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ABSTRACT

Accurate remote detection of plant health indicators such as moisture, plant pigment concentrations, photosynthetic flux, and other biochemicals in canopies is a major goal in plant research. Influencing factors include complex interactions between wavelength dependent absorbing and scattering features from backgrounds as well as canopy biochemical and biophysical constituents. Accurately controlling these factors in outdoor field studies is difficult. Early testing of a porous tube plant culture system has indicated that plant biomass production, biomass partitioning, and leaf moisture of plants can be controlled by precisely managing the root water potential. Managing nutrient solution chemistry can also control plant pigments, biochemical concentrations, plant biomass production, and photosynthetic rates. A test bed was developed which utilized the porous tube technology with the intent of evaluating remote sensing systems, spectral analyses procedures, gas-exchange, and fluorescence measurements for their ability to detect small differences in plant water status. Spectral analysis was able to detect small differences in the mean leaf water content between the treatments. However these small differences were not detectable in the gas-exchange or fluorescence measurements.

INTRODUCTION

Many types of plant research can benefit from development of technologies to precisely control, alter, and monitor plant growth and physiological processes. The ideal test plant growth system would allow for control of the spectral quality and intensity of the light environment, the water and nutrient contact with the root system, the temperature and humidity of the growth area, and the concentration of gases in the atmosphere. Today's high quality plant growth chambers can be used to address the atmospheric gas, temperature, and humidity concerns. Advances in lighting technology such as light-pipe, LED and other innovative lamps as light sources are addressing issues associated with control of the photosynthetically active radiation environment. Creation of a reliable simple system for controlling the flow of water and chemicals directly available to plant roots provides tremendous opportunities for plant research in areas such as remote sensing of plant stress, toxicology testing, tissue culture of plants, and plant physiology. By accurately controlling the plants growth environment to minimize variance resulting from environmental responses we can begin to more accurately define treatment effects and minimum detectable differences in physiological characteristics as they relate to sampling strategies and procedures. For this research we are evaluating configurations of the porous tube plant nutrient delivery system (PTPNDS) and assessing our ability to detect plant responses to the various configurations.

The PTPNDS (Dreschel 1990) was originally designed for NASA for growing plants in the microgravity of space. In microgravity, plant rhizospheres tend to become anoxic because gravity-mediated convection is non-existent. To avoid these problems, the delivery of water and dissolved nutrients to roots grown in microgravity must be done in a precise fashion. The PTPNDS utilizes a controlled fluid loop to supply nutrients and water to plant roots growing on a ceramic surface moistened by capillary action. See Berry and others (1992) for a detailed description of the PTPNDS.

The PTPNDS has been specifically developed to overcome many of the challenges in providing water and mineral nutrients to the roots of plants in microgravity (Dreschel 1990) and in certain ground systems (Dreschel and Brown 1993). Preliminary studies with the PTPNDS have centered around testing the system with various crop plants (Dreschel et al. 1988, Dreschel et al. 1992, Bubenheim et al. 1987), developing hydraulic pressure control systems for laboratory-scale crop tests (Dreschel 1992), measuring the effects of hydraulic pressure, pore size, and root zone volume on plant growth (Dreschel et al. 1989a, Dreschel et al. 1989b, Dreschel et al. 1994, Peterson et al. 1989, Berry et al. 1992) developing physical and mathematical models to describe the operation of the system (Tsao et al. 1992, Tsao et al. 1996) and utilizing the system to grow crop plants in the confines of ground-based spaceflight plant growth unit such as the one patterned after the Russian SVET hardware (Chetirkin et al. 1994). The PTPNDS has also been used as a research tool to evaluate the response of plants to varying degrees of water and nutrient stress (Dreschel and Sager 1989, Dreschel et al. 1989b) and to investigate the hydrotropic response of plant roots to moisture gradients (Takahashi et al. 1992).

The porous tube technology avoids problems related to a lack of oxygen in the rhizosphere. Roots grow directly on the surface of the nutrient-supplying porous tubes and are surrounded by an air space contained within an outer root-encompassing barrier (Figure 1). Thus there is a

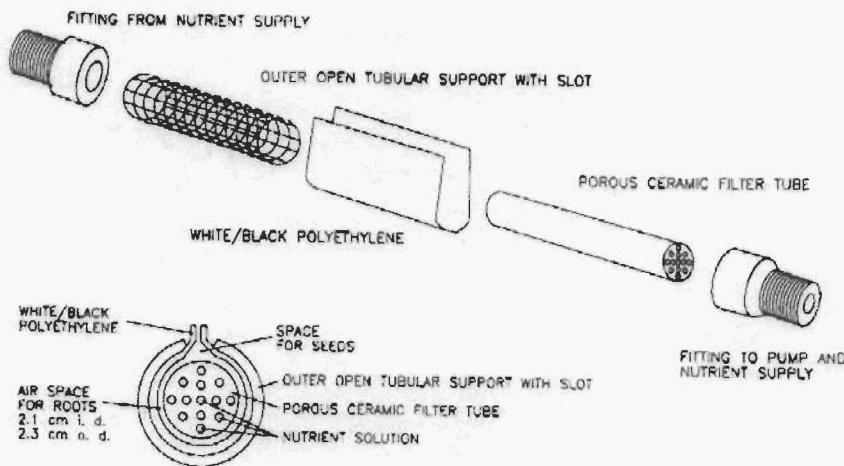


Figure 1. A Diagram of the Porous Tube Component of the PTPNDS.

constant supply of oxygen in the immediate vicinity of the roots. This has been demonstrated by a series of studies in which the PTPNDS was compared with agar-based and floral foam plant

culture systems developed for space flight applications. In these investigations alcohol dehydrogenase (ADH) levels in the roots were used to indicate water-logging (oxygen-deprivation) stress. Of the three systems tested, the PTPNDS grown plants produced the lowest amount of ADH in their roots and the highest shoot biomass, indicating the lowest level of stress (Porterfield 1996).

In ground-based studies water flux measurements were made with a series of gravimetric tests and absorbent material (germination paper) on various pore size tubes (Figure 2). Results indicated a dependence of water flux on applied water potential (suction pressure) and pore size. Other studies (Dreschel et al. 1993) have indicated that water flux for various pore sizes differs according to the magnitude of the gravitational force.

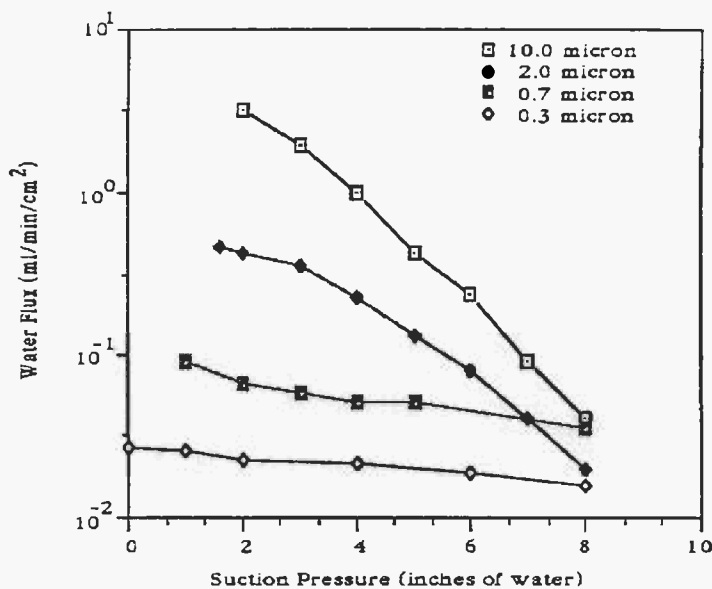


Figure 2. A semi-log plot of the water flux rate versus suction pressure (applied water potential) (Tsao et al. 1992).

