ANNUAL RESEARCH REPORT TO THE USGA - NOVEMBER, 1989

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An overview of progress and accomplishments in 1989, and directions for upcoming research in 1990 for the project "**A Realistic Whole Plant Microculture Selection System for Turfgrasses**".

Overall Goals for 1989:

To implement and test parallel solution batch culture and microculture screening systems for thorough evaluation of turfgrass response to a saline environment.

Summary of Progress to Date (1989):

Although the program was partially crippled by the lack of USGA funding support for 1989, our research late in 1988 and in 1989 resulted in development and thorough testing of parallel solution batch culture and microculture test systems for three turfgrass types. We completed all the required tests for Cynodon datylon L. Pers. [bermudagrass; cvs. Tifway (salt tolerant) and Tifway II (salt-intolerant)], Agrostis palustris Huds. [creeping bentgrass; cvs. Seaside (salt tolerant) and Penneagel (salt-intolerant)], and Stenotaphrum secundatum (Walt) Kuntze [St. Augustinegrass; cvs. Seville (salt tolerant) and Floratam (salt intolerant). The relative ratings for resistance/susceptibility on these selections were made based on previous field and greenhouse tests completed by Dr. Ki Sun Kim (formerly of Texas A&M University) and Dr. Al Dudeck (University of Florida).

Salinity effects on St. Augustine provided the model system for development of a tailored solution batch culture system. This novel system maximizes assessment of turfgrass stress response and quantification of resistance characteristics, since it quantitatively monitors growth characteristics and physiological responses of grasses on many fronts, simultaneously. The model system was developed using St. Augustinegrass cvs. Seville (salt tolerant) and Floratam (salt intolerant) and saline stress imposed by incorporating increasing levels of NaCl into a modified Hoaglands nutrient solution (Meyer et al., 1989- see attached reprint). Previously reported solution culture experiments designed to test salt-tolerance on grasses and other crops have focused on one or two destructive measurement parameters, and have invariably required the root systems to be embedded in gravel or other matrix. The matrix not only hides the root growth progress, but can retain cations and disable conductivity controls. The solution batch culture method, alternatively, was developed in collaboration with Dr. S. L. Knight (involved with the NASA space program's implementation of solution culture testing methods for the CELLS program). This unique hydroponic system allows more complete control of aeration, nutrients, and conductivity, while allowing root growth observation without interference of an aggregate matrix. Conductivity treatments were 2.4, 12.4, 22.4 and 32.4 decisiemens per meter.

With this system, we were able to measure growth (increases in length, area, and density) of shoot and root systems simultaneously and over the time course of the experiment, as well as determine RGR (relative growth rate) and monitor osmotic adjustment variations between susceptible and resistant grass lines. Color analysis of grass blades in response to stress was also achieved. These multiple parameters helped us to isolate and identify the mechanisms that were responsible for the superior tolerance of one grass line over another. In addition, the solution batch culture system serves as the direct in vivo comparison for the microculture stress screening system. An in vivo system was required to validate observations made in the microculture system, to assure that the results in vitro were relevant to turfgrass responses in nature, and field tests were too variable to provide the required correlations. After completion of initial work with St. Augustinegrass, sequential runs were completed with bermudagrass and bentgrass.

The microculture system was developed in direct parallel to the batch culture. Vegetative plugs of bermudagrass and St. Augustinegrass and seedlings of creeping bentgrass were adapted to whole plant microculture using established procedures (Pieper and Smith, 1988). The four treatments incorporated were based on those used in the batch nutrient culture experiment - again, 2.4, 12.4, 22.4 and 32.4 dSm. The in vitro medium conductivity was measured prior to the addition of Gelrite gellan gum (used as the solidifing agent instead of agar, to insure clear visibility of roots).

For the creeping bentgrass microculture experiments, 90 uniform explants (2.5 cm shoot, 1-1.5 cm root) of each line were transferred to the maintenance of Control medium. This number of plants allowed for ten replicates with two subreplicates per treatment. After the first week, just as for the solution culture experiments, 3/4 of the replicates were moved up to a higher conductivity level (by subculture to a medium with NaCl at a conductivity of 12.4 dSm, and 1/4 of the replicates were resubcultured to control medium. After an additional week, the control cultures were resubcultured to control medium, 1/4 of the replicates were resubbed on 12.4 dSm (the second treatment conductivity level), and the remaining cultures were moved to higher conductivity until, at the end of 3 weeks, 4 distinct conductivity levels were established in parallel treatments. The shoots and roots were trimmed to the uniform length (2.5 cm and 1.5 cm respectively) at each subculture in order to allow clean transfer to new medium. A gradual incorporation of high salt levels is necessary to avoid osmotic shock, hence the gradual increase to maximum conductivity treatment levels. Ten replicates/treatment were harvested at the onset, and at 4 and 8 weeks into the experiment to test for osmolarity. Ten additional plants per treatment were also harvested for image analysis and RGR analysis at each of these periods.

