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A method for cultivating plants under controlled redox intensities in hydroponics

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Abstract

An automated, low-cost hydroponic system was developed for growing single plants at controlled redox potentials (Eh) for extended periods. The system features a millivoltmeter with high and low set-point relays and offers the investigator full control of Eh within the entire redox potential range encountered in natural soils (−300 to +700 mV) with an accuracy of ±50 mV around the set-point value. Eh is lowered in the nutrient solution by adding the reducing agent titanium citrate. Conversely, Eh is elevated by bubbling the nutrient solution with air. The system's ability to control Eh is demonstrated using data from a test experiment involving *Phragmites australis* plants grown at three redox levels (−150, +150 and +550 mV). Some frequently encountered factors that were potentially responsible for erroneous control are discussed. The buffer capacity and the relationship between pH and Eh were determined for the nutrient solution. These parameters, along with plant-mediated changes in pH, not only determine the time interval between pH adjustments, but also affect the Eh fluctuations in the continuously aerated experimental units. The variability and drift in readings from the platinum (Pt) electrodes, used to measure Eh, was investigated in order to relate electrode reliability to system performance. The variation in Eh readings for clean Pt electrodes was lower (S.E. = 4.3 mV, $n = 23$) than that of the calomel reference electrodes (S.E. = 6.8 mV, $n = 23$), the difference fixed across solutions of different poise. The response time (drift) of Pt electrodes in hydroponics (low-poised nutrient solution) was compared to pH-buffered quinhydrone (medium-poised) and flooded alluvial silt (well-poised). Drift increased from the low-poised nutrient solution, where Eh readings stabilized within minutes, to high-poised flooded soil, where Eh readings showed a downward drift for ca. 50 h. Overall, it is concluded that both the Pt electrodes and the calomel reference electrodes perform well in hydroponics. The extent to which the high

Abbreviations: ORP (Eh), oxidation–reduction (redox) potential

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NaCl salinity of titanium citrate (ca. 84‰) may limit the growth of plants is also discussed relative to the proportion of titanium citrate in the nutrient solution.

Keywords: Titanium citrate; ORP; Nutrient uptake; Platinum electrodes

1. Introduction

In plant nutrition studies it is often difficult to control or quantify nutrient availability when soil is present because availability is regulated by complex sorption, precipitation and solubilization processes. Soil redox potential (Eh) intensity and pH are among the factors that strongly influence the mobility of many nutrients in biologically and chemically complex soil environments (Gambrell and Patrick, 1978). Hydroponic systems provide a convenient means of studying plant uptake of nutrients free of confounding and uncontrollable changes in soil nutrient supply to the roots. An additional advantage of water culture is that secondary effects of low redox, such as accumulation of soil toxins, are likely reduced. Most hydroponic studies of the responses of wetland plants to anaerobic root environments have involved the removal of oxygen by purging the solution with nitrogen. However, such a system would have a redox potential of +350 to +400 mV, much higher than the −150 to −200 mV typical of flooded soils. Thus, an oxygen depleted solution does not reflect the high oxygen demand commonly found in wetland soils. In fact, it has been shown that plants grown at highly reduced conditions respond differently than plants grown at moderately reduced conditions (DeLaune et al., 1990; Kludze et al., 1994). To create a strong oxygen demand in hydroponic solution similar to that of flooded soils, organic matter, or alternatively, an artificial reducing agent, must be added. Organic matter is not suitable for obtaining fixed redox treatments because the relative capacity and intensity of reduction will change upon its consumption by microorganisms. Another drawback of organic matter is that its presence alters the nutrient status of the nutrient solution.

There have been only a few short-term studies that have dealt with the effects of strong oxygen demand on the physiology of wetland plants in hydroponic systems using an artificial redox buffer. DeLaune et al. (1990) developed a system with manual Eh control in order to study the photosynthetic activity of *Spartina patens*. A similar system was used for sampling phosphorus uptake over a period of 24 h (DeLaune et al., 1999). Eh was monitored every hour and titanium citrate was manually injected into the low redox treatment (Eh < -200 mV) whenever needed. Moderately reduced conditions +100 to +300 mV were obtained by bubbling the solution with helium without adding reduced titanium citrate. Unfortunately, Eh is responsive to small changes in titanium citrate concentrations. In the concentration range 10^{-3} to 10^{-7} M Ti^{3+} citrate, Eh changed approximately 50 mV within each order of magnitude change (Zehnder and Wuhrmann, 1976). In practical experiments this means that frequent additions of titanium citrate are required by the investigator. The present study estimates that addition is needed every 20 min–6 h to maintain Eh within a 100 mV window, making manual control impractical for long-term studies involving many replicates. Another drawback of manual addition is that overshooting may occur, causing extremely reduced conditions, which may be detrimental to the study. Culturing plants at low pH may lessen the

impact of overshooting due to the relationship between Eh and pH. DeLaune et al. (1999) cultured *Typha domingensis* plants at pH 4.9–5.0 so that Eh levels could be maintained at an average of ca. −200 mV.

In this study we present a design for an oxidation–reduction potential (ORP) controlled system suitable for long-term hydroponic studies. Test data have been included to demonstrate the ability of the system to control Eh. Growth data have also been included involving the long-term growth responses of *Cladium jamaicense* and *T. domingensis* to Eh and phosphate availability. Three redox treatments were chosen: (1) highly reduced (−150 mV), (2) moderately reduced (+150 mV), and (3) oxidized (+550 mV). The nutrient solution of the oxidized treatment was continuously bubbled with air to maximize Eh, making ORP controllers unnecessary. However, since Eh and pH are coupled, plant nutrient uptake mediated pH change is expected to influence the Eh of the oxidized treatment. This study, therefore, determined the relationship between pH, Eh and buffer capacity for the nutrient solution within the plant physiological pH range of 5–8.