OBJECTIVE

Determination of biomass, water status, photosynthetic rates, chemical concentrations, and biophysical features of vegetation are recognized as important objectives for remote sensing in agriculture, terrestrial ecology, and earth system science. Successful algorithm development for feature characterization requires development of a quantitative understanding of complex interactions that occur between photons and the numerous absorbing and scattering components of targets (Bostater et al. 1994, Asner 1998). For plants, these wavelength dependent interactions involve tissue optical properties, canopy biophysical attributes, bottom reflectance (soil and litter), and illumination characteristics.

In remote sensing, two regions of the infrared reflectance spectra, the near- (700-1300 nm) and middle- (1300-3000 nm), have been identified as being especially useful for the detection of vegetation water stress or water content (Ripple 1986, Tucker 1980) and other plant constituents

such as cellulose, lignin, starch and nitrogen compounds (Jacquemoud et al. 1995). Organic bonds found in foliar mass exhibit vibrational stretching modes that absorb radiation in these regions of the spectrum. Water absorption bands occur at around 760, 970, 1450, 1940, and 2950 nm in response to the OH bond (Grant 1987). Changes in the curvature or inflection of the reflectance signature in the 970 nm region have been found to have high correlation with water potential and percent moisture of scrub oak leaves (Bostater et al. 1994).

Under water-stressed conditions, plants are expected to exhibit a decrease in photosynthesis and conductance while experiencing an increase in fluorescence at higher light intensities. It has been found that water-stress does not directly affect the biochemistry of photosystem II (Lu and Zhang 1998). If photosystem II was directly affected, differences in fluorescence should be detected in the dark adapted state. However, studies have found that water stress does not affect fluorescence measurements in the dark adapted state (Monje et al. 2001, Lu and Zhang 1998). Instead, water-stress increases the potential for damage caused by high light intensities, otherwise referred to as photoinhibition (Lu and Zhang 1998).

The objective of this study is to investigate the potential for using remote sensing instrumentation, infrared gas exchange system, or a fluorometer to detect small differences in the water status of plants being grown on the PTPNDS at varying water potentials.

METHODS AND MATERIALS

Eight porous ceramic tubes, 2 cm in diameter and 80 cm in length were placed on each of three laboratory carts. These tubes were connected to a standpipe using a manifold at the upstream end and connected to a peristaltic pump at the downstream end. The nutrient solution for each cart was contained in an insulated 80-liter covered container (cooler) and the solution was pumped to the standpipe using a submersible (magnetically coupled impeller) pump. One-half inch vinyl hose carried the solution to the standpipe and three-eighths inch vinyl hose carried the overflow from the standpipe back to the reservoir. Solution was supplied from the standpipe to the manifold via another one-half inch vinyl hose. Two laboratory-scale peristaltic pumps, each with four heads, draw the solution through each ceramic tube and return it to the reservoir at a rate of nine milliliters per minute, per porous tube. Each cart can be independently moved relative to the others, allowing ready access to any of the plants being grown on the cart (Figure 3).

Metal-halide lamps provided the photosynthetically active radiation for the plants and were hung on a structure that allowed the carts to be moved underneath the lights. Nutrient solution was maintained by adding water daily to the reservoir and periodically adding concentrated nutrients to maintain solution conductivity. A bracket system allowed the standpipes to be placed at the level of the porous tubes down to about 40 cm below the tubes. This creates a pressure difference between the tubes and the standpipe and was used to establish the initial three treatments: near zero pressure; -16.0 cm of water pressure; and -32.0 cm of water pressure on the nutrient solution within the porous tubes. The entire system was constructed and maintained within a controlled temperature chamber, a walk-in refrigerator (3 m x 4 m x 2.5m). Chamber temperature was typically 22°C with lights on and 18°C with the lights off. Within the plant



Figure 3. Photograph of one of three PTPNDS used for the study.

canopy, with the lights on, the temperature was typically about 25°C. Humidity was not controlled, but was typically about 55 % ± 5%. A 12-hour photoperiod was used to grow the plants until day 60 when a 24-hour photoperiod was implemented. This was used to provide a relatively constant environment for the plants while a series of gas-exchange and fluorescence measurements were conducted.

Super-dwarf wheat plants (*Triticum aestivum*, cv. Perigee) were grown for a complete life cycle. Wheat seeds were planted dry on each of the tubes (approximately 40 seeds per tube) and covered with a section of moistened paper laboratory wipe. Planting was conducted on June 6, 2002 with solution flowing through each tube. The variable nutrient solution pressure, from now on to be referred to as applied water potential, was established at the initiation of the study. The seed and plants on the porous tubes of the three carts were subjected to applied water potentials of -0.1 cm of water (-0.8 Pa), -16 cm of water (-1,570 Pa), and -32 cm of water (-3,140 Pa). Plant height measurements began seven days after planting (DAP) with measurements thereafter being taken on a weekly basis. Spectral reflectance as well as fresh and dry weights was measured on five plants taken from each cart at 25, 32, 40, and 57 DAP. Beginning week 3, the carts were rotated within the growth chamber each week to ensure consistent lighting for the three carts.

Spectral data were collected using an Analytical Spectral Devices (ASD) Fieldspec Pro spectrograph (ASD 1999). Downwelling radiance (as digital number) was estimated using a Spectralon white reflectance panel. Upwelling radiance was measured as the average of 30 spectral samples of each leaf. Reflectance was calculated as the ratio of the upwelling to the downwelling values. All spectral measurements were made using a tungsten light source set at a constant output level with a rheostat. All angles of measurement, illumination and distances between target leaves, light source and ASD optical probe were held constant to minimize bi-directional influences. For each treatment, flag leaves from five randomly selected plants were used in the spectral measurements. Leaf spectral reflectance was measured immediately after fresh weights of the plants were determined (Ohaus Analytical Plus Balance; ± 0.001 g). The plants were then dried at 70°C for 72 hours and reweighed to determine the plant dry weight.

Data collected with the ASD spectrograph were sub-sampled at 5nm intervals to reduce issues associated with correlations between adjacent spectral bands (Demetriades-Shah et al. 1990). Analyses focused on data from DAP 57, a period that demonstrated significant differences in leaf percent moisture for the three applied water potentials. A simple correlation analysis was run between the spectral reflectance data and the percent moisture to define first order relationships between reflectance values and leaf moisture. To assess possible relationships between leaf percent moisture and spectral curvature or inflection a three band inflection ratio (Grew 1980, Bostater et al. 1994) was computed. Spectral smoothing was utilized to reduce influences of noise and maximize the signal (Demetriades-Shah et al. 1990). The inflection estimator was defined as:

$$I_{(\lambda) m, i, n} = R_{(\lambda) i}^2 / [R_{(\lambda) i-n} \times R_{(\lambda) i+m}] \quad (1)$$

where $I_{(\lambda)}$ is the inflection or curvature estimator centered at band i calculated from the reflectance signature. M and n are forward and backward operators, respectively. For this analysis m and n were 9 band steps or about 50 nm after smoothing. Correlograms were generated to identify areas of strong correlation between the inflection estimator and leaf percent moisture. Analysis of variance was used to test for significant differences between inflection values in the regions of highest correlation associated with the three levels of root water potential. The Bonferroni pairwise mean comparison test was used to test between individual treatment differences. A least squares linear model was calculated to describe the strength of the relationship between leaf percent moisture and the three band inflection estimator computed from reflectance data. Analyses and data processing were conducted with Systat 9 (SPSS 1999).

