SCREENING FOR WATER CONSERVING TURFGRASS AND IMPROVED WATER USE EFFICIENCY IN KENTUCKY BLUEGRASS

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

by

Jeffrey S. Ebdon

August 1995
In the future, as the competition for a finite, limited supply of potable water increases, there will be a need to conserve limited water resources applied to irrigated turfgrass. The identification of water conserving Kentucky bluegrass (Poa pratensis L., KBG) is important in reducing irrigation requirements. However, alternative methods that are less labor intense than weighing lysimeters need to be found for evaluating turfgrass evapotranspiration rate (ET). In warm-season turfgrass, turfgrass morphology that increase canopy resistance to ET and reduce total leaf areal evaporative surface has been used successfully in selecting for low ET. In KBG, studies have been unable to specify the criteria to be used in selecting low-ET genotypes. The objectives of this study were two-fold, i) develop a method that would allow breeders to identify KBG selections with a low-ET pattern based on simple morphological measurements, and ii) determine the relationship between water use efficiency (WUE, ratio of carbon gained to water loss by the plant) and carbon isotope discrimination (Δ13, derived from stable carbon isotope ratio). WUE and Δ13 have been shown to be negatively correlated in C3 plants. Using cluster analysis, 61 KBG cultivars were categorized as either low- or high-water use cases based on ET rate evaluated in the growth chamber at three temperatures (25, 30, and 35°C). Fourteen morphological characteristics were assessed in the greenhouse using unmown space plants and mowed turfgrass (20cm diam. lysimeters). Using discriminant analysis, classification rules were developed for classifying KBG cultivars (cases) into low- or high-water use groups. Turfgrass morphology required the
fewest predictors (measurements) to perform classification. Based on turfgrass morphology, two- and three-variable functions were identified that correctly classified an estimated 75.4% (based on cross-validation) of the cases into their true water use groups. A 75.4% correct classification rate was the best achieved. Leaf angle, a component of canopy resistance, was the most important discriminator of water use group, predicting actual group membership in 72.1% of the cases. WUE measured as the ratio of biomass to water loss (ET) in 11 KBG cultivars over two 4 day harvest periods was correlated with A ($r = -0.55, P < .01$), and cultivar ET was significantly correlated with $t_a$ ($r = 0.64, P < .05$). Carbon isotope analysis ($t_a$) appears to be a promising tool for screening KBG selections for improved WUE and water conserving patterns.
BIOGRAPHICAL SKETCH

Scott was born and raised in Connecticut and lived most of his adult life in New England. Although he was an average student in high school he performed well at institutions of higher education. Scott decided on a career in turfgrass science as a young adult, and received a B. S. degree (cum laude) in Agronomy from the University of Connecticut and a M. S. degree in Plant and Soil Science from the University of Rhode Island with emphasis in turfgrass management. He then worked for 8 years in the turfgrass industry before corporate down-sizing and a case of terminal vacation encouraged him to return to school full time at age 38. Scott learned from personal experience that employment and associated financial gains can be temporary while an education will last a life time. He moved to Ithaca NY in August of 1991 hopeful of turning a negative experience in his life into a positive one by pursuing a Ph. D degree in turfgrass science at Cornell University.
Dedicated in loving memory of my father, and to my mother.
ACKNOWLEDGMENTS

I wish to thank my major advisor Dr. A. Martin Petrovic for providing the financial support that made this research possible. Additionally, I appreciated the latitude he afforded me in selecting a research project of my own choosing. I would also like to thank my committee members; Drs. Todd Dawson, Steven Schwager, and Rich Zobel for their comments in reviewing this manuscript.

Without the funding from the Lofts Seed Co. the costs of this project for materials, supplies, and growth chamber would not have been possible. Also, the help of Dr. Robert Langhans in providing the much needed use of his growth chamber is greatly appreciated.