We also investigated some characteristics of titanium citrate in order to evaluate its usefulness as a reducing agent. Titanium citrate is regarded as a non-toxic redox buffer (DeLaune et al., 1990). However, it does contain appreciable amounts of sodium and chloride, which could lead to possible salinity effects on plant growth. Therefore, the NaCl salinity of titanium citrate was calculated to evaluate to which extent it is likely to impose undesirable growth reductions for salt-sensitive plant species. Also, the lifespan of freshly made titanium citrate, under the experimental conditions, was investigated to elucidate how often titanium citrate should be renewed in the addition containers due to time dependent loss of potency.

Finally, the performance of platinum (Pt) and calomel electrodes was compared for systems differing in poise, i.e. systems differing in resistance to changes in redox potential upon additions of a reductant or oxidant. More specific, it was investigated whether Eh measurements in a low-poised hydroponic nutrient solution are less precise compared to other substrates such as medium-poised pH-buffered quinhydrone solution and high-poised flooded soil. The substrates were also tested for differences in response time as lag time effects may impede the control of Eh. Equally important for control is the accuracy of Pt and calomel electrodes. A test was performed to compare the standard deviations of readings from a large number of electrodes. The Pt electrodes were of the welded type using epoxy as an insulator. However, under continuous exposure to water, epoxy may become unstable, thereby limiting the lifespan of the electrodes (Cogger et al., 1992; Patrick et al., 1996). Therefore, the performance of the Pt electrodes over time was also monitored in order to determine whether these types of electrodes are suitable for long-term experiments.

2. Materials and methods

2.1. ORP controlling system

A truly replicated system to cultivate single plants hydroponically under controlled redox potential was designed (Fig. 1). Eh was controlled using ORP controllers (Cole-Parmer,

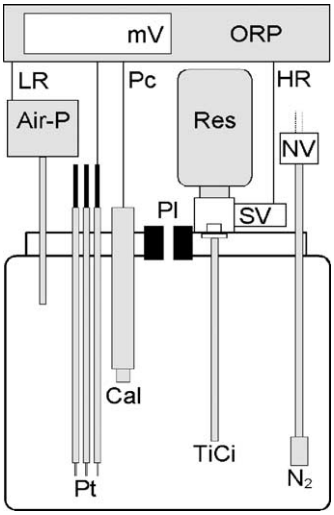

Fig. 1. A schematic diagram showing a single ORP controlling system in which a plant can be grown under different redox treatments. ORP: millivoltmeter (mV: display); LR: low set-point relay; HR: high set-point relay; Air-P: air pump; Res: titanium citrate (TiCi) reservoir; NV: needle valve to control flow of N_2; SV: solenoid valve; Pl: central hole to fasten plant; Pc: probe connections; Pt: platinum electrodes; Cal: calomel electrode.

Model 57000-00, Vernon Hills, IL, USA) which activated and deactivated control devices according to high and low Eh set-points. The Eh of the hydroponic solution was controlled either by adding the reducing agent, titanium citrate, or by injecting compressed air, which was used as the oxidizing agent. Each experimental unit consisted of a 4 l wide-mouth plastic bottle serving as a nutrient solution tank. The bottle was primed and painted black to eliminate light transmission and coated with silver paint to minimize heat absorption. The lid was furnished with a central hole 40 mm in diameter for mounting a plant using commercial pipe insulation foam. To provide a gas outlet the seal was not made tight, allowing the shoot base to thicken during the course of the experiment. Holes along the fringe of the lid were drilled to allow insertion of: (1) a calomel electrode (Cole-Parmer, P-05990-50, US std jack), (2) three replicate platinum electrodes of the welded type (Patrick et al., 1996), (3) an inlet pipe terminating in a bubble stone carrying air or nitrogen gas for mixing and purging, and (4) an inlet pipe from an air pump (Tetratec Whisper, USA) connected to an ORP controller. Self-sealing rubber septa were used to hold the electrodes and tubing at desired depths and created gas tight seals. One additional hole was used for adding nutrient solutions, replace transpired water, and for pH adjustment. To control titanium citrate addition, a two-way, normally closed, 3.2 mm NPT(F) stainless steel solenoid valve was screwed on to the top of the lid by inserting a pipe adapter (3.2 mm NPT(M) × 1.6 mm tubing i.d.) with a nylon flat washer (13 mm × 11 mm × 1.6 mm) from the underside, using an O-ring to seal the valve/lid junction. A 15 cm piece of 0.3 mm i.d. Microbore PTFE tubing (Cole-Parmer, Vernon Hills, IL, USA) was attached to the pipe adapter using 1 cm pieces of Tygon tubing of different diameter as reducers. A second pipe adapter (3.2 mm NPT(M) × 4.8 mm tubing i.d.) was screwed in to the body of the valve from the top. A single hole silicone stopper was attached upside down on the pipe adapter. The taper size of the stopper