Physiological parameters were measured with a LiCor 6400 Portable Photosynthesis System (LiCor; Lincoln, NE) on flag leaves of plants from each treatment. Measurements of photosynthesis, stomatal conductance, and fluorescence were taken on one or two plants from each cart 15, 26, 32, 34, 36, 39, 41, and 55 DAP. Leaves were dark adapted for 20 minutes using the LiCor 6400 with the leaf chamber fluorometer attachment. Fluorescence and gas-exchange measurements were made on the dark adapted leaf. After dark adapted measurements were taken, fluorescence light curves were made on each leaf. Measurements were taken at eight light levels (1200, 1000, 800, 600, 300, 100, 50, and 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Data was graphed and compared for trends between the treatments.

Regular additions of water to the reservoirs were recorded to provide a rough estimate of evaporation and water uptake by the plants. The number of plants that had produced tillers was counted on DAP 60 to determine differences between treatments.

In order to determine if the different applied water pressures affected the various growth parameters (% moisture, height, # tillers, fresh weight, dry weight, etc), analysis of variances were conducted. If a significant difference was found, then Games-Howell Post Hoc tests were conducted to determine which treatments differed from the others.

RESULTS

Following planting of the wheat seed, approximately 85% germination was observed over the next two days. No trend in plant height growth persisted through the entire study (Figure 4). Plant height growth was statistically different between all three treatments for DAP 7, 14 and 21, however the order of the treatments did not remain the same. By DAP 21 height growth in the -0.1 cm treatment was significantly lower than in the other two treatments. This trend persisted until DAP 69, when the -0.1 cm treatment increased dramatically relative to the other two treatments so that by DAP 69 the height was statistically similar to that of the plants in the -16.0 cm treatment. The height growth of the -32.0 cm treatment was significantly lower than the other two treatments by DAP 69.

Tiller production varied between the three treatments (Figure 5). The -0.1 cm treatment demonstrated a much higher production of tillers at DAP 60 and 69 than did the other two treatments, such that 38% and 49% of the stalks were tillers respectively. The difference in tiller production between the -16.0 cm and -32.0 cm treatments was greater at DAP 60 than at DAP 69 (10% and 2% respectively).

No trend in photosynthesis between the treatments was observed (Figure 6). In fact little variation was observed in either respiration or photosynthesis under low light ($300 \mu\text{mol s}^{-1}$). However, photosynthesis under high light ($1200 \mu\text{mol s}^{-1}$) was more variable, but no treatment consistently had higher rates of photosynthesis.

Unlike photosynthesis, trends were observed in stomatal conductance when the plants were light adapted (Figure 7). The -0.1 cm treatment consistently had higher stomatal conductance than did the other two treatments. This increase in stomatal conductance without a corresponding increase in photosynthesis, indicates that the plants in the -0.1 cm treatment were less water use efficient (WUE) than the plants in the other two treatments. No consistent trend was found in conductance between the -16.0 cm and -32.0 cm treatments. Interestingly, trends in the amount of water added to the reservoirs were not in agreement with the conductance data (Figure 8). Both the -0.1 cm and the -16.0 cm trays had more water added to the reservoir late in the experiment where as the -32.0 cm treatment had less water added. This would suggest that conductance of the two stressed treatments should differ considerably. This difference was not observed.

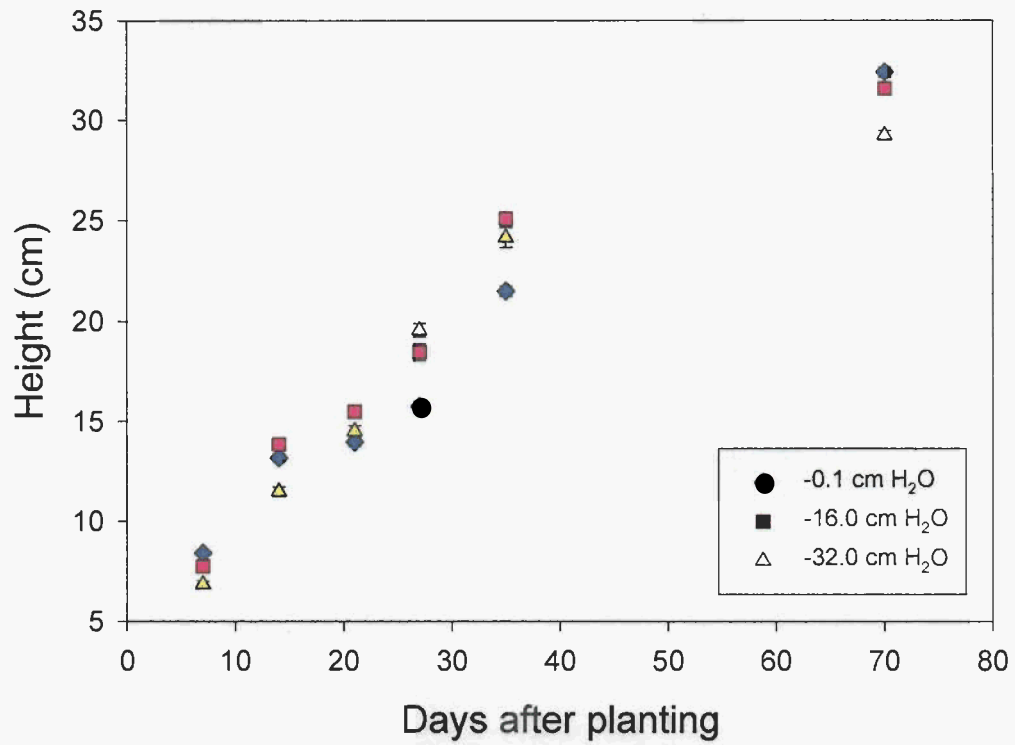


Figure 4. Measurements of plant height for the wheat grown at three applied water potentials on the PTPNDS.

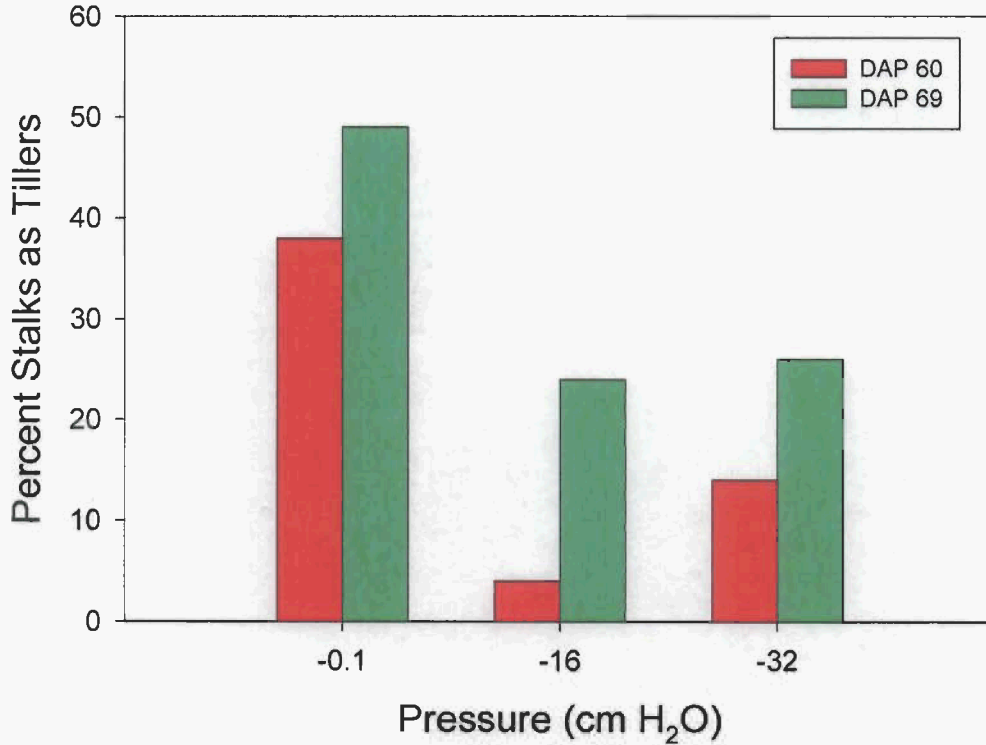


Figure 5. The percent stalks as tillers in wheat plants grown at three different applied water potentials on the PTPNDS for two different days after planting (DAP 60 and 69).