Insufficient replicates (less than 10) were available for extraction of cell sap in the 2 high conductivity treatments for bentgrass, due to a severe stunting reaction, especially for the susceptible line. As a result, the number of subreplicates (a total of 35 plants per treatment) was increased in treatments 3 (22. dS m^{-1}) and 4 (32.4 dS m^{-1}) in subsequent experiments. In addition, the initial microculture tests with bentgrass suggested that the roots were slightly inhibited in the strength of Gelrite used to solidify the medium. As a result, the concentration was reduced to provide a slightly softer matrix in remaining experiments. In related work, the video image analysis measurements were optimized and compared to manual (FW, length) measurements in microculture. Through custom-adapted staging techniques, we were able to achieve correlations

of .9 or higher between i.a. and manually-extracted data in microculture (Smith et al., 1989).

The results of these trials have demonstrated that microculture provides a worthy parallel to solution batch culture screens, while utilizing simpler and more standard facilities, and a smaller experimental scale. Potential problems with inadequate in vitro rooting response and insufficient foliar material for cell sap extraction were easily eliminated following the initial test run of the system. The completed experiments are currently being prepared as an M.Sc. thesis in partial fulfillment for the degree requirements for M. J. Meyer, the student whose stipend was in part (one year) provided by USGA funding (Meyer, 1989).

Future directions:

In the near future, we intend to refine and enhance both the microculture and solution batch culture systems, by focusing in on improved stress-imposition techniques. Once a new student to continue the project is identified and funding is placed on account, we intend to test alternative salt formulations in both microculture and batch culture systems. This component of the work is required since some reports indicate differences in plant response to different salt stress agents, and in fact, NaCl is not the factor responsible for salt stress in nature.

Recent reports have indicated an enhanced interest in cell-level research with turfgrass germplasm (Asano, 1989; Horn et al., 1988; van der Valk et al., 1989; Zaghmout and Torello, 1989). Some of these investigations (esp. Torello's group) have attempted cell-level selection for tolerance, but have had limited success due to difficulties with plant regeneration, and selection screens at the cell level which fail to isolate tolerance traits that are expressed at the whole plant level. The whole plant microculture selection screen we have developed provide a needed link between this cell-level/biotechnological research, and practical screening of whole grass crops for salt tolerance. Our future work will hopefully involve a Ph.D. level graduate researcher to carry out a complete system from regeneration of novel germplasm through practical microculture prescreening prior to field introduction of new lines.

Continued Funding Requirements:

Annual stipend for one dedicated graduate research assistant (Ph.D. candidate), and related supplies = $9,000.

Support recently granted by the USGA will allow a new research associate to be hired this coming spring. A Ph.D. candidate usually represents a 3-year funding commitment on the part of the P.I. For this reason, it is crucial that support be continued, if at all possible, to allow uninterrupted progress on the project. As noted in a previous (1988) report, a Ph.D. candidate is the preferred recruit for continuation of the research initiative, since the research will require in depth analysis of key cellular and whole plant responses to stress, as well as implementation of some biotechnological routes of investigation. We were simply not able to recruit a candidate due to the uncertain funding status of the project, but hope to make up for lost time in future months.

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EXECUTIVE SUMMARY TO THE USGA - NOVEMBER, 1989

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An overview of progress and accomplishments in 1989 for the project "**A Realistic Whole Plant Microculture Selection System for Turfgrasses**".

This past year, our research has adapted parallel salt-tolerant and salt-susceptible lines of bermudagrass, creeping bentgrass, and St. Augustinegrass to both solution batch culture and microculture test systems, in order to thoroughly evaluate the symptoms and responses that characterize reaction to saline environments in the root zone.