allowed tight fastening of a 125 ml HDPE bottle (Nalgene, Rochester, NY, USA) serving as a titanium citrate reservoir (100 ml). The titanium citrate in the reservoir was gravity fed to the solenoid. An 18 gauge needle inserted in a rubber septum on top of the reservoir provided pressure equalization between headspace and growth chamber. The length and inner diameter of the drip tubing, in conjunction with the level of titanium citrate in the reservoir, were selected to provide an adequate flow rate of titanium citrate. The reservoirs were purged with nitrogen when filled. Subsequent to a filling event, the headspaces were pressurized for 15 s using a syringe (solenoid valve open) to squeeze out air bubbles that otherwise would have been trapped in the drip tubing blocking the flow of titanium citrate. The calomel and one platinum electrode were connected to the ORP controller using cables with alligator clips. The platinum electrode that exhibited medium reading was chosen as the lead electrode. The two unused platinum electrodes were compared to the in-use electrode daily, and served as indicators for electrode fouling. In-use electrodes producing incorrect readings were replaced by one of the replicate electrodes and cleaned by scraping the platinum tips using a stainless steel razor blade. ORP controllers were calibrated using 0.05 g of quinhydrone ($C_{12}H_{10}O_4$) in 30 ml of pH 4 and pH 7 buffer (Patrick et al., 1996). The nutrient solution of the −150 and +150 mV treatments were continuously purged with oxygen free nitrogen gas while the +550 mV treatment was continuously purged with compressed air. Air and nitrogen were distributed via commercial needle valves connected to an air pump or a regulator of a nitrogen canister. Air and nitrogen lines were made of 5 mm PTFE tubing and were color-coded for easy identification. Nitrogen and airflow rates were set to 35 ml min^{-1} using variable area flow meters calibrated for nitrogen and air. The pressure gradient over the valves was set to 10 bar in order to minimize the influence of flow meter resistance on the gas flow. Flow meters were only inserted during flow rate control events undertaken twice weekly. When Eh was greater than the high set-point, the solenoid valve was automatically activated, and titanium citrate was added to the solution (ca 0.2 ml min^{-1}) causing Eh to decrease. Whenever Eh was less than the low set-point, the air pump was activated, causing Eh to drift back up. Usually, the drift continued 10–20 mV after the relays switched off and as a consequence, the hysteresis bands of the controllers could be set at 0 mV. Experimental units in the fully oxidized +550 mV treatment were continuously aerated and therefore not furnished with ORP controllers.

The nutrient solution was prepared using deoxygenated solution for the medium and low Eh treatments. Reduced titanium citrate must be used to adjust the Eh level of the nutrient solution to the treatment level before renewal, otherwise it may take several hours before enough titanium citrate has passed the drip tubing to reach the treatment level. Oxidized titanium citrate was added to the +150 and +550 mV treatments in order to achieve the same total concentration of titanium citrate across treatments. To demonstrate the precision of the system to control Eh, a datalogger (Campbell, CR10X, Logan, UT, USA) was used to record Eh from three Pt electrodes, every 10 min, for 3 days, across all three redox treatments. *Phragmites australis* (Cav.) Trin. ex Steud. served as the study plant. Plants with shoot heights of ca. 50 cm and with dry weights of ca. 10 g were propagated from rhizome cuttings collected from a stand fringing the Rio Grande at Hot Springs, Big Bend, TX, USA. The cuttings were pre-cultured in the nutrient solution for 20 days to encourage root development in the experimental growth chamber (Environmental Growth Chambers, model M-75, Chagrin Falls, OH, USA). The chamber was operated with a photosynthetic flux

density of 1200 μmol m^{-2} s^{-1}, 12 h photoperiod, and day (28 °C)/night (20 °C) temperature cycle. This study used a nutrient solution specially formulated for wetland plants (Lorenzen et al., 2001). Phosphate, the limiting nutrient, was maintained at 100 μg PO_4-P l^{-1} by daily KH_2PO_4 additions. To demonstrate that the system can be used in long-term experiments, studies were performed involving *C. jamaicense* Crantz and *T. domingensis* Pers. plants propagated from seeds collected at the central area of Water Conservation Area 2A, South Florida, USA. Plants were grown at Eh levels of −150, +150 and +600 mV with phosphate availability as the second treatment factor. Phosphate levels were adjusted twice daily to 10 μg l^{-1} (P10), 80 μg l^{-1} (P80) and 500 μg l^{-1} (P500). The two species were grown for 9 weeks (*Cladium*) and 4.5 weeks (*Typha*) in separate, factorial experiments and treatment effects on final biomass were recorded.

2.2. Titanium citrate and nutrient solutions

Reduced titanium citrate (Ti^{3+} citrate) was prepared under an N_2 atmosphere according to the method of Zehnder and Wuhrmann (1976). Nine hundred milliliters of deoxygenated, deionized water was added to 52.95 g sodium citrate to give 0.2 M sodium citrate solution. Then 90 ml of 8.9% (w/v) $TiCl_3$ in 30% (w/v) HCl (Aldrich, Germany) was added to the sodium citrate solution. The pH of the solution was adjusted to 5.6 using ca. 210 ml of saturated sodium carbonate to give a final volume of ca. 1200 ml. The Ti^{3+} complex forms a violet-blue solution but becomes colorless upon oxidation. The color thus serves as a visual indicator of reductive potency.

The headspaces of the titanium citrate containers were connected to the atmosphere via the 18 gauge syringe needle used for pressure equalization. Oxygen entering the headspace may increase the oxidation rate of titanium citrate thereby shortening the time interval between renewals. The rate of oxidation for titanium citrate kept in the containers ($n = 7$) in the growth chamber under experimental light and temperature conditions was compared to titanium citrate kept in stoppered bottles ($n = 7$) by measuring absorbance at 527 nm at 1-day intervals during a 10-day period.