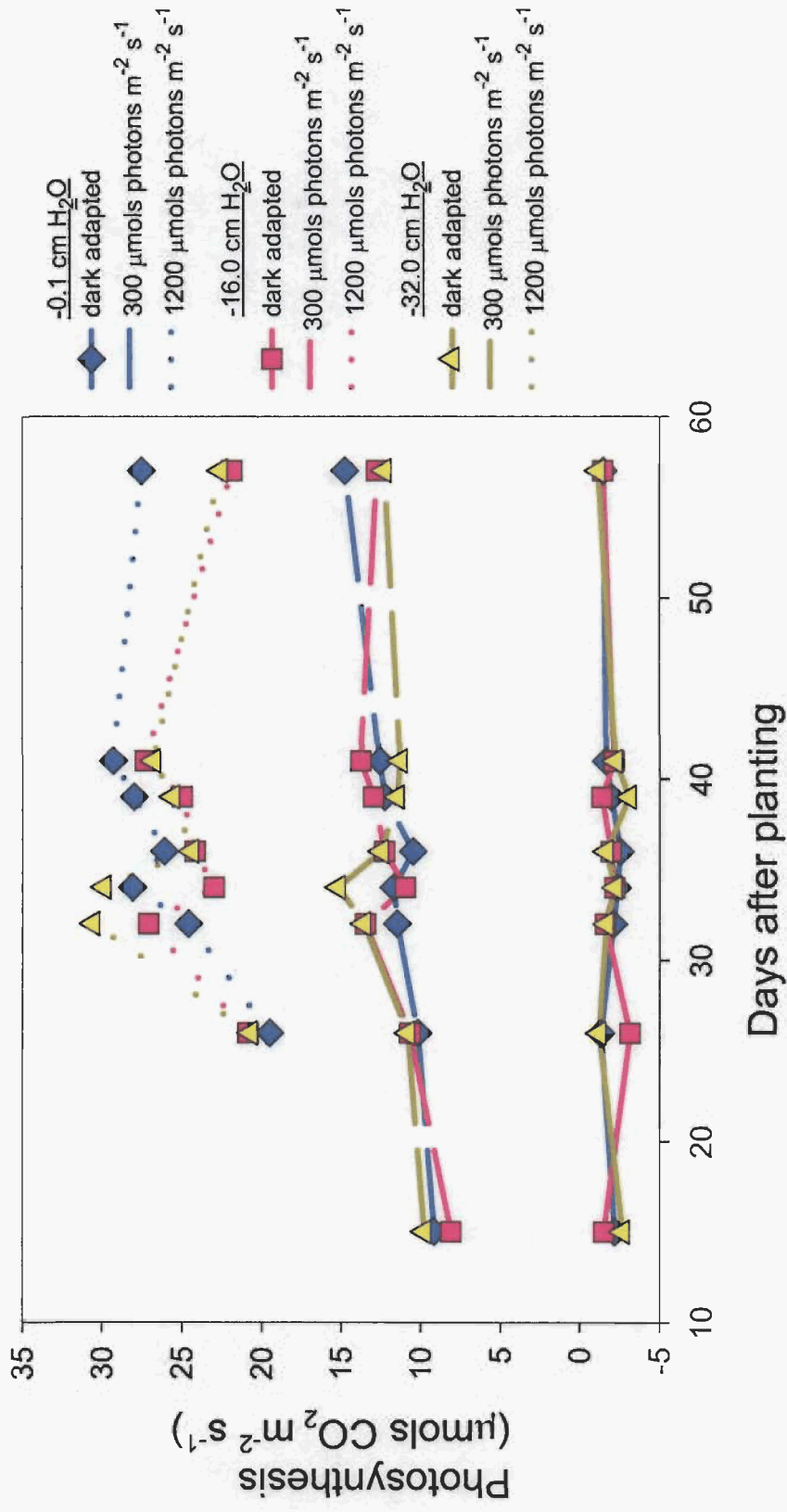


Figure 6. Photosynthesis rates of wheat grown at three root water potentials on the PTPNDS.

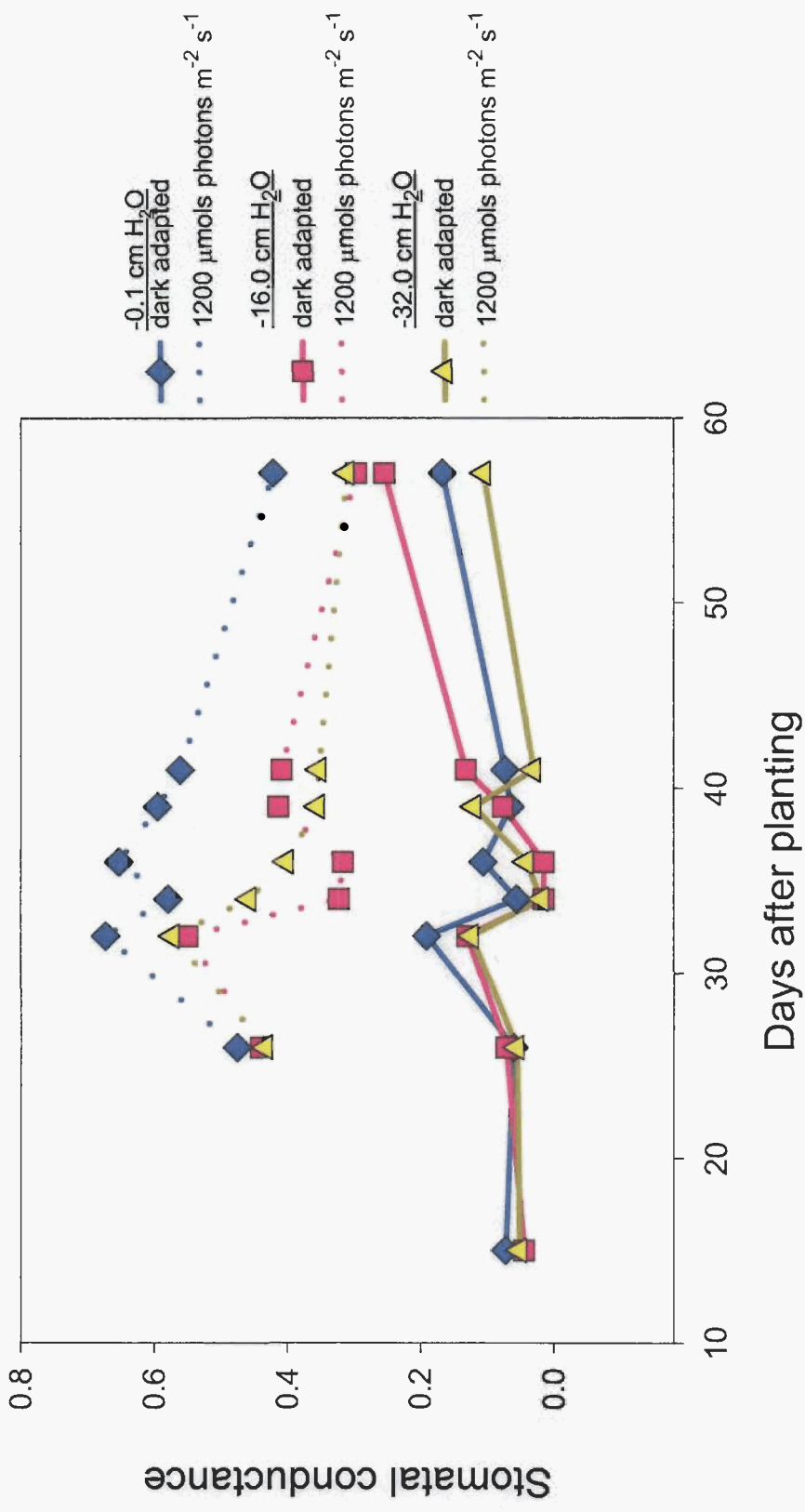


Figure 7. Stomatal conductance rates of wheat grown at three root water potentials on the PTPNDS.

