once seeds were germinated seedlings were left in the germination trays to grow into established seedlings. The water in the hydroponic tray was maintained at one centimetre deep.

Figure 2. Established roots on cotton plants used in both experiments.

Plant growth

Once seedlings were established they were transferred into pots and placed into the lid of hydroponic boxes. These Styrofoam boxes were lined with large garbage bags and filled with distilled water. Manutec complete hydroponic source was added at half strength in order to provide nutrients to the seedlings and the water was aerated. Lids were placed on top of the hydroponic boxes and sealed tightly. Once the roots on the cotton plants were established (at least ten centimetres long) and the plant had the primary leaves they were used in the experiments (see image 1.).

Experiment set up

Established cotton plants were placed into the lid of a sealed pot that had been filled with the designated treatment water. Oxygen in each treatment was monitored through a PSt3 O2 sensitive fibre optic mini sensor with a Fibox-3 oxygen meter (PreSens Gmbh, Germany) and a data logger. A thermocouple was used to monitor the temperature of each treatment. Each pot had an oxygen probe and a thermocouple probe inserted into the lid and sitting in the treatment water throughout each experiment (see image 2.). A Minolta SPAD meter was used to measure the chlorophyll content of the leaves of the plants. An ADC BioScientific Ltd LCi IRGA was used in order to measure and record the leaf photosynthesis rate, leaf transpiration rate and the stomatal conductivity of each plant.

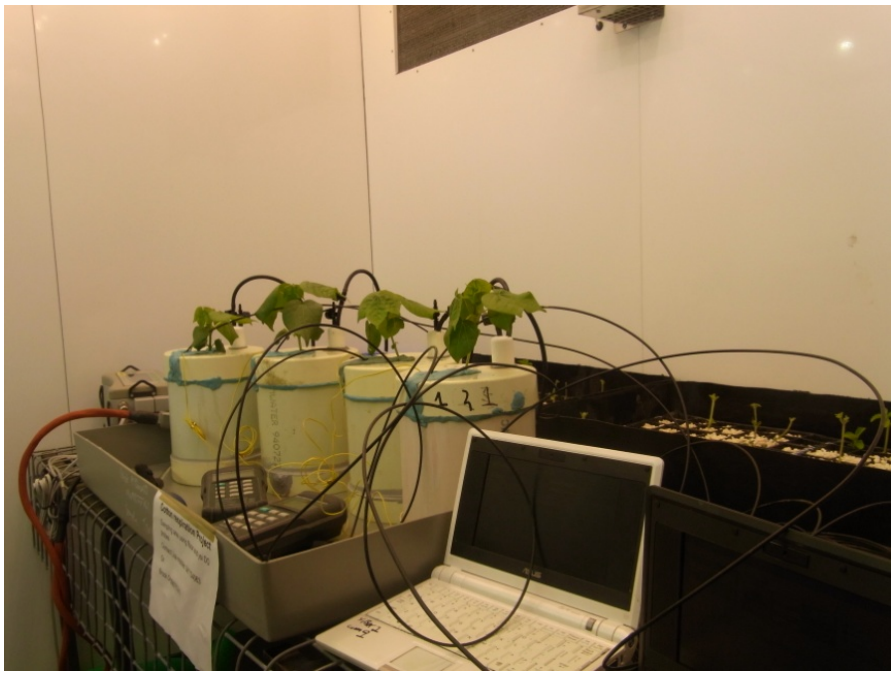

Figure 3. Plants subjected to four different treatments in pots (3.5L sealed PVC containers).

Four treatments were used in both experiments; these included tap water, micro bubble water (Seair treated water), hydrogen peroxide and low oxygen water (deoxygenated water). The microbubble water was taken from a Seair water tank. The hydrogen peroxide treatment was prepared using 50% hydrogen peroxide solution at half a millilitre per litre of water dilution rate. In order to prepare the low oxygenated water sodium sulphite was used to lower the oxygen of tap water to below 2 ppm. Each of the treatments had manutec added to them in order to reduce nutrients deficiency symptoms in the plant.

Experiment 1

Four cotton plants were each subjected to one of the four treatments, so there were four plants and four treatments used each run. The oxygen and temperature in the solution were recorded at the start of the experiment and at least every hour during the experiment. Chlorophyll content and IRGA readings were also taken at least every hour throughout the experiment. The experiment was run until the oxygen levels in all pots were below 3 ppm. After the experiment was finished for that day the plants were put back into the hydroponic boxes for the night to recover. The same four plants were used for the next days in a block; however they were exposed to different treatments for each run (Table 1). The experiment was repeated for three times. Once the cotton plants did not recover they were sacrificed and sorted into leaves, stems and roots. The plant parts were then weighed (fresh weight) and the leaves were scanned in order to get the leaf area in mm^2. The plant parts were then

dried for 72 hours in a drying oven that was set at seventy degrees Celsius. Once dry the plant parts were again weighed for their dry weight.