The Na and Cl salinity of titanium citrate was calculated from the concentrations and volumes of the various compounds used for preparation. The concentration of Na in saturated sodium citrate was determined by oven-drying a known volume and then weighing the residual crystals, subtracting the calculated weight of citrate.

The relationship between Eh and redox potential for the nutrient solution was determined by plotting Eh as a function of pH for 1 l of solution containing 12.5 ml of titanium citrate. Using magnetic stirring, the pH of the solution was adjusted to an initial value of 6.00. Then 0.05 ml of base (1 M NaOH) was added incrementally while pH and Eh were recorded until pH reached 10.00. The procedure was repeated with acid (1 M HCl) until pH reached 4.00. Using the same data, a titration curve was constructed in order to describe the relationship between buffer capacity and pH within the plant's physiological pH range.

2.3. Electrodes

Low-cost Pt electrodes were manufactured from 13 mm pieces of 18 gauge platinum wire and 10 gauge copper wires using the welding procedure of Patrick et al. (1996). Commercial

epoxy resin was used to keep the copper from coming into contact with the solution. Drift and electrode variability for three different substrates (nutrient solution, a pH-buffered quinhydrone solution and Mississippi alluvial silt) each differing in poise were investigated using 35 different platinum electrodes. Electrodes were cleaned by scraping with a stainless steel razor blade and placed in deionized water for 24 h before each change of substrate. For the soil test, 20 l of fine-grained alluvial silt, with no visible organic material, was collected in the Mississippi River Delta, LA, USA, from an unvegetated channel discharging into the mouth of Southwest Pass. The sediment was transferred to a 25 l bucket and flooded with 5 cm of water. In the laboratory the sediment was carefully homogenized, keeping oxygen from mixing with the sediment. The bucket was then furnished with a lid and left undisturbed, in the laboratory, for 2 weeks prior to the start of the experiment. A cluster of 35 parallel Pt electrodes with the tips spaced ca. 1 cm apart was inserted 20 cm below the surface. Eh was measured for all substrates at specific time intervals from 0 to 120 min but was extended to 115 h for the alluvial silt since readings did not stabilize. A portable voltmeter was used to read Eh (Digi-Sense, Cole-Parmer, Vernon Hills, IL, USA).

The relative precision of platinum electrodes compared to calomel electrodes was estimated using 1 l of nutrient solution mixed with 12.5 ml oxidized titanium citrate and pH-buffered quinhydrone solution (pH 7) as test solutions. The variance around the mean value was calculated from the readings of 23 platinum electrodes using one calomel reference electrode. Similarly, the variance around the mean value was calculated from readings of 23 calomel electrodes using one Pt electrode as “reference”. Pt electrodes were cleaned by scraping the platinum tips with a stainless steel razor blade and placed in deionized water for 24 h before change of solution.

Possible time related deterioration of Pt electrode performance was investigated by comparing standard deviations for readings of the same batch of electrodes ($n = 23$) 8 and 16 months after manufacture using pH-buffered quinhydrone as the test solution. Cleaning the electrodes prior to testing eliminated any effects from possible fouling of the Pt surface on electrode performance.

2.4. Data analysis

Standard deviations of calomel and platinum electrodes were compared for each solution using *F*-tests for comparison of two samples (null hypothesis: standard deviation for calomel and platinum electrodes are the same). Standard deviations within each of the three substrates were compared for both calomel electrodes and platinum electrodes using Bartletts’ test (null hypothesis: standard deviation within each solution is the same). The data used for comparison of standard deviations was tested for normality using the Kolmogorov–Smirnov goodness-of-fit test. Because of temporal heteroscedasticity, test of equality of means for the time series of Eh in the different substrates were carried out using the Games–Howell method (Sokal and Rohlf, 1995). The biomass variable was analyzed using analysis of variance (ANOVA). The model applied for the factorial random block design was $Y = f(\text{block, redox intensity, phosphate level})$. Logarithmic transformation was performed to ensure normality of error terms prior to testing. The tests were carried out using Statgraphics Plus for Windows (version 4.1), Manugistics, Rockville, MD, USA.

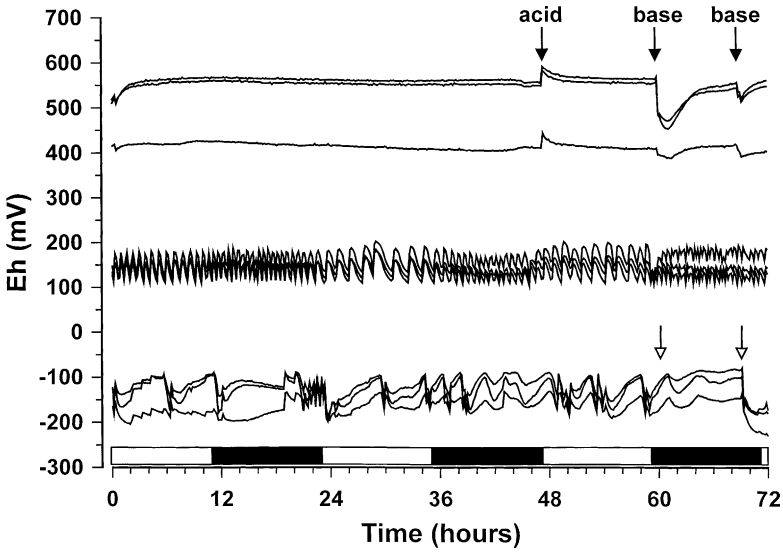

Fig. 2. Time course of Eh readings from nutrient solutions with *P. australis* plants. Triplicate Pt electrodes were used for each of three different redox treatments (−150, +150 and +550 mV). Open and solid bars designate day and night periods, respectively. Solid arrows designate points in time for acid or base additions for the +550 mV treatment. Open arrows designate time interval with improper Eh control for the −150 mV treatment due to low potency of the reductive agent, titanium citrate.