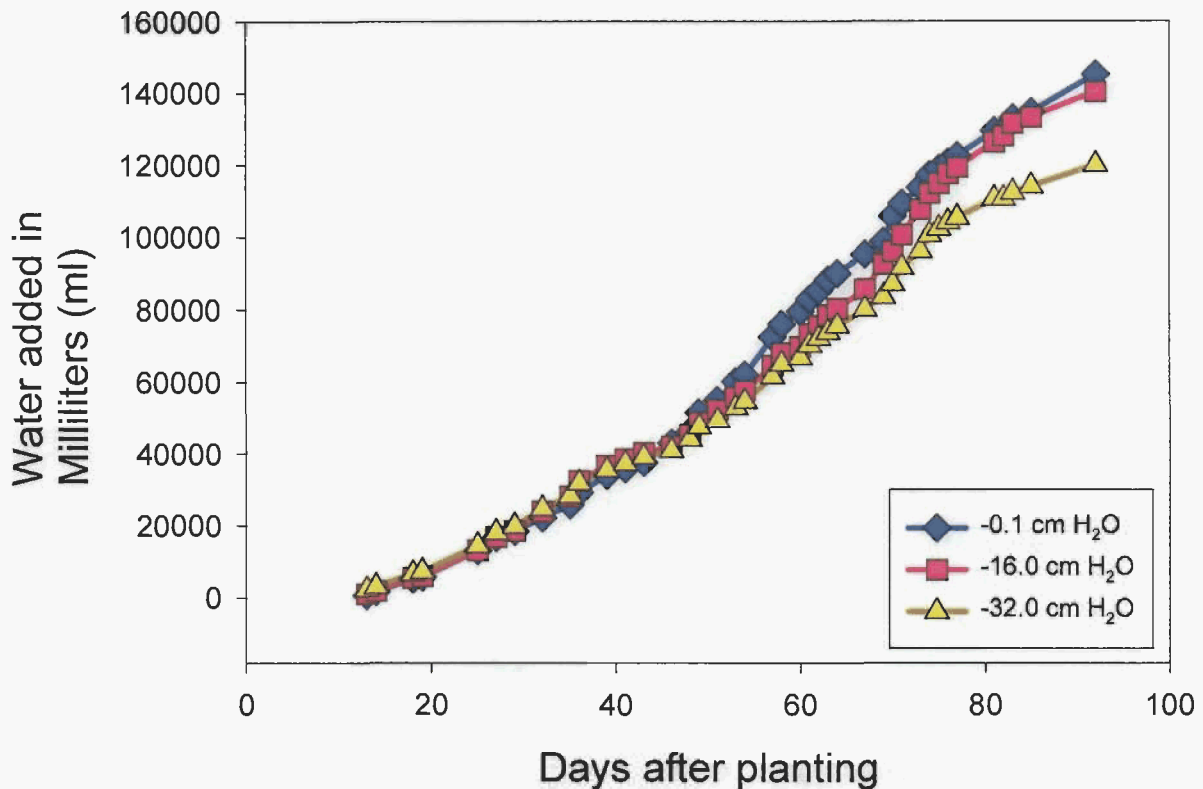


Figure 8. Water added to each of the three reservoirs representing the three applied water potentials.

Plant moisture between treatments was similar for the early part of the study but after DAP 40, the percent moisture between the -32.0 cm treatment and the other treatments began to diverge (Figure 9). For DAP57, percent leaf moisture was significantly different between treatments ($F=10.67$, $p=0.002$). Leaf percent moisture in the -32.0 cm H₂O treatment at DAP57 was significantly lower than percent moisture in the -0.1 cm and -16.0 cm treatments (78.7%, $p=0.006$ and 0.004 respectively). However no significant difference between leaf percent moisture in the -0.1 and -16.0 cm H₂O treatments (82.6% and 82.4% respectively). The percent moisture level in the above-ground biomass of the -0.1 cm treatment was significantly higher than that of the other two treatments but there was no difference between the -16 cm and the -32 cm treatment levels.

No trends between the three treatments were exhibited in the fluorescence measurements. For example, the light harvesting efficiencies (Figure 10) as well as the non-photochemical quenching (Figure 11) of plants on all three treatments were similar during both dark and light adaptation.

Reflectance and percent leaf moisture are not significantly correlated at any wavelength. However percent leaf moisture and the estimate of inflection were significantly correlated at 6

inflection points across the 800 nm near-infrared region (Figure 12 and Table 1). Trends between the treatments can be seen in the mean reflectance signatures for the region 750 to 1000 nm, during the time frame when significant differences in leaf percent moisture were exhibited (Figure 13). Interestingly the absorption area centered around 860 nm, when converted from reflectance to the inflection estimate, is strongly related to leaf percent moisture (Figure 14). The treatments explain a large amount of the variability in the inflection estimate ($R^2=0.75$, $p=0.000$). The -32.0 cm treatment had significantly different inflection estimates than the -0.1 cm and -16.0 cm treatments ($p=0.000$ and 0.003 respectively). No significant difference was found in the inflection estimates of the -0.1 cm and -16.0 cm treatments.