The solution batch culture system (similar to systems now used in testing for tolerance by the NASA 'Plants in Space' program) was specifically developed to accommodate the grasses from small plug stage (start of experiment) to 8-week old plant (termination). The batch culture system allowed multiple replicates to be grown in control (2.2 dSm) nutrient solution, or, for the appropriate treatments, very gradually brought up to encounter high-salinity levels similar to what might be found in saline soils. The systems approach allowed the **whole plant** to be continuously monitored for response to saline conditions - not just a single feature like clippings growth or size as in previous research. Microcomputerized video image analysis was adapted to analyze parameters of shoot growth and density, blade color, root length, and root system area over time. The technique yielded highly quantitative data on overall growth response. In addition, measurements of dry weight facilitated RGR analysis, and osmotic adjustment response was quantified using a vapor pressure osmometer. The unique attributes of this batch system approach have been recently reported (Meyer et al., 1989, J. Plant Nutrition 12:893-908). The microculture system parallel was designed to make some of the same analyses on tolerant and susceptible grass responses in a compact, efficient screening test. While dry weight (RGR) analysis was not feasible in microculture, additional advantages of the microculture system include smaller scale, and very clear presentation and observation of root system responses in the gellan-gum tissue culture matrix.

Both systems have been quite effective at separating traits of grasses in terms of their relative tolerance to saline environments; most notable the overall growth response (stunting), ability of tolerant plant root systems to increase in length proportionate to level of stress, and osmotic adjustment. Given the high degree of correlation between solution culture and microculture screening tests, the more efficient and scaled-down microculture screen is envisioned as a practical tool for screening untested germplasm for use in roadside erosion control, or for turfgrasses to be irrigated with saline water.

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SALINITY EFFECTS ON ST. AUGUSTINEGRASS: A NOVEL SYSTEM TO QUANTIFY STRESS RESPONSE[1]

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ABSTRACT

An objective and quantitative batch nutrient culture system was developed to assess responses of St. Augustinegrass (Stenotaphrum secundatum [Walt.] Kuntze) cultivars 'Seville' (saline tolerant) and 'Floratam' (saline sensitive) to salt stress. Vessels containing individual grass plugs growing in solution culture were randomized in a walk-in growth chamber. Nutrient solutions were supplemented weekly with NaCl to gradually increase the conductivity over a three week period to treatment levels (2.4 [control], 12.4, 22.4, and 32.4 dS m^{-1}). Plants were sampled at the onset, and at four and eight weeks during the experiment. Cell sap was extracted from grass blades and measured with a vapor pressure osmometer to determine osmolarity and osmotic adjustment. Length and area of sample shoot and root systems and foliage color changes were measured using an adaptation of video image

[1]This research was supported by a grant from the United States Golf Association - Green Section. Our sincere thanks to Dr. A. Dudeck for donation of the grass lines and helpful advice on their relative salt tolerance, and Dr. L. Art Spomer for constructing the staging apparatus for image analysis.

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analysis, and dry weight (DW) was measured to determine relative growth rate (RGR). 'Seville' responded to increasing salt concentration with uniform stepwise increases in cell osmolarity, whereas 'Floratam' showed strong depression of cell osmotic potential only in the highest conductivity treatment. At high salt levels, overall shoot development was initially more inhibited for 'Floratam', although RGR analysis indicated recovery in the susceptible cultivar after long salt stress duration. 'Seville' reacted to saline stress with increased root length, whereas roots were actually stunted in saline treatments for 'Floratam'. Stress induced an increase in the optical density of grass blades for both cultivars although foliage color was not visibly affected. A positive linear correlation ($r = 0.81 - 0.97$) was obtained between shoot area as determined by image analysis versus DW measurements. Use of this novel system maximizes assessment of turfgrass stress responses, and quantification of resistance characteristics.

INTRODUCTION

Selection of salt resistant turfgrass cultivars is the long-term objective of many breeding programs (1-3). Soil and climatic conditions, as well as uncontrolled environmental fluctuation, often prevent the identification of resistant germplasm in field studies (4,5). Controlled environment tests using artificially imposed stress are an improvement, but often fail to adequately control the plant growth environment (6) in order to quantify levels of resistance or clarify the mechanisms of resistance (7-9). Relative top growth (clipping yields) data and highly subjective ratings of quality or color are typically used to rank selections for response to stress, fertilization, or environmental factors (1,4,10), while root growth data may be discounted due to the difficulty of precise measurement in the root zone matrix.

This report describes the use of a batch nutrient culture system to induce NaCl stress on St. Augustinegrass, an important warm season turfgrass especially popular in coastal areas (11,12). In the model application, two cultivars with observed differences in salt tolerance

(13) are compared. This type of hydroponic system was used because it allowed satisfactory control of aeration, nutrients, and conductivity levels for each treatment for the duration of the experiment, and facilitated root growth assessment without interference from an aggregate matrix. The osmotic adaptation response of leaf blade cells to increasing levels and duration of salt stress was examined. Quantitative video image analysis measurements of area and length, dry weight (DW) and relative growth rate (RGR) calculations provided assessment of the overall growth response. Image analysis also contributed a quantitative evaluation of foliar shifts in visual density in response to increasing salt stress.