3. Results

The Eh readings for the three redox treatments over the 3-day test period are shown in Fig. 2. Eh ranged between +560 and +580 mV for the continuously aerated treatment discounting the readings of one faulty electrode which had an 100 mV offset. Additions of acid and base at 48, 60 and 72 h resulted in initial jumps in Eh after which Eh stabilized after 2–4 h. Since the *P. australis* plants had relatively small root biomass, plant nutrient uptake did not significantly influence pH of the nutrient solution to a degree exceeding the buffer capacity. As a consequence, pH was stable throughout the recording period. All Pt electrodes were tested in pH-buffered quinhydrone before the onset of the recording period and were found to give proper readings. However, this was not the case in the more weakly poised nutrient solution where one platinum electrode deviated more than 100 mV from the two other replicate electrodes (Fig. 2).

Eh tended to decrease just by bubbling with N_2 for the ±150 mV treatment. A rapid increase in Eh due to activation of the air pump was followed by slow decrease in Eh until the next aeration event. While the three replicate platinum electrodes gave similar readings at the onset of the test experiment, one electrode gradually began to deviate from the other two. At around hour 60, the readings no longer exhibited overlapping curves. It is interesting to note that the frequency of control events for the +150 mV treatment was markedly lower during daytime hours.

Eh was controlled differently at −150 than at +150 mV. Eh tended to increase for the −150 mV resulting in a frequent need for titanium citrate additions. The elapsed time between control events ranged between 20 min and 6 h and was more erratic than for the +150 mV treatment. The titanium citrate had become partly oxidized after 60 h and as a consequence of reduced potency there was a gradual increase in Eh despite continuous addition of titanium citrate. Manual injection of freshly made titanium citrate, between 68 and 70 h, was used to quickly restore the Eh level back to the treatment range.

Eh was related to pH in a linear fashion within the physiological pH range (1.5 pH units around pH 6.5) (Fig. 3a). Eh was found to decrease 50 mV for each unit increase in pH. The buffer capacity (moles of acid added to 1 l of nutrient solution to change pH by one unit) increased 20 times from 0.067 mmol HCl at pH 8 to 1.111 mmol at pH 5 (Fig. 3b).

Titanium citrate is a strong oxygen scavenger and the oxygen that found its way through the syringe needle used for pressure equalization in the addition container decreased the half-life of titanium citrate. The decrease in absorbance was linear during the 1-week test

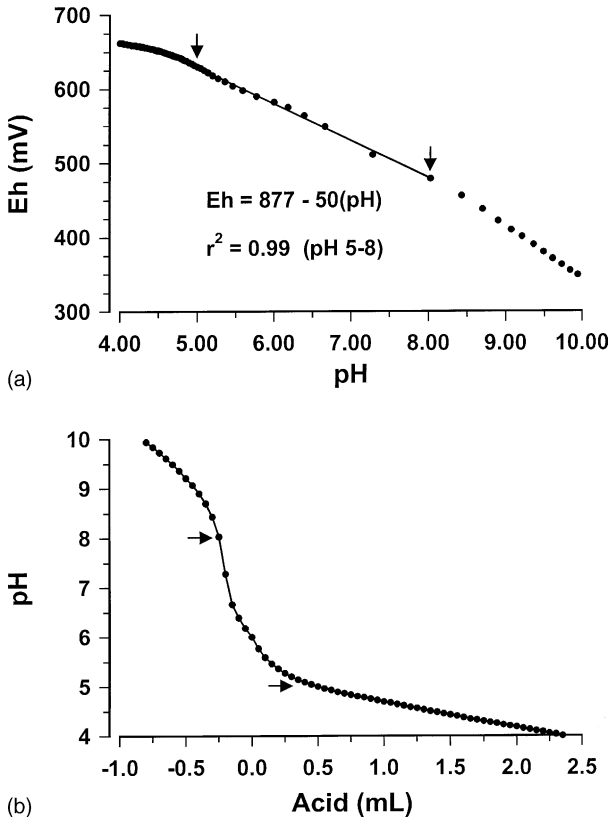

Fig. 3. (a) Relationship between pH and redox potential for a nutrient solution designed for wetland plants. Regression was run for solution pH between 5 and 8 (arrows). This range is assumed to include the optimum pH for most plant species. (b) Buffer capacity for the same solution.

Table 1
Na and Cl salinity of titanium citrate

Source	Na (‰)	Cl (‰)
$TiCl_3$	–	5.49
HCl	–	25.14
$Na_3C_6H_5O_7 \cdot 2H_2O$	10.35	–
Na_2CO_3	45.35	–
Total	53.70	30.62
Total NaCl salinity	84.3‰	

Based on the use of 90 ml of 8.9% (w/v) $TiCl_3$ in 30% (w/v) HCl (density 1.192 g cm^{-3}), 900 ml of 0.2 M sodium citrate and 210 ml of saturated sodium carbonate for pH adjustment.