I would also like to thank the greenhouse staff for their friendship; John Kumpf, Bob McBride, Barbara Stewart, Peter Podaras, Amy Roberts, Bob Spaulding, and Siri Kalsa. Last to Cait Morse for her emotional support..
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biographical Sketch</td>
<td>iii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>v</td>
</tr>
<tr>
<td>Table Of Contents</td>
<td>vi</td>
</tr>
<tr>
<td>List Of Tables</td>
<td>ix</td>
</tr>
<tr>
<td>List Of Figures</td>
<td>xiii</td>
</tr>
<tr>
<td>Chapter 1</td>
<td></td>
</tr>
<tr>
<td>Introduction And Literature Review</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Screening For Water Conserving Kentucky Bluegrass Using Morphological</td>
<td></td>
</tr>
<tr>
<td>Attributes And Discriminant Analysis</td>
<td>3</td>
</tr>
<tr>
<td>Plant characteristics and water use</td>
<td>3</td>
</tr>
<tr>
<td>Discriminant analysis</td>
<td>5</td>
</tr>
<tr>
<td>Carbon Isotope Discrimination And Water Use Efficiency In Kentucky</td>
<td>9</td>
</tr>
<tr>
<td>Bluegrass Turfgrass</td>
<td></td>
</tr>
<tr>
<td>Chapter 2</td>
<td></td>
</tr>
<tr>
<td>Stability Of Evapotranspiration Rates In Kentucky Bluegrass Cultivars Across Low And High Evaporative Environments</td>
<td></td>
</tr>
<tr>
<td>Abstract</td>
<td>18</td>
</tr>
<tr>
<td>Introduction</td>
<td>19</td>
</tr>
<tr>
<td>Materials And Methods</td>
<td>21</td>
</tr>
<tr>
<td>Water Use Analysis</td>
<td>21</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>24</td>
</tr>
<tr>
<td>Results And Discussion</td>
<td>25</td>
</tr>
<tr>
<td>Conclusions</td>
<td>38</td>
</tr>
</tbody>
</table>
Chapter 3

Turfgrass Morphological and Growth Characteristics of Low- and High-Water Use Kentucky Bluegrass Cultivars

Abstract..........................................................42
Introduction.........................................................43
Materials and Methods...........................................45
Results and Discussion..........................................51
  Unmown Space Plants........................................52
  Mowed Turfgrass (Lysimeters)...............................55
  Space Plant/Mowed Turf Comparisons......................60
Conclusions.......................................................61
References........................................................63

Chapter 4

Identification of Low- and High-Water Use Kentucky Bluegrass Cultivars Using Morphological Attributes and Discriminant Analysis

Abstract..........................................................66
Introduction.........................................................67
Materials and Methods...........................................69
  Comparative Water Use Groups...............................69
  Plant Measurements............................................70
  Discriminant Analysis........................................72
Results and Discussion.........................................75
References........................................................85
Chapter 5
Carbon Isotope Discrimination And Water Use Efficiency In Kentucky Bluegrass Turfgrass

Abstract ................................................................. 88

Introduction ........................................................... 89

Materials And Methods ............................................. 92

Results And Discussion ............................................ 97

References ............................................................. 106
LIST OF TABLES

Table 2.1. Mean squares and sum of squares from AMMI analysis of ET rate in 61 Kentucky bluegrass at three temperatures ...........................................26

Table 2.2. Average evapotranspiration rate in 61 Kentucky bluegrass cultivars across three temperature environments and regression coefficients and lAS1 from AMMI analysis of interaction ...........................................27

Table 2.3. Mean squares and sum of squares from linear regression analysis of interaction of ET rate in 61 cultivars of Kentucky bluegrass at three temperatures ...........................................................................34

Table 3.1. Fifteen attributes measured on 61 Kentucky bluegrass grown as unmown space plants (n=6) under greenhouse conditions and their evapotranspiration (ET) group ...........................................................................53

Table 3.2. Mean squares from ANOVA of leaf characteristics in 61 Kentucky bluegrass cultivars grown as unmown space plants under greenhouse conditions ...........................................................................54

Table 3.3. Mean squares from ANOVA of shoot and root characteristics in 61 Kentucky bluegrass cultivars grown as unmown space plants under greenhouse conditions ...........................................................................54
Table 3.4. Fourteen attributes measured on 61 cultivars of Kentucky bluegrass maintained as mowed turf in 20 cm diam. lysimeters (n=4) and their evapotranspiration rate (ET) group .......................................................... 56