MATERIALS AND METHODS

Plant Material. Nodal segments (3.5 cm) of 'Seville' and 'Floratam' were obtained from stolons of plants growing in 1:1:1 (by volume) soil:peat:perlite mix in plastic flats in a greenhouse (29°C day, 18°C night). After segments were dipped in Horm's CUTstart XX (Vitamin Institute, North Hollywood, CA), they were planted in wooden flats containing 1:1 (by volume) peat:vermiculite mix. The flats were placed under mist in another greenhouse (24°C day, 22°C night) during the 2.5 week period of root establishment. Immediately prior to placement in batch nutrient culture, plants were rinsed with distilled water from a spray bottle to remove adhering media. Shoots were trimmed to a length of 3.5 cm, and roots to 1.5 cm.

Culture System. A batch nutrient culture system based on the design of Hershey and Merritt (14) was assembled in a Conviron (model PGW36) walk-in growth chamber (3.34 m^2). Modifications to this static culture system were made to accommodate 80 one-liter vessels (wide-mouth glass Mason jars). Individual plants were placed in pretreated white foam plugs (15) and five plants were then inserted into fitted holes in the plastic lid of each vessel. Uniform aeration between vessels was supplied through bubblestones by an oil-less diaphragm pump and regulated by a pressure gauge.

Each vessel initially contained a modified Hoagland's nutrient solution #1 (16) containing Sequestrene 330 (Ciba Geigy) as the source of Fe (17), at a pH of 5.5 +/- 0.5 and conductivity of 2.4 dS m^{-1}. Solution pH was maintained by addition of 5mM 2 (N-morpholino) ethanesulfonic acid buffer (18), and conductivity was monitored with a Cole-Parmer meter (model 71200). Gradual build-up to final conductivity levels was required to avoid osmotic shock to the plants. After one week the initial solution was rapidly decanted and replaced with the same solution for 1/4 of the vessels. The remaining vessels were replenished with NaCl-supplemented solution at a conductivity level of 12.4 dS m^{-1}. At the end of the second week, vessels were again decanted and 1/4 replaced with the original 2.4 dS m^{-1} solution, 1/4 replaced with the 12.4 dS m^{-1} solution, and 1/2 replaced with solution increasing the conductivity to 22.4 dS m^{-1}. This process was repeated until at the end of three weeks final treatment conductivity levels of 2.4 [control], 12.4, 22.4, and 32.4 dS m^{-1} were reached. Treatments were maintained for the remaining five weeks at the appropriate conductivity level by periodic decantation of the vessels and replacement with fresh solution. Vigorous plant growth in the latter weeks of culture rapidly depleted solutions, necessitating frequent (every 3-4 days) replacement.

Transplants were exposed to 50% input wattage from high-pressure sodium lamps (Sylvania Lumalux 1500W) and 50% from metal halide lamps (Sylvania Leviton 1500W). Lamps and plants were separated by a 1/4" acrylic barrier. Photosynthetic photon flux (PPF) was measured with a quantum radiometer (LI-COR model LI1776, Lincoln, NE) at 1.58 m below the lamps (at the top of the plant canopy). During the first 24 hour cycle after transplant, PPF was kept lower (269 +/- 25 µmol m^{-2} s^{-1}) in order to allow plants to adjust to the environment. After the first day, PPF was increased to 576 +/- 50 µmol m^{-2} s^{-1} and this level was maintained for the duration of the experiment. Photoperiod was 15 hr on a 24 hr light/dark cycle. A light/dark temperature of 28 +/- 2°C was maintained throughout the

experiment. Relative humidity was 55 +/- 5% as determined with a Psychro-dyne psychrometer (Environmental Tectonics Corp., Southhampton, PA).

Measurement Techniques. Ten sample plants per treatment were harvested for each measurement after zero, four, and eight weeks of growing in solution culture. For analysis of cell sap osmolarity, fresh cut upper leaf blades were collected prior to the end of the dark cycle, and packed in the barrel of a 1 cm^3 tuberculin syringe (Becton Dickinson) to the 0.4 cm^3 mark. The syringes were inserted into rubber stoppers and placed in a freezer at -78°C for one hour. The samples thawed at room temperature, then cell sap was dispensed into capped 13 x 75 mm test tubes. Samples (10 µl) were measured using a Wescor 5500 Vapor Pressure Osmometer (Logan, UT).