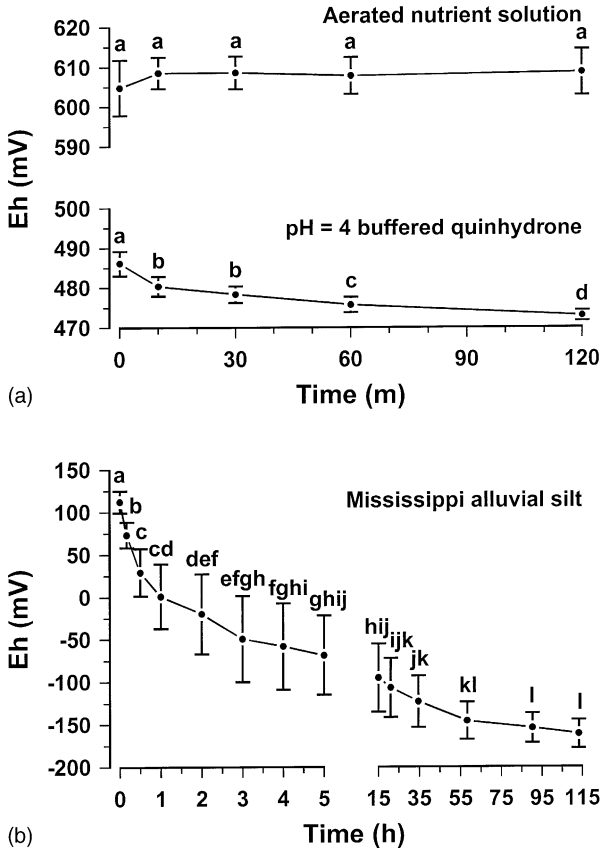

Fig. 4. Time course of Eh readings from substrates differing in poise. (a) Nutrient solution (pH adjusted to 6 before each time of measurements) and pH 4-buffered quinhydrone. (b) Mississippi alluvial silt (pH 7.12 throughout the recording period). Results are presented as average and standard deviation of 35 replicate electrodes. Any two means which share the same letter do not differ at the $P < 0.05$ probability level.

Table 2
Standard deviations of electrode readings ($n = 23$) for nutrient solution and quinhydrone dissolved in pH 7-buffer

	Pt (mV)	Calomel (mV)
Nutrient solution	4.44 a	6.99 b
Quinhydrone		
8 months	4.06 a	6.66 b
16 months	4.29 a	6.98 b

Pt electrodes were tested in quinhydrone 8 and 16 months after manufacture. Standard deviations sharing the same letter do not differ at the $P < 0.05$ probability level.

period with a calculated half-life of 5.4 days (slope $b = -0.1396$, for regression equation $Y = a + bX$, $r^2 = 0.97$). When oxygen was prevented to enter the headspace there was only little loss of potency resulting in a calculated half-life of 160 days ($b = -0.0046$, $r^2 = 0.99$). Total Na and Cl salinity for titanium citrate was calculated at 84.3 g l^{-1} (Table 1). It is noteworthy that $TiCl_3$ and sodium citrate make only minor contributions to the salinity and that the dominant sources were the $TiCl_3$ solvent, HCl, and the Na_2CO_3 used for pH adjustment.

Platinum electrodes equilibrated fast in the nutrient solution with no significant drift in meter reading (Fig. 4a). Eh readings for pH-buffered quinhydrone (Fig. 4a) decreased with time (7.5 mV between 10 and 120 min), and may not have stabilized at the time of the last measurements. The theoretical potential in pH-buffered quinhydrone (pH 4) should

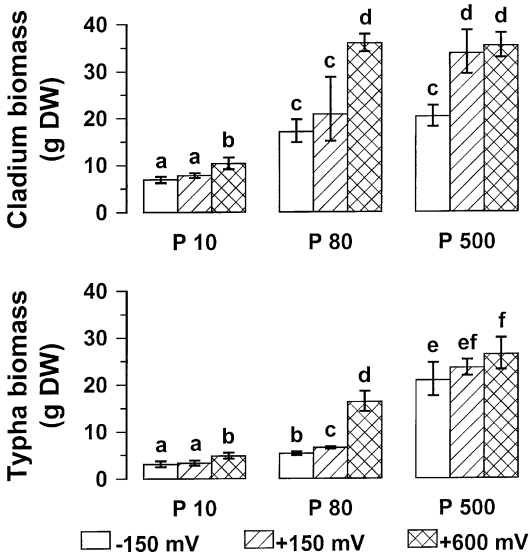

Fig. 5. Biomass produced for *C. jamaicense* and *T. domingensis* plants grown at phosphate levels of 10, 80 and 500 μg l^{-1} and redox intensities of −150, +150 and 600 mV. *C. jamaicense* was grown for 9 weeks while the faster growing *T. domingensis* was grown for 4.5 weeks. Backtransformed means ±95% asymmetric confidence limits ($n = 4$). Any two means which share the same letter do not differ at the $P < 0.05$ probability level.

be 464 mV at 23 °C (extrapolated from data tabled by Bohn (1971)). In this experiment, it averaged 472 mV ($n = 35$) after 120 min, thus differing by 8 mV from the theoretical value. The Eh readings for the alluvial silt (Fig. 4b) decreased much more gradually over time by ca. 250 mV and may not have reached equilibrium after 115 h. Values recorded between 58 and 115 h, however, did not differ statistically. The variance around the mean for this substrate was initially relatively small but gradually increased to a maximum at 3–4 h, after which the variance gradually decreased. The variance for the calomel electrodes were higher than for the platinum electrodes for both the nutrient solution and the pH-buffered quinhydrone (Table 2). Variances for platinum electrodes of 8 and 16 months of age did not differ (Table 2).