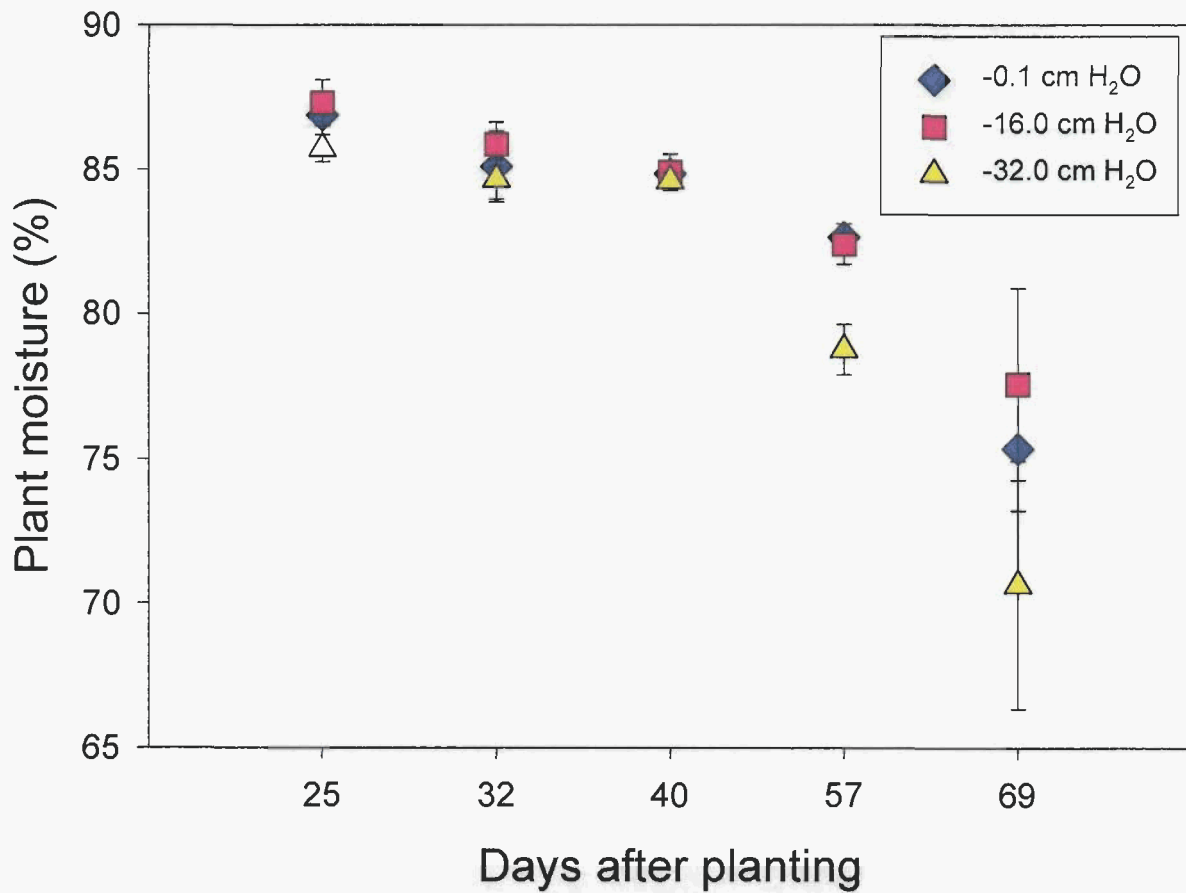


Figure 9. Percent leaf moisture of wheat plants grown at three different applied water potentials on the PTPNDS.

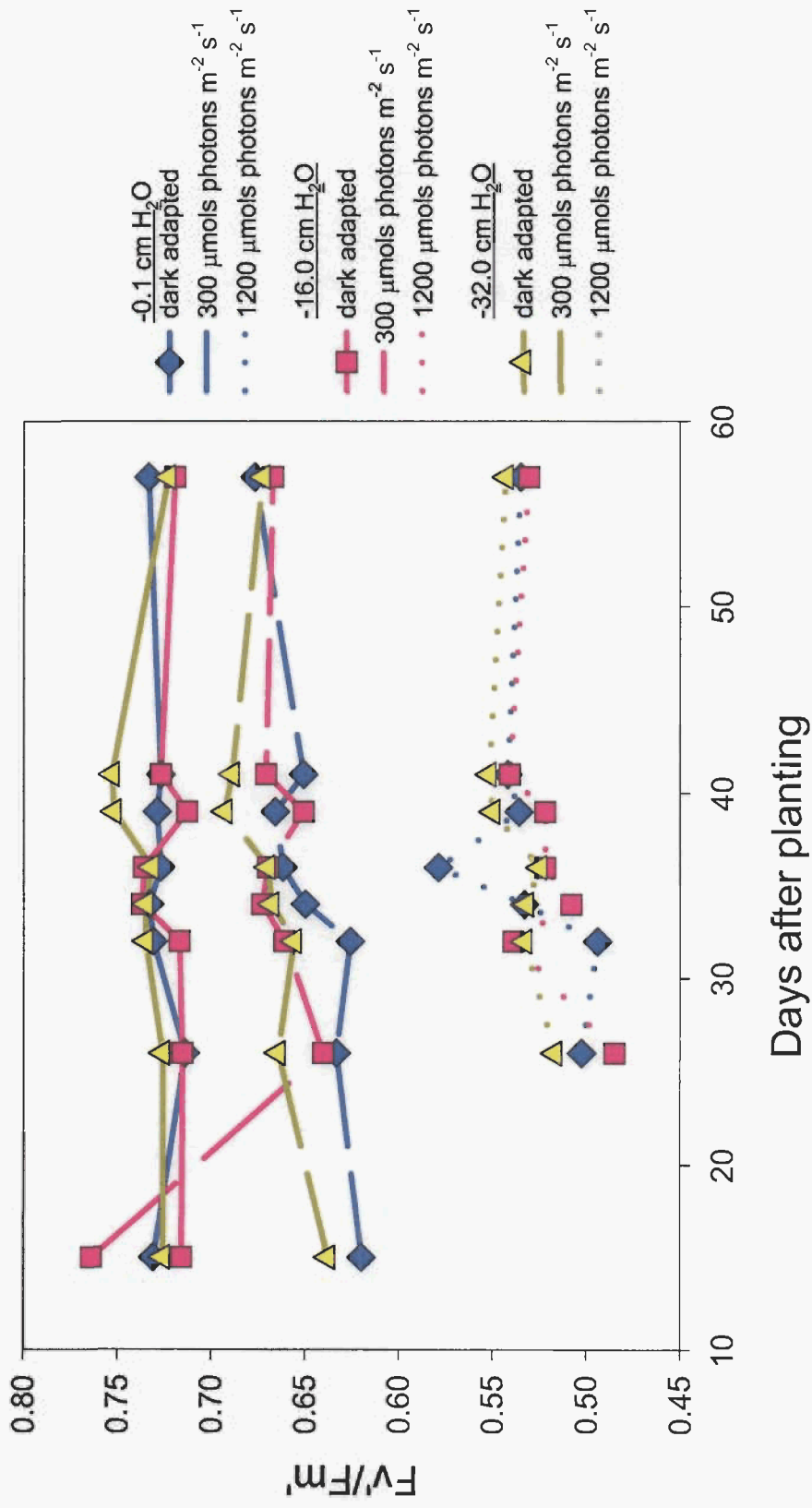


Figure 10. Light harvesting efficiency (F_v'/F_m') measured on wheat grown at three root water potentials on the PTPNDS.