Plants were staged for video image analysis by placement atop a light box surfaced with a 0.32 cm thick flashed opal glass diffuser and containing 12 side-by-side 122 cm long cool-white fluorescent lamps (Phillips Econ-o-watt 34W). The opal glass surface was protected by a sheet of 0.64 cm thick cool grey plate glass, which also acted as a neutral density filter. Emission from the light box surface was 49 +/- 1.5 µmol m^{-2} sec^{-1} PPF (measured with a Li-Cor Li-190SB Quantum sensor). Specimens were held flat for imaging by placement under an additional sheet of 0.64 cm thick clear plate glass and viewed from 1.8 m through a 0.28-0.85 m focal length Tokina lens with a Sony AVC-D1 CCD camera rigidly mounted on a copy stand. Lens aperture was set at f16. Light transmission images of individual shoot and root systems were captured and digitized (Fig. 1) with an Imaging Technology FG-100-AT digitizer (Woburn, MA) housed in an IBM PC/AT microcomputer and operated with Image Pro software (Media Cybernetics, Silver Spring, MD). Each pixel (image point comprising the digitized image) had a resolution of 0.635 mm^2, with 1560 cm^2 in the total field of view. Shoot length, root length, shoot area, and root area were calculated at these settings. Leaf color/optical density was then measured by placing a representative sample onto the surface of a

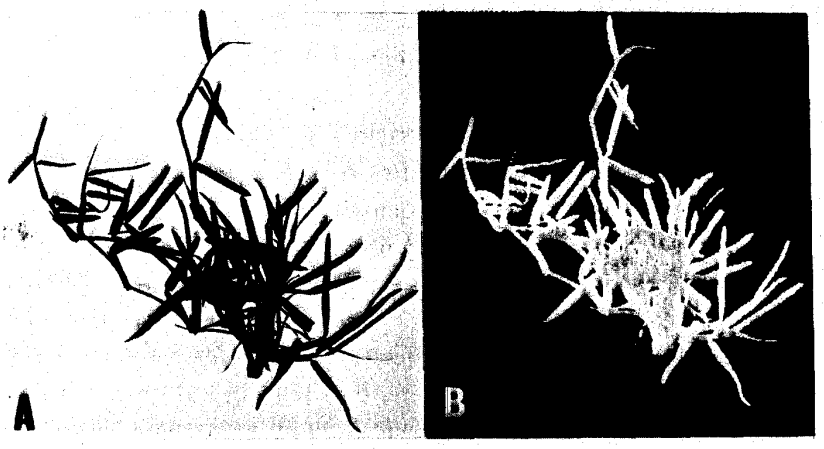

Figure 1. A. Video image of 'Seville' St. Augustinegrass staged for image analysis. B. Corresponding digitized image, from which precise area measurements were calculated from the number of pixels (calibrated image points) corresponding to the plant image.

light box containing a convoluted white neon lamp (Aristo Grid Lamp Products, Inc., Port Washington, N. Y.; model V-56) emitting a uniform PPF of 56-57 μmol m^{-2} s^{-1} and located at a 25-cm focal distance from the camera lens. A 4 diopter close-up lens was attached for close focusing and the aperture set to f11 for optimal exposure. The captured image was posterized to 25 brightness levels and the relative proportion of area within each value was recorded to determine visual density/color of sample foliage. Image analyzed samples were subsequently dried for two days at 75°C in a forced-air oven.

Experimental Design and Statistical Analysis. The experimental design was completely randomized. There were ten vessels per treatment per cultivar arranged in four rows of fourteen vessels and two rows of

twelve distributed around the air sample intake pipe in the center of the floor. Each vessel contained five subsamples.

Correlation coefficients were determined between plant area as estimated by video image analysis and DW. Analysis of variance was used to identify statistically significant trends and to identify differences between means (19). RGR was calculated by regressing ln whole shoot or root DW with time of treatment. Differences in slope of RGR plots were tested for significance using a t-test for comparison of regression coefficients.

RESULTS AND DISCUSSION

The osmotic adjustment responses of the two cultivars to increasing saline stress were consistently different over the course of the experiment. 'Seville' St. Augustinegrass responded to increasing salt level treatments with correspondingly uniform, stepwise increases in leaf cell sap osmolarity. The osmotic adjustment of 'Floratam' was more subtle at the 12.4 and 22.4 dS m^{-1} treatments, then cell osmolarity increased sharply at the highest level of solution conductivity (Fig. 2). Variability in the measurement data was low and uniform (SD = 33 - 56 mOsm/kg) except at the two highest salt treatments for 'Floratam', where SD ranged between 79 - 193 mOsm/kg. These trends were consistent at both the four and eight week measurements (Fig. 2). The erratic response of 'Floratam' at high salt levels may be indicative of cellular breakdown in response to stress (20).