Table 1: Allocation of plants in different treatments in sequence in order to evaluate the effect of hypoxia in cotton root respiration

Sequences	**Plant 1**	**Plant 2**	**Plant 3**	**Plant 4**
Exp 1.1	Seair	Deox	HP	Tap
Exp1.2	Deox	HP	Tap	Seair
Exp 1.3	HP	Tap	Seair	Deox

Experiment 2

Four plants were subjected to one of each of the four treatments in a way so there were four plants and four treatments. The volume of each of the pots was measured at the start and end of the experiment. The oxygen and temperature of each of the treatment waters was measured and recorded at the start of the experiment and periodically throughout the experiment. The chlorophyll content of the leaves was measured as well as IRGA readings on each plant at the beginning of the experiment and periodically throughout the experiment. The experiment was repeated for three times. Once the cotton plants showed signs of wilting; they were sacrificed and sorted into leaves, stems and roots. The plant parts were then weighed (fresh weight) and the leaves were scanned in order to get the leaf area in mm^2. The plant parts were then dried in a drying oven that was set at seventy degrees Celsius for 72 hours. Once dry the plant parts were again weighed to get the dry weight.

Results and Discussion

Experiment 1

Young cotton plants subjected to hypoxia at the beginning recorded significant set back in terms of root and plant growth. In fact plants exposed to hypoxia at the beginning even did not fully recovered even when the plant was later on transferred to aerated medium (Table 2). Highest plant growth was noted on plants which was not exposed to deoxygenation at al and on the plants which experienced the deoxygenation treatment only at the end of the other treatment in cycles.

Table 2: Response of young cotton plants to various cycle exposure to hypoxic root environment in the soil less culture system.

Plant/ treatment	Root (g)	Stem (g)	Leaf (g)	Total wt (g)	Leaf Area (cm2)	Leaves/ plant
1 (S-D-HP)	11.81	25.35	29.58	66.74	1233	28
2 (D-HP-T)	9.24	18.37	18.25	45.86	798	18
3 (HP-T-S)	19.33	28.27	36.22	83.82	1479	26
4 (T-S-D)	18.76	29.5	36.12	84.38	1399	29
SE	3.7	4.2	4.5	6.13	126	5.1

With respect to the leaf photosynthetic rate, plant subjected to the deoxygenated water dropped it's photosynthesis within few hours, and did not recover at all suggesting permanent damage to root membrane.