The responses of *C. jamaicense* and *T. domingensis* to long-term Eh treatments are shown in Fig. 5. Plants of both species produced significantly more biomass at high phosphate availability. Within each phosphate level, biomass was lower at −150 than at + 600 mV. Both species had significant Eh × phosphate interaction ($p < 0.01$).

4. Discussion

The ORP controlled hydroponic system described here was used to study effects of Eh on plant physiological responses without the interference from confounding soil factors. Using titanium citrate as a reducing agent, plant response was evaluated with respect to redox intensity and not redox capacity, which have been found to influence plant growth differently (Kludze and DeLaune, 1999). The test data obtained in this study demonstrated a clear temporal separation of Eh for each of the three redox treatments selected. Eh was found to fluctuate within ±50 mV of set-point values for the two reduced treatments. Eh remained fairly stable (+560 to +580 mV) in the fully oxidized solution since the NH_4^+ uptake activity of the *P. australis* roots was not high enough to modify the pH, and hence Eh, of the nutrient solution. However, when the system was applied to *C. jamaicense* and *T. domingensis* grown at the same redox levels, there were significant effects of plant nutrient uptake on Eh in which, after 2 months, the oxidized treatment exhibited the same ±50 mV fluctuation range as the two reduced treatments. It is difficult to buffer the pH of solutions when roots occupy the greater part of the containers, which typically is the case towards the end of a growth experiment. If diurnal pH fluctuation is not desired, a pH controller can be added to the design presented here and would operate in concert with the Eh controller. The system is not suitable for evaluating the response to oxidized treatments since Eh shows only small changes in the region where there is gaseous O_2 (+400 to +700 mV). Within this aerated range it may be better to evaluate plant responses by purging with mixtures of oxygen and nitrogen and measuring aeration as O_2 concentration (Gambrell and Patrick, 1978).

Control frequency, and the way in which controlling was achieved, differed between the −150 and +150 mV treatment. The consumption of reduced titanium citrate was higher at −150 mV, which may have been related to the much higher electron activity, giving rise to electron transfer to other solutes (e.g. sulfate). Since Eh generally tended to drift down at +150 mV, only a small amount of titanium citrate was consumed between solution renewals, indicating that Ti^{3+} did not play an important role in eliminating inputs of O_2.

The difference in Ti^{3+} citrate usage should be accounted for when the investigator attempts to maintain the same total concentration of titanium citrate across treatments in order to avoid different titanium, citrate, and salinity concentrations that can confound the treatment effects. It is possible that the oxygen consumption capacity of microorganisms metabolizing citrate and/or root exudates was high enough at the +150 mV treatment to eliminate the O_2 entering the system. Up to 30% of plant assimilated carbon may be lost as root exudates (Meharg and Killham, 1990) suggesting that exudates may constitute a significant electron source. Diurnal variations in oxygen release due to convective gas flow may explain the phenomenon of fewer control events during daytime hours. For *P. australis*, light enhanced, convective flow increases oxygenation of below-ground organs as well as in the rhizosphere (Armstrong and Armstrong, 1990). Diurnal variation in control frequency may also relate to higher NH_4^+ uptake during daytime hours conferring a faster rate of overall concomitant pH decrease/Eh increase thus resulting in a slower rate of Eh decrease and extending the periods between control events.

Proper electrode performance is crucial for optimum control. A quinhydrone test only detects bad electrodes, while a less-poised solution may reveal additional unsatisfactory electrodes. For each batch of Pt electrodes made, typically less than 5% had to be discarded due to unacceptable readings in pH-buffered quinhydrone (±10 mV from the theoretical value). However, an additional 5–10% of the electrodes deviated more than 10 mV from the mean value in the nutrient solution despite working well in quinhydrone. Only electrodes that give proper readings in the nutrient solution should be selected for experimental use. This is exemplified by Fig. 2, where a Pt electrode deviated more than 100 mV from the two other replicate electrodes despite all three produced proper readings in quinhydrone. In an actual experiment such a faulty electrode would have been cleaned, re-tested and discarded if cleaning failed to improve performance.

This study concludes that Pt electrodes perform well in hydroponics if the investigator routinely inspects readings among replicate electrodes to identify the ones that are fouling. A typical example of gradual electrode fouling is shown in Fig. 2 where one electrode gradually deviates from the two replicate electrodes. The gradual loss of accuracy is easily corrected by cleaning the electrode. Calomel electrodes are seemingly resistant to contamination and are therefore unlikely to cause any major problems in this respect but still need to be monitored in respect to KCl flow.

In addition to allowing precise control of nutrient levels, a hydroponic system also facilitates more accurate and faster control of Eh, since the Pt electrode signal converged much quicker to that of the actual redox potential in solutions (minutes to hours) than in flooded Mississippi alluvial silt (days). The Pt electrodes had to be left 75 h in the alluvial silt before it became apparent that the soil was sufficiently reduced (−150 mV) for sulfate to be unstable (Etherington, 1985). A short response time was also found for Pt electrodes in an oxygen-free nutrient solution spiked with 2.5 ml titanium citrate per liter of nutrient solution. Eh decreased 400 mV within 1 min of addition then dropped an additional 40 mV during a 20 min period before equilibration (data not shown). Thus, the slow response in the alluvial silt is not related to this substrate having the lowest Eh of the tested substrates. The higher variability among electrodes in the silt may be partly related to micro-site differences in the soil (Cogger et al., 1992), while the temporal variability in standard deviation more likely reflects the different speed at which individual electrodes converge to the actual Eh.