Both cultivars reacted to increasing salt stress with a corresponding reduction in shoot area (Table 1). Although the susceptible 'Floratam' in control or 12.4 dS m^{-1} treatments produced substantially more top growth than 'Seville' (both in length and area), the trend was reversed under salt stress. At the highest salt level, shoot area for the resistant cultivar exceeded that of the susceptible cultivar by almost 2x at the four week measurement. After eight weeks, however, 'Floratam' shoot systems, although still inhibited at high salt levels,

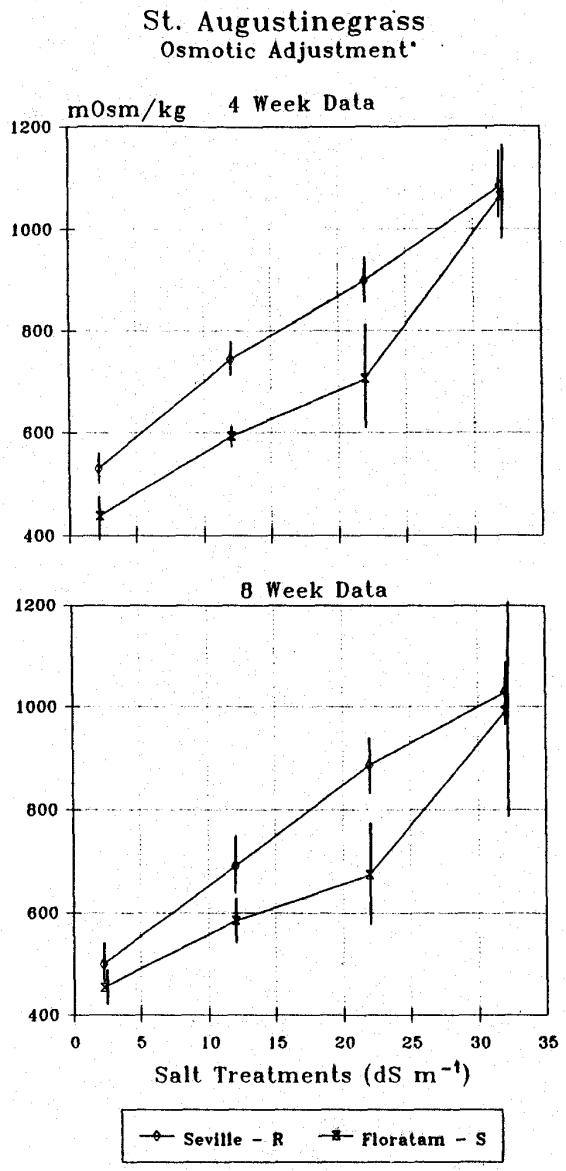

Figure 2. Osmotic cell sap concentration measurements of the saline-susceptible 'Floratam' and resistant 'Seville' St. Augustinegrass cultivars in response to increasing levels of NaCl in nutrient culture. Data points are the mean of ten measurements/treatment; bars indicate +/- SD.

TABLE 1. Response of shoot area, shoot length, root area, and root length for 'Seville' and 'Floratam' St. Augustinegrass cultivars to increasing levels of saline stress after four and eight weeks in nutrient batch culture. Conductivity level for Treatment 1 (control) = 2.4 dS m^{-1}, Treatment 2 = 12.4 dS m^{-1}, Treatment 3 = 22.4 dS m^{-1}, Treatment 4 = 32.4 dS m^{-1}. Data was collected using video image analysis. Ten samples per cultivar/treatment/time were analyzed.

