

## Evaluating Turfgrass Response to Water Stress Using Moisture Release Curves

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### ABSTRACT

A temperature-controlled hydroponic system is described for culture of turfgrasses for physiological studies. Rate of water stress development is controlled using polyethylene glycol in nutrient solution. A technique using a combination of pressure-volume and moisture release curves is <sup>used</sup> described to evaluate turfgrass response to water stress. Leaf parameters that can be ascertained from this technique before and after water stress treatments include: maximum turgor pressure and relative water contents, turgid weight:dry weight ratios, osmotic potentials at full turgor and incipient plasmolysis, and elastic moduli. Implications of how these parameters may affect overall water use requirements of selected Kentucky bluegrass (Poa pratensis) genotypes are discussed.

### INTRODUCTION

As water for turfgrass irrigation has become more limited, current turfgrass research emphasis is being focused on plant factors that enable turfgrasses to survive and grow during periods of limited soil moisture (2). Although much effort is being placed on evaluating turfgrasses for their water use requirement, subsequent research is being conducted to elucidate factors

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which control, or at least influence, that water use rate. A fundamental issue that must be addressed is the identification of plant factors at a tissue or single plant basis <sup>rather than</sup> ~~versus~~ those that are characteristic of a turfgrass population or sward. Plant factors at the ~~tissue or single plant~~ level might include stomatal control, cuticular resistance to water loss, and turgor maintenance by osmotic adjustment in response to drought, while shoot density, canopy resistance to water loss, and rooting behavior may represent plant factors at a population or sward level. This paper concerns the former.

Osmotic adjustment refers to the lowering of tissue osmotic potential arising from the net accumulation of organic and inorganic solutes in response to water deficits (13). By actively accumulating organic and inorganic solutes, plants can lower their osmotic potentials more than can be attributed to solute concentration due to decreased cell volume alone. Plants that osmotically adjust can, therefore, maintain turgor-mediated processes, such as growth and stomatal conductance, longer and to a greater degree than plants that do not possess this characteristic. Several grasses are capable of osmotic adjustment including sorghum (8), corn (5), wheat (4), rice (12), and wheatgrasses (6).

Evidence for osmotic adjustment is gained by comparing leaf osmotic potentials at full turgor or incipient plasmolysis before and after water stress treatments. Osmotic potentials at full turgor and incipient plasmolysis have been estimated using pressure volume (14) or moisture release curves (3, 10). The turgor pressure at any given water potential, however, depends not only on the osmotic potential of the tissue, but tissue elasticity as well (13), which can be characterized by as bulk elastic modules (7). This paper describes techniques to administer a controlled rate of water stress and evaluate turfgrass response based on leaf parameters including: maximum

turgor pressure and relative water contents, turgid weight:dry weight ratios, osmotic potentials at full turgor and incipient plasmolysis, relative water content at incipient plasmolysis, and bulk elastic modulus.

#### MATERIALS AND METHODS

To study the response of turfgrasses to water stress, a system was needed with the capacity to grow large numbers of single plants whose stress levels could be controlled independently and accurately. This was accomplished with a temperature-controlled hydroponic system which utilized polyethylene glycol (PEG 8000) in nutrient solution to induce water stress osmotically. Because the level of water stress that a specific PEG solution exhibits is temperature dependent (9), a series of insulated water baths were constructed. Four 76 X 63 X 19 cm stainless steel pans filled with approximately 70 liters of water and insulated on all sides with 2.5 cm thick styrofoam were used. A constant 23 C ( $\pm 0.5$ ) was maintained in each pan by opposing a thermostatically-controlled heating element (Fig. 1) against chilled ethylene glycol (50% V/V) being circulated through each pan in approximately 15 meters of copper tubing (Fig. 1). A circulating pump moved the chilled ethylene glycol through the copper coils in the pans and returned it to a reservoir in a refrigerator (Fig. 2). Water in the pans was constantly circulated within each pan by non-submersible pumps (Fig. 1).

Single plants were grown in silver-painted nutrient culture jars (Fig. 3) that were positioned in a 2.5 cm layer of styrofoam floating on the surface of the baths (Fig. 4). Constant aeration of the nutrient solution was provided by pressure-regulated PVC manifold connected to a compressed air line (Fig. 4). Light was provided by a 1000-watt metal halide lamp over the bath (Fig.

5). Approximately  $650 \pm 100$  micromoles  $\text{sec}^{-1}\text{m}^{-2}$  PAR light intensity was achieved. Each of the four baths could support 30 nutrient culture jars.