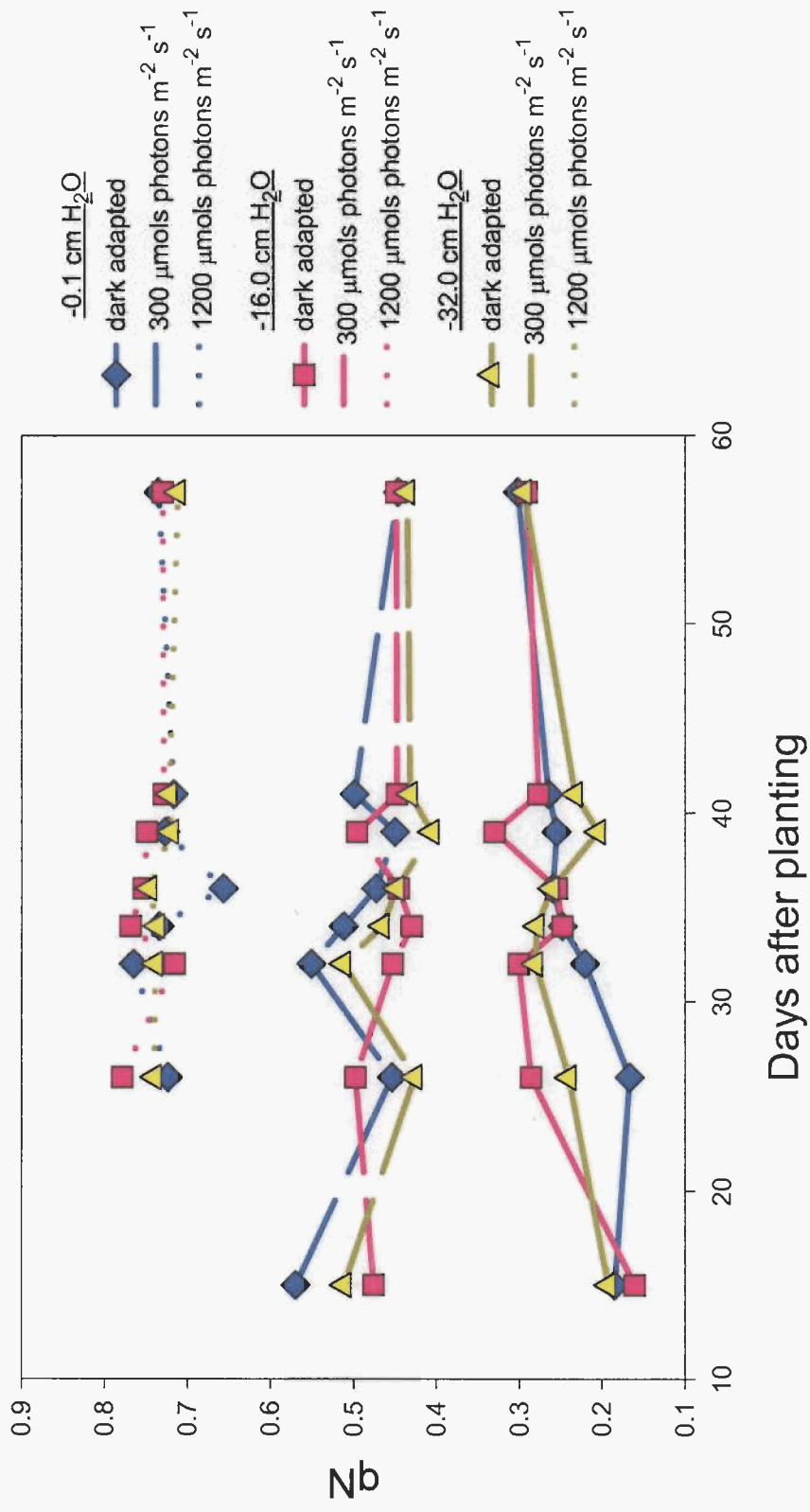


Figure 11. Non-photochemical quenching (qN) of wheat grown at three root water potentials on the PTPNDS.

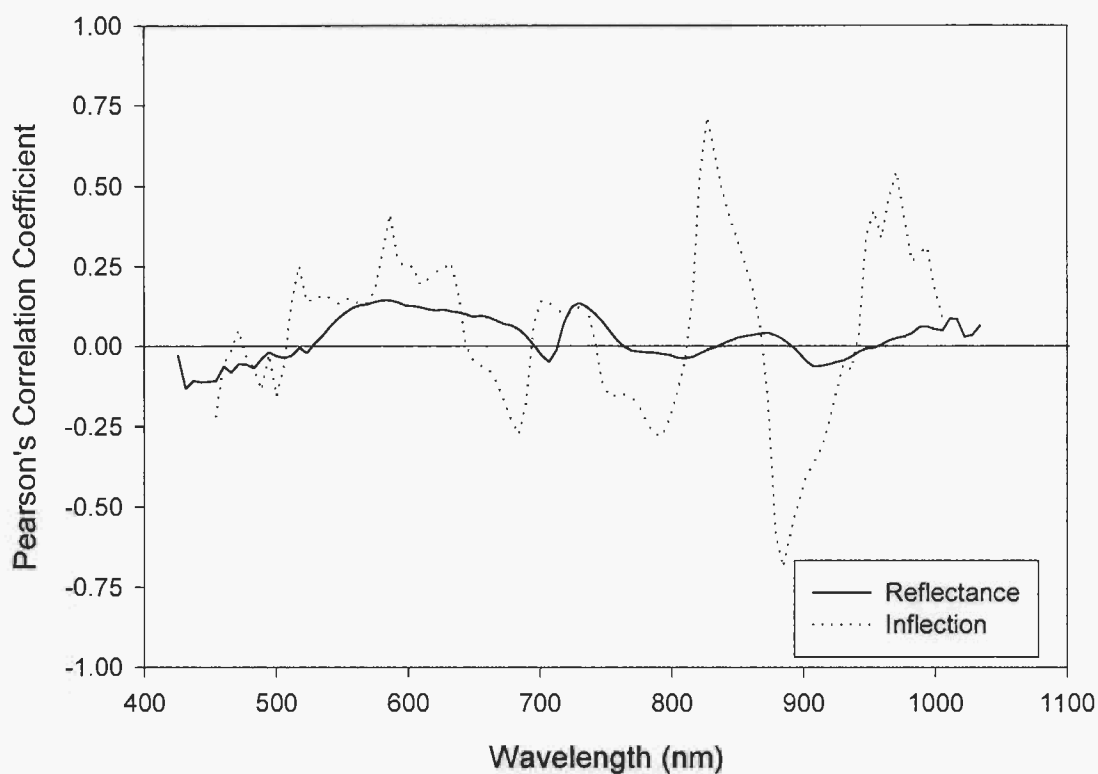


Figure 12. Correlation between leaf percent moisture, measured reflectance and calculated inflection estimates for leaves collected on DAP 57.

Table 1. Correlation between estimates of inflection and percent leaf moisture for six bands. Mean inflection estimates of 1.0 represent a flat line; values above 1.0 are concave down.

Inflection Bands	Mean Inflection Estimate	Pearson Correlation Coefficient	Probability
769.9, 821.6, 873.3	0.9935	0.53047	0.04192
775.7, 827.4, 878.9	0.9747	0.71373	0.00281
781.5, 833.1, 884.7	0.9551	0.59977	0.01811
827.4, 878.9, 930.6	0.9966	-0.58545	0.02185
833.1, 884.7, 936.4	1.0283	-0.68611	0.00470
838.8, 890.5, 942.1	1.0531	-0.57531	0.02480

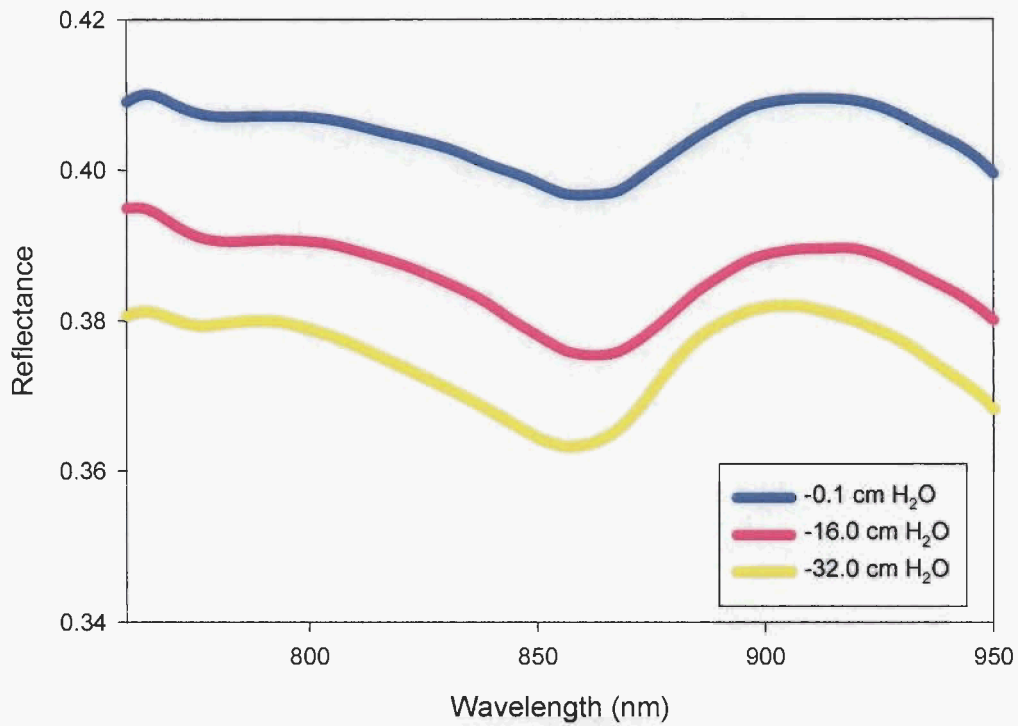


Figure 13. Average reflectance signatures for leaves harvested at DAP 57 for each applied water potential.