	Week 4		Week 8	
	Seville	Floratam	Seville	Floratam
Treatment		Shoot Area (cm^2)		
1	108.5 aA[z]	128.2 aA	329.3 aB	433.6 aA
2	99.5 aA	127.0 aA	353.2 aA	415.2 aA
3	64.8 bA	67.6 bA	197.1 bA	218.6 bA
4	62.0 bA	33.7 cB	147.7 bA	121.9 bA
		Shoot Length (cm)		
1	24.1 aA	31.1 aA	55.5 aB	81.0 aA
2	22.5 abA	26.5 aA	54.9 aB	65.9 bA
3	15.4 cA	18.3 bA	38.7 bA	43.8 cA
4	15.9 bcA	11.4 bB	34.1 bA	28.2 dA
		Root Area (cm^2)		
1	50.6 aB	69.2 aA	59.2 aA	85.4 aA
2	50.0 aA	64.8 abA	68.8 aA	74.2 aA
3	37.7 aA	49.4 bcA	52.5 aB	75.9 aA
4	47.8 aA	36.9 cA	55.2 aA	62.1 aA
		Root Length (cm)		
1	31.4 bA	28.5 aA	36.6 bA	39.8 aA
2	32.8 bA	29.8 aA	43.5 abA	36.0 aA
3	35.0 bA	26.4 aB	40.8 abA	41.9 aA
4	44.5 aA	26.9 aB	51.8 aA	41.6 aA

[z]Mean separation by Tukey's studentized range test (HSD), 5% level (lowercase for treatment differences, uppercase for cultivar differences).

apparently had initiated partial recovery. The between-cultivar difference in both shoot length and shoot area evident at four weeks had narrowed (was not significant) by the end of the test.

While root area of 'Seville' was not affected by stress treatment, root length gradually extended with increasing level of conductivity as measured at both observation times (Table 1). This was indicative of a change in root morphology (root systems elongated but were less branched) in response to higher conductivity. In contrast, root development was clearly inhibited by increased conductivity for 'Floratam' at the four week measurement. By the end of the salt stress test, 'Floratam' exhibited less dramatic differences in both root area and length in response to high levels of salt stress relative to 'Seville'.

No net differences occurred in RGR between cultivars at the first three conductivity levels. At the highest salt level, RGR of 'Seville' (for shoots) was 28% greater than that of 'Floratam' from zero to four weeks, but was 17% less than that of 'Floratam' from four to eight weeks (Fig. 3). A similar trend was found for the root RGR (data not shown). Overall RGR was not significantly different between treatments from zero to eight weeks at the highest conductivity treatment, although 'Seville' tended to perform better than 'Floratam'. This finding suggests that evaluating short-term as well as long-term effects of increasing root-zone conductivity levels on plant growth rate is very useful in understanding how plants change in response to their environment over time. The results from RGR analysis were consistent with an apparent recovery in 'Floratam' after long salt stress duration. The observed trend in RGR at 32.4 dS m^{-1} intimates that shoot and root development of 'Floratam' may have paralleled or even exceeded that of 'Seville' if treatment had been extended beyond eight weeks. Alternatively, the trend may be a consequence of a stronger inhibition of 'Seville' to limiting growth space available to roots during the latter weeks of the study.

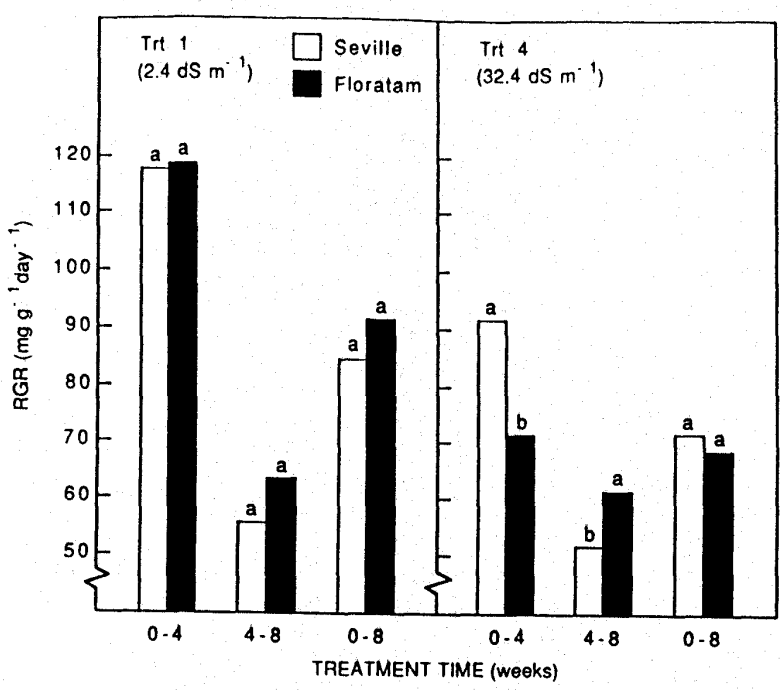

Figure 3. Effect of solution conductivity on shoot relative growth rate (RGR) of 'Seville' and 'Floratam' St. Augustinegrass. Different lower case letters indicate significant differences according to t-test, 5% level. RGR analysis results at the 12.4 and 22.4 dS m^{-1} conductivities showed similar trends to the control. The trends for root RGR were very similar to that of the shoot system over all four treatment regimes.