Water stress was induced at a controlled rate by replacing nutrient solution with nutrient solution containing progressively more PEG (Fig. 6) each day at the end of the 12-hour photoperiod (See reference 11 for PEG 8000 versus nutrient solution osmotic potential standard curve and specifics of the nutrient solution). Osmotic potential of nutrient solution without PEG added equaled 0.06 MPa. Rate of stress development was  $0.1 \text{ MPa day}^{-1}$  until nutrient solution osmotic potentials equaled  $-0.96 \text{ MPa}$  (9 days). At the end of the stress period, the second fully-expanded leaf blade from stressed and non-stressed plants were excised near the end of the light cycle and placed immediately in vials containing distilled water and allowed to rehydrate in the dark for at least 8 hours.

After rehydration in the dark, moisture release curves were established using a method similar to that described by Brown and Tanner (3). Leaves were weighed and water potentials were determined using a PMS pressure bomb and a pressurization rate of  $0.02 \text{ MPa sec}^{-1}$ . After endpoints were determined, the pressure was relieved slowly (approximately  $0.03 \text{ MPa sec}^{-1}$ ). Leaves were weighed again, water potentials determined, and the process was repeated until leaf water potentials were below  $-3.5 \text{ MPa}$ . Data were plotted as leaf water potentials versus leaf weight (Fig. 7A). The linear portion of the curve was extrapolated back to the Y axis to estimate leaf weight at full turgor. After curves were established, leaves were oven-dried and weights recorded. Turgid weight:dry weight ratios were calculated as comparative estimates of cell wall thickness and elasticity (15). Relative water contents (1) were calculated for each datum point and data were replotted as leaf water potential versus relative leaf water content (the moisture release curve; Fig. 7B). The point

of intersection between the linear and curvilinear phases of the curve was used as an estimate of incipient plasmolysis and osmotic potentials and relative water contents at this point were recorded. An extrapolation of the curvilinear phase of the curve to the Y axis yields the leaf osmotic potentials at full turgor (3). By using the linear phase and an extrapolation of the curvilinear phase of the moisture release curve, maximum turgor (predawn) and an estimate of the bulk elastic modulus can be obtained (Fig. 8). Assuming the volume of bound water in the leaf is small, the bulk elastic modulus can be approximated by the change in turgor pressure divided by the change in relative water content (7). Maximum turgor can be calculated by the difference in leaf water potential and osmotic potential (the extrapolated curvilinear phase) assuming matric effects are insignificant. The change relative water content from the initial leaf water potential determination to the relative water content estimate at incipient plasmolysis yields the total change in relative water content from maximum (predawn) to zero turgor (Fig. 8), and the bulk elastic modulus can be estimated accordingly.

Predictability of the osmotic potential at full turgor using moisture release curves was greatly reduced in water stressed leaves because of dramatic shifts toward lower relative water contents and leaf water potentials after stress treatments. To circumvent this problem, advantage was taken of the linearity of pressure volume curves at leaf water potentials where positive turgor is lost (14). By extrapolating the linear phase of the pressure volume curve back to the Y-axis, osmotic potentials at full turgor could still be estimated. Osmotic potentials at full turgor generated in this way were plotted on moisture release curves and the curvilinear phase was drawn so that maximum turgor and bulk elastic moduli could be estimated according to the technique just described.

## DISCUSSION

Water use requirements of plants depends on transpiration rates. Although transpiration is the sum total of stomatal and nonstomatal water loss, stomatal closure is generally recognized as the main cause for transpiration decline as water stress develops (13). Control of stomatal aperture, therefore, is a very important factor in a plants ability to survive periods of limited moisture. If stomates close when bulk leaf turgor is near zero and turgor maintenance depends on the plants ability to osmotically adjust, any enhanced ability to osmotically adjust may be coupled to a higher water use rate. Table 1 shows the effect of a water stress treatment on leaf parameters of 'Sydsport' Kentucky bluegrass. Water stress resulted in significant reductions in all leaf parameters tested. It appears that Kentucky bluegrass is not only capable of osmotic adjustment in response to water stress, but cell wall elasticity is also affected. A study is underway utilizing Kentucky bluegrass cultivars that have exhibited a range of water use requirements. It will be valuable to test whether any consistent patterns emerge concerning leaf parameters obtainable from techniques described here.

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LEAF PARAMETERS

	MEAN MAXIMUM TURGOR (MPA)	MEAN MAXIMUM RWC (%)	MEAN OSMOTIC POTENTIAL AT FULL TURGOR (MPA)	MEAN OSMOTIC POTENTIAL AT INCIPIENT PLASMOLYSIS	MEAN RELATIVE WATER CONTENT AT PLASMOLYSIS (%)	MEAN BULK ELASTIC MODULUS (MPA)	MEAN TURGID WEIGHT:DRY WEIGHT RATIOS
BEFORE STRESS	0.9	98.6	-1.15	-1.26	93.5	0.185	4.42
AFTER STRESS	0.7*	88.2*	-1.77*	-1.92*	80.8*	0.087*	3.46*

(10 REPLICATIONS OF 'SYDSPOUR' KENTUCKY BLUEGRASS)

\*SIGNIFICANTLY DIFFERENT (0.05) THAN BEFORE STRESS