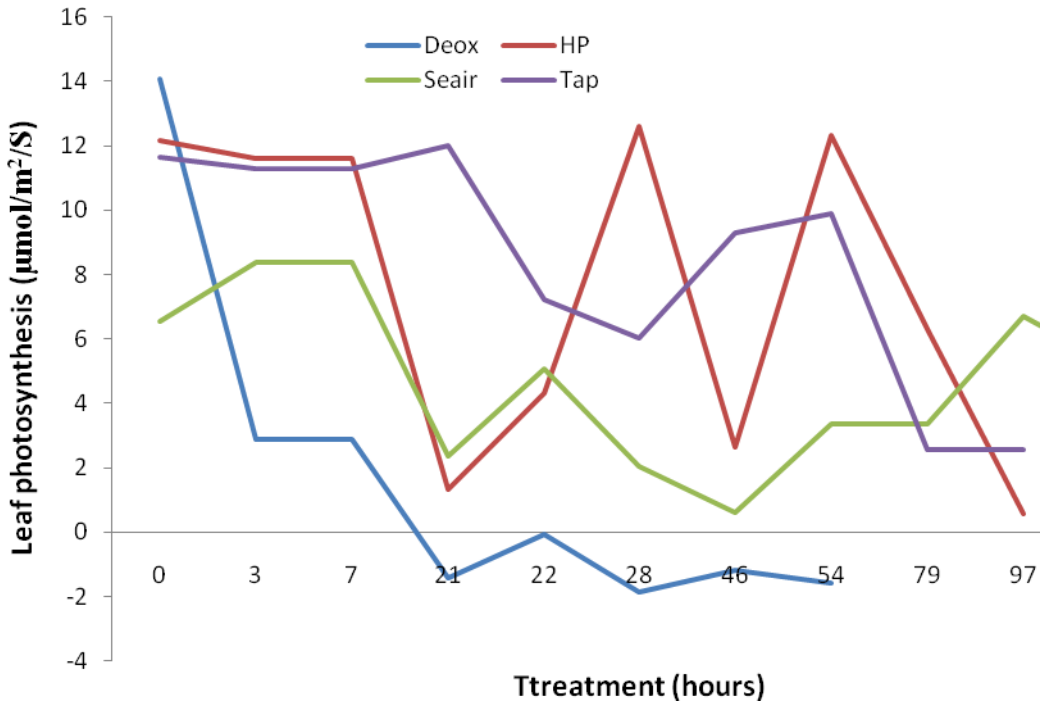

Figure 4: Leaf photosynthetic rate over four days in different treatments

The plants first exposed to seair recorded greater photosynthetic rate, but up on exposure of this plant to deoxygenated water the leaf photosynthetic rate declined markedly and took sometime to recover even after transfer to the hydrogen peroxide solution culture. This sustained period that took to recover lead to slow plant growth in this treatment compared to other treatments where plants were not exposed to

deoxygenated water at all or had the exposure but only at the end of the treatments cycle. The HP-T-S treatment showed a cyclic pattern of leaf photosynthesis, where plant maintained greater photosynthetic rate for 24 hours then started to drop, but picked up after transfer to Tape water, dropped again, pickup after the transfer to seair water and finally dropped again. The T-S-D treatment showed initial high rate, followed by decline, increased by seair and finally plummeted by the exposure in the deoxygenated water (Figure 4). The results suggested that the early exposure on the roots to deoxygenated water exert greater negative effect, followed by exposure in the mid turn and least at the end of the exposure cycle.

Experiment 2

Cotton plants subjected to the deoxygenated water showed a quick drop in leaf respiration rate in this experiment. The cotton plants subjected to the Seair water in this experiment also maintained a high leaf transpiration rate. Wilting in plants is a good indicator of plant exhaustion and the first sign of wilting is curling or sagging leaves (Salisbury & Ross 1992). In this experiment the cotton plants in the low oxygen water treatment were the first to show signs of wilting (see table 3) and they used a considerable amount of water for the amount of time they were subjected to the treatment. The cotton plants subjected to tap water showed a faster drop in leaf transpiration when compared to the plants in the hydrogen peroxide and Seair treatments (see table 3.). The amount of water used in the hydrogen peroxide treatment plants was however less than those plants subjected to the Seair treatment.

Table 3. Water volumes in pots (ml), time taken to show first signs of wilting, and leaf gas exchange parameters for cotton with different oxygation treatments.

Treatment	Start volume	End volume	Amount transpired (ml)	Wilting symptoms(hrs)
Deoxygenated water	3550	3160	390	45
Tap water	3500	1580	1920	104
Hydrogen Peroxide	3500	2295	1205	130
Seair	3500	1838	1663	130
SE	0	237	197	13.3

The results from both experiments indicate that cotton plants subjected to low oxygenated water and tap water treatments have a quick drop in leaf transpiration rate. The cotton plants subjected to the hydrogen peroxide treatment maintained a higher level of leaf transpiration for a longer period of time; however cotton plants

subjected to the Seair treatment indicated that the plant was more active for longer and maintained the highest transpiration rate. The maintenance of plant activity with the Seair treatment indicates that the root transpiration was also maintained.

Table 4: Fresh and dry weight of the plant component part with respect to different oxygation treatments in the soil less culture system

Treatment	Root (g)	Stem (g)	Leaf (g)	Total (g)	Leaf Area (cm2)	Leaf (#)
Tap water	17.03	41.34	49.94	108.31	2103.6	41
Hydrogen peroxide	16.11	38.49	33.42	88.02	1437.1	26
Deoxygenated water	32.1	49.97	38.66	120.73	1881.8	39
Seair	25.65	53.04	45.93	124.62	1757.6	32
SE	3.14	4.05	3.79	6.18	197.2	3.98

Oxygen levels and other chemical parameters can have an effect on root development, which in turn can have an effect on crop performance (mid). Previous studies have shown that by aerating water in subsurface drip irrigation crop yields can improve (Ninghu Su & Midmore, D 2005). The results from this experiment indicate that subjecting cotton plants to microbubble water can maintain oxygen levels at the roots. Further research is needed in order to gain more information on subjecting cotton plants to Seair water through a Sub-surface irrigation system in the field. Given positive results, delivering microbubbles directly to the roots of cotton plants through Sub-surface drip irrigation may help to overcome associated problems with growing cotton in waterlogged conditions in Australia.