Neither grass cultivar exhibited an obvious color shift (chlorosis) in response to salt treatment, however, image analysis revealed that the visual density of grass blades increased as conductivity increased for both cultivars (Table 2). Tougher, thicker foliage texture was characteristic in the slower-growing, stressed plants. Although no significant chlorosis or color change characterized the response of these St. Augustinegrass cultivars to saline stress, ratings of turfgrass color

TABLE 2. Percent leaf area within three visual density indices for 'Seville' and 'Floratam' St. Augustinegrass cultivars under different levels of salt stress. Index #1 corresponds to highest visual density; index #2 indicates medium density; index #3 corresponds to low visual density. Conductivity level for Treatment 1 (control) = 2.4 dS m^{-1}, Treatment 2 = 12.4 dS m^{-1}, Treatment 3 = 22.4 dS m^{-1}, Treatment 4 = 32.4 dS m^{-1}. Ten samples/treatment were analyzed, and data is shown for the eight week measurements.

	Seville			Floratam		
	Index					
	1	2	3	1	2	3
Treatment						
1	3	82	14	6	90	3
2	19	77	3	16	71	12
3	34	63	3	33	65	2
4	24	74	2	32	67	2

changes are very commonly used as part of quality evaluations (1,10), and invariably rely on subjective observation alone. A strong advantage of this test system is that it includes an objective, rapid, and quantifiable means of rating turfgrass foliage color.

Good correlations were established between image analysis measurements of shoot area, and the corresponding DW of the sample shoot systems (Table 3). Similar results also were found between root area and corresponding root DW measurements (data not shown).

Extreme care was taken to provide adequate and uniform aeration of solution as well as even distribution of salt in the rhizosphere. In an aggregate culture, establishing these conditions would be extremely difficult, and entire root systems would not be easily removed for

TABLE 3. Correlations between shoot area (cm^2, measured using video image analysis) and DW (g) for 'Seville' and 'Floratam' St. Augustinegrass cultivars under different levels of salt stress. Conductivity level for Treatment 1 (control) = 2.4 dS m^{-1}, Treatment 2 = 12.4 dS m^{-1}, Treatment 3 = 22.4 dS m^{-1}, Treatment 4 = 32.4 dS m^{-1}. Ten samples/-treatment were measured using each method, and correlated using the Pearson Product Moment Coefficient of Correlation test.

	Week 4		Week 8	
	Seville	Floratam	Seville	Floratam
Treatment		r value		
1	0.96	0.94	0.87	0.92
2	0.92	0.97	0.86	0.89
3	0.82	0.92	0.85	0.84
4	0.94	0.92	0.81	0.90

evaluation (21). An additional problem with aggregate culture, circumvented by use of the described batch culture system, is the potential for salt residue to adhere to gravel or other substrates over time, preventing accurate maintenance of desired conductivity.

SUMMARY AND CONCLUSIONS

This nutrient batch culture system has demonstrated several advantages for in depth analysis of turfgrass stress responses. As a non-aggregate hydroponic technique, it expedites removal of entire root systems for measurement, and allows more precise control over salinity levels within a treatment. Multiple comparisons within a single experiment could be evaluated. Through the adaptation of image analysis, shoot and root length and area measurements could be made very

rapidly (less than 30 seconds/sample) and objectively, and color or visual density analysis, normally a highly subjective evaluation, can be objectively assessed. The morphometric and photometric accuracy of the imaging data has already been confirmed (22-24), and is also validated by the strong correlations between image analysis shoot area and DW data in these tests. The vapor pressure osmometer technique allowed accurate analysis of microliter quantities of extracted cell sap, to reveal distinct differences in the osmotic adjustment responses between cultivars, whereas extraction would not necessarily give reliable results for plants grown outdoors or in the greenhouse (20,22). RGR analysis indicated an apparent recovery response in the susceptible cultivar that would not be detected by conventional data on clipping yields or appearance.

The batch culture technique is currently being compared to a parallel whole plant microculture stress selection system for a range of turfgrass species. Both systems have used NaCl to impose saline stress (based on previous studies), but upcoming tests will screen alternative ionic species (i.e. KCl, Na_2SO_4, KNO_3, $CaSO_4$) to determine if similar results are obtained. The strong correlations established between image analysis area measurements and DW are particularly relevant for the microculture system, since it is more difficult to extract roots from the in vitro medium for DW analysis.

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