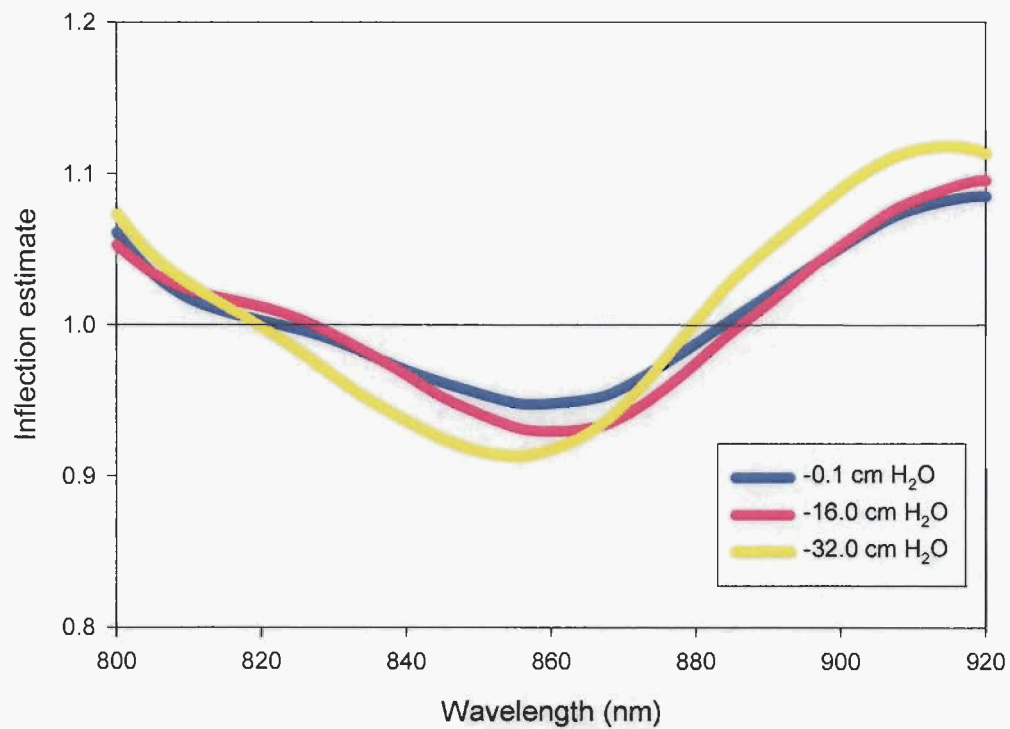


Figure 14. Mean inflection estimates between 800 and 920 nm for leaves harvested at DAP 57 for each applied water potential.

DISCUSSION

The porous tube nutrient delivery system performed well and wheat plants were cultured using three different applied water potentials in the rhizosphere; -0.1 cm of water (-8 Pa), -16 cm of water (1,570 Pa), and -32 cm of water (3,140 Pa). In a previous study, Dreschel et al. (1990) found that wheat plants grown to maturity under three different water potentials demonstrated differences in yield relative to the water potential. For example, with a decrease in applied water potential (becoming more negative), the resultant yield was decreased. Therefore it was expected that the -0.1 cm treatment would exhibit superior growth compared to the other two applied water potentials. It was evident after the first week post planting that this was not the case. Instead these plants appeared to be under water-logged conditions early in their life cycle and until the amount of leaf transpiration was sufficient to overcome this condition, the plant roots were oxygen stressed. When the leaf transpiration rates were sufficient, the growth rate of the plants accelerated and matched or exceeded the growth rate of the plants grown with the other two applied water potentials (Figures 4 and 5). Plants grown on the PTPNDS, when compared to other growth techniques, have been found to exhibit lower levels of stress due to oxygen deprivation in the rhizosphere (Porterfield 1996). However the conditions were such in the early stages of development for the -0.1 cm treatment, that oxygen-deprivation stress was exhibited. The other two treatments grew as anticipated, with the plants on the -16.0 cm treatment growing faster and with a higher moisture content and fresh weight than their counterparts on the -32.0 cm treatment.

The amount of applied water potential that the roots were exposed to was small when compared to commonly accepted field capacity values (soil at near water saturation) of around -1/3 atmosphere. Even the largest of our three applied water potentials (-32 cm of water = -0.03 atmosphere) was only about one-tenth that of an accepted value for field capacity. Yet these differences were detectable using spectral analysis.

Two regions of the infrared reflectance spectra, the near- (700-1300 nm) and middle- (1300-3000 nm), have been identified as being useful for the detection of vegetation water stress or water content (Tucker 1980, Ripple 1986). Organic bonds found in foliar mass exhibit vibrational stretching modes that absorb radiation in these regions of the spectrum. Pure water absorption bands occur at around 760, 970, 1450, 1940, and 2950 nm in response to the OH bond (Grant 1987). Bostater et al. (1994) found that changes in the curvature or inflection of the reflectance signature in the 970 nm region was highly correlated with water potential and percent moisture of scrub oak leaves. In this study, changes in the shape of the spectral curve between 790 and 945 nm displayed strong significant correlations with percent moisture in the super dwarf wheat plant leaves. An especially encouraging result was the ability to detect, in a statistically significant fashion, the small change in mean leaf water content of 5% that occurred between treatments. This feature could not be detected utilizing standard reflectance data values that displayed no significant correlations with percent moisture in leaves.

Although these small differences in leaf water content were detected in the spectral analysis, they were not detected in the gas-exchange or fluorescence measurements. Under water-stressed conditions, plants are expected to exhibit a decrease in photosynthesis and conductance while experiencing an increase in fluorescence at higher light intensities. Berry et al. (1992) found that

wheat plants grown under various applied water potentials on the PTPNDS demonstrated differences in net carbon dioxide uptake and water use efficiency. However, in this study, the expected trends were not observed for most parameters. The plants on the -0.1 cm tray were expected to be the least water stress, thus exhibiting higher photosynthesis and conductance while having the least amount of non-photochemical quenching. The plants on the -32.0 cm tray were expected to have the lowest rates of photosynthesis and conductance, while exhibited high levels of non-photochemical quenching particularly at high light intensities. However in most cases, no distinguishable trends were found for the parameters, with the exception of conductance. The -0.1 cm tray consistently had the highest conductance of the three treatments. Since photosynthesis was similar between all treatments, this indicates that plants on the -0.1 cm tray were the least water-use efficient. The most probable reason for the discrepancy between the expected and observed results is that the water potentials applied using the PTPNDS were not sufficient to induce severe water-stress in this species. Lu and Zhang (1998) found that a cultivar of wheat (*Triticum aestivum* L. cv. Shannong 229) when exposed to high light it was only the more severely water-stressed plants that exhibited a difference in measures of fluorescence. The most severely stressed plants (-1.8 and -2.1 Mpa) exhibited lower light harvesting efficiencies (F_v'/F_m') as well as increases in non-photochemical quenching (qN).

CONCLUSIONS

The rhizospheric water potential of super dwarf wheat plants (*Triticum aestivum*, cv. Perigee) was maintained at three different pressures using the Porous Tube Plant Nutrient Delivery System. Varying this water potential was found to impact the growth and development of the plants and altered the water content of the leaves. The applied water potentials used in this study are small when compared to field capacity values reported in the literature, and yet there was a measurable effect on plant moisture content, growth, and development. The difference in water content was detectable using spectral analysis, but was too small for gas-exchange or fluorescence measurements to detect. However there is still a great deal of work to be done to develop robust algorithms or methods for precisely estimating plant biochemical and biophysical properties utilizing remote sensing approaches.

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