The leaf photosynthetic rate in the plants in experiment 2 for the deoxygenated water dropped within a day and became negative. The seair treatment maintained consistently higher photosynthetic rate up until 4 days and HP treatment maintained the moderate photosynthetic rate. These results are in agreement to plant biomass and transpiration data, suggesting that the micro bubble in the seair system can provide cotton root with extra oxygen for extending the period of root respiration under limited oxygen concentration in the rhizosphere of the irrigated plants.

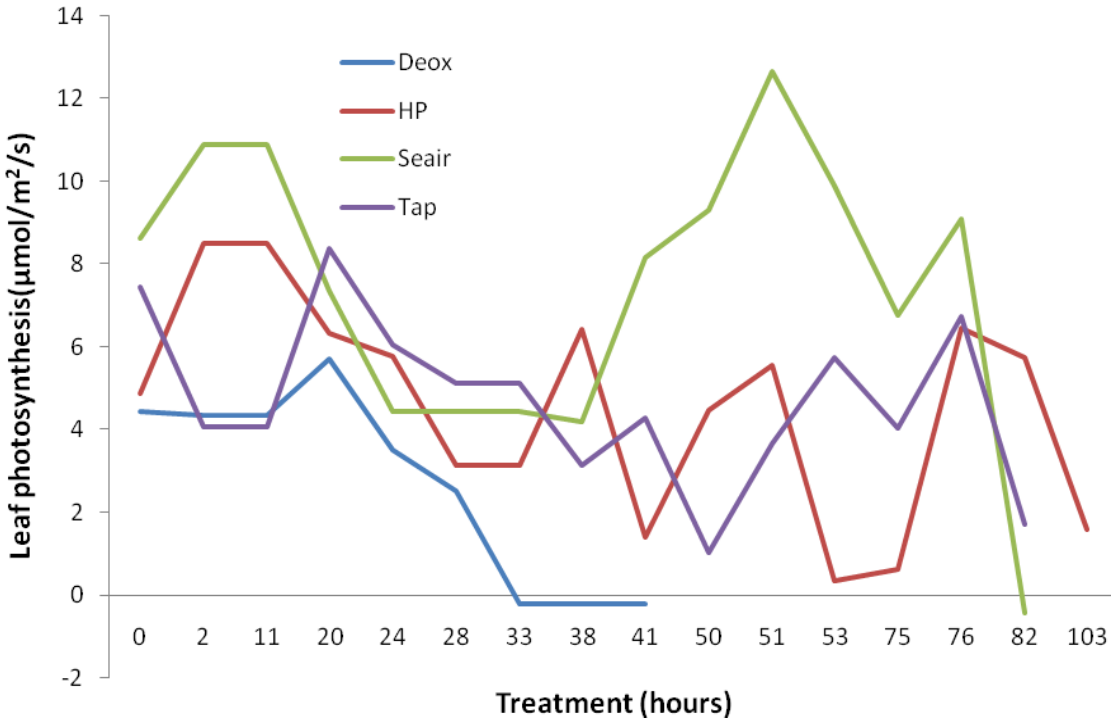

Figure 5: Leaf photosynthetic rate over five days in different treatments for aeration of water for soil less culture

Conclusion

Cotton is a great contributor to the Australian economy; however poor soil quality can contribute to waterlogging. This can have an effect on crop yields. Seair water pumps can pump microbubbles through a sub-surface drip irrigation system as an alternative to traditional aeration methods. With further research this method may be a viable way to increase oxygen levels at the roots and help farmers to produce higher yielding crops.

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References

Bange, M, Milroy, S & Thongbai, P 2003, 'Growth and yield of cotton in response to waterlogging', *Field crops research*, vol.88, pp. 129-142.

Bhattarai, S, Su, N & Midmore, D 2005, 'Oxygation unlocks yield potentials of crops in oxygation-limited soil environments', *Advances in Agronomy*, vol. 88, pp.313-377.

Department of Primary Industries and fisheries 2008, *Cotton – an overview*, viewed 20 May 2010,
http://www.dpi.gov.au/26_3431.htm

Ninghu Su & Midmore, D 2005, 'Two-phase flow of water and air during aerated subsurface drip irrigation', *Journal of Hydrology*, vol.313, pp. 158-165.

Salisbury, F & Ross, C 1992, *Plant Physiology*, 4th edn, Wadsworth Publishing Company, California.

Thongbai, P, Milroy, S, Bange, M, Rapp, G, Smith, T, 2001, 'Agronomic responses of cotton to low soil oxygen during waterlogging', Proceedings of the 10th Australian Agronomy Conference Hobart, Tasmania,
http://www.regional.org.au/au/asa/2001/2/thongbai.htm