It can be concluded that a soil-less culture offers the advantage of relatively fast control of Eh over the entire Eh range encountered in wetlands. Redox potential of quinhydrone is not a mixed potential and the observed decreasing trend is therefore a cause for worry, especially if quinhydrone readings are used to calculate individual correction factors for Pt and calomel electrode pairs. The 7.5 mV drop during the 120 min test period is too large to be explained by temperature effects as Eh only decreases about 1 mV $^{\circ}C^{-1}$ (Patrick et al., 1996). Despite the drop, Eh was still 8 mV higher than the quinhydrone derived theoretical value (220 mV at 23 °C, equal to 464 mV when corrected, 244 mV for calomel potential) indicating that convergence to the theoretical value takes more than 120 min. While this offset to the theoretical value may have further lessened beyond the 120 min due to the decreasing trend of Eh, the 8 mV offset may also wholly or partly be ascribed to electrode offsets. The accuracy, defined as the closeness of measured value to its true value for Pt electrodes, seems therefore to depend on several important factors, including the individual half-cell electrode potentials, the chemical composition of the test solution and the time elapsed after the electrodes where inserted into the solution. These factors makes the Eh of the nutrient solution difficult to quantify exactly. However, if one considers that the variation around the mean value is consistent with that of quinhydrone (Table 2) and that the actual Eh readings in quinhydrone generally deviates less than 15 mV from the theoretical value, it is likely that Eh readings in hydroponics lies within ±15 mV of their true values. In the present study, this level of accuracy is acceptable as there was 300 mV between the treatments.

Monitoring the system requires that the investigator understands the possible factors responsible for Eh drifting out of set-point range. Erroneous control is frequently related to faulty electrodes giving higher Eh readings than actual. If not corrected, excess consumption of titanium citrate will take place resulting in lower Eh than desired, as well as an increase in salinity. Contamination of the platinum surface, caused by, for example, absorption of organic substances or platinum sulfide reactions, sometimes occur and will result in bad readings (Bohn, 1971). Usually, it takes days or even weeks of continual immersion in hydroponics before electrodes become contaminated and unresponsive. In some instances, gently shaking the Pt electrode may cure the problem. At other times cleaning is necessary to restore the electrode's response. Contamination is a difficult problem to deal with; however, comparing readings from replicate electrodes can reveal suspect electrodes. It is advisable to compare readings of replicate electrodes at least once daily and use no less than triplicate Pt electrodes, as also recommended for soil (Patrick et al., 1996). For Pt electrodes 8 and 16 months after manufacture, no difference in mean and variance was detected for Eh readings in pH-buffered quinhydrone. It is therefore concluded that this inexpensive, easily constructed type of Pt electrode is useful in long-term experiments.

The relationship between pH and redox potential was found to be linear with a pH change of one unit for every 50 mV change in Eh. The relationships between buffer capacity, pH and redox potential are important with respect to the acceptable Eh window set by the investigator, who should attempt to attain the same range of Eh fluctuations for all redox treatments.

The high salinity of titanium citrate (84‰ NaCl) may impose a confounding treatment effect on salt-sensitive plant species even when added in small amounts. In the long-term experiments with *C. jamaicense* and *T. domingensis*, the salinity of the nutrient solution

increased from 1.0 to 2.5‰ NaCl between weekly solution renewals. These salinity levels may cause growth reduction for moderately salt-sensitive plants such as rice (*Oryza sativa* L.). This species groups with crop species that have a maximum threshold salinity level of 3 dS m^{-1} (Ashraf, 1994), which is equivalent to ca. 2‰ salinity (Bernstein, 1975). To reduce the impact of salinity, the investigator can increase the frequency of solution renewals or use organic buffers instead of sodium carbonate for the pH adjustment of titanium citrate (Sorrell et al., 1993).

Titanium citrate has previously been used to colorimetrically quantify radial oxygen loss, exploiting the fact that the complex is decolorized upon oxidation (Chabbi et al., 2000; Sorrell et al., 1993). These studies have used a relatively high proportion of titanium citrate in the nutrient solution. Some of these studies may therefore have imposed a sudden salt stress resulting in water loss, as well as growth inhibition, making oxygen loss estimates questionable. Since the amount of titanium citrate added to the solution increases as potency is reduced, it is important to use titanium citrate of maximal potency to minimize salinity increases between solution renewals. It was found that enough air could enter an 18 gauge syringe needle to decrease the reducing capacity of titanium citrate considerably within a few days. As a consequence, titanium citrate in the reservoirs must be renewed twice weekly. It therefore seems worthwhile to use the extra tubing and nitrogen to continuously purge the headspace of the titanium citrate reservoirs.

The method presented her was designed to mimic long-term waterlogging and to test the ability of plants to cope with such conditions. The results of this study indicated that *C. jamaicense* and *T. domingensis* produced less biomass at low Eh and that the effect of Eh was modified by phosphate availability. As nutrient levels can be precisely controlled in hydroponics, the method may also show itself useful to investigate nutrient uptake in response to Eh.

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