Table 3.5. Mean squares from ANOVA of leaf characteristics in 61 Kentucky bluegrass cultivars maintained as mowed turf in 20 cm diam. lysimeters ........................................................................................................ 57

Table 3.6. Mean squares from ANOVA of shoot and root characteristics in 61 Kentucky bluegrass cultivars maintained as mowed turf in 20 cm diam. lysimeters ........................................................................................................ 57

Table 4.1. Discriminant analysis correct classification rates based on variables from unmown space plants as predictors of low- and high-water use groups ...................................................................................................... 76

Table 4.2. Predictor variables from unmown space plants ordered by their F-ratio from oneway ANOVA with water use group used as the independent factor ...................................................................................... 77

Table 4.3. Discriminant analysis correct classification rates based on variables from mowed lysimeters as predictors of low- and high-water use groups ...................................................................................................... 78
Table 4.4. Predictor variables from mowed lysimeters ordered by their F-ratio from oneway ANOVA with water use group used as the independent factor

Table 4.5 Coefficients for standardized linear discriminant functions using leaf angle and leaf width from mowed lysimeters as predictors of water use group

Table 4.6. Summary of classification with cross-validation using leaf angle and leaf width from mowed lysimeters as predictors of water use group

Table 4.7. Summary of classification with cross-validation using leaf angle and leaf extension rate from mowed lysimeters as predictors of water use group

Table 5.1. Mean squares for water use efficiency (WUE) and C isotope discrimination ($L_l$) from ANOVA for individual harvest periods and combined data conducted in the greenhouse under well watered condition

Table 5.2. Cultivar means for water use efficiency (WUE) and C isotope discrimination ($L_l$) at individual harvest periods

Table 5.3. Cultivar means at individual harvest periods for evapotranspiration rate (ET) and canopy temperature (CT)
Table 5.4. Mean squares from ANCOVA with canopy temperature as the covariate for evapotranspiration rate measured under well watered conditions in eleven KBG cultivars at individual harvest periods and combined data conducted in the greenhouse ........................................102

Table 5.5. Correlation matrix for mean evapotranspiration rate (ET), water use efficiency (WUE), and C isotope discrimination (A) for the first harvest (below diagonal) and second harvest (above diagonal) periods (n=11).

........................................................................................................................ 103.
LIST OF FIGURES

Figure 2.1. Regression lines showing the relationship of ET rate and three temperature environments for two cultivars having average stability. LSD (.05) bars are shown. Cultivar ET ranking are shown in brackets. ................................................................. 29

Figure 2.2. Regression lines showing the relationship of ET rate and three temperature environments for two cultivars different in their stability (interaction). LSD (.05) bars are shown. ET ranking are shown in brackets................................................................. 30

Figure 2.3. A generalized interpretation of ET response in Kentucky bluegrass cultivars across low evaporative- and high evaporative-environments when cultivar regression coefficients are plotted against cultivar mean ET rate............................................................................................................................... 32

Figure 2.4. The relationship of cultivar stability (regression coefficient) and cultivar mean ET rate for 61 Kentucky bluegrass cultivars. For explanation of individually labelled cultivars see figures and text................................................................. 32

Figure 2.5. Biplot of the AMMI model for ET rate in 61 Kentucky bluegrass cultivars. For explanation of individually labelled cultivars see figures and text .................................................................................. 35
Figure 3.1. Dendogram using complete linkage between groups. Grouping variables are standardized cultivar mean evapotranspiration rate measured at 25, 35, and 35°C in 61 Kentucky bluegrass cultivars.

Figure 5.1. Relationship between ET rate and turf canopy temperature at two harvest periods obtained in the greenhouse for KBG. ET and temperature values for each harvest (n=29) represent observations for 11 cultivars.

Figure 5.2. Relationship between water use efficiency (WUE) and carbon isotope discrimination (%, 0/0) for Kentucky bluegrass at the first harvest period. Individual observations (n=29) represent 11 cultivars.

Figure 5.3. Relationship between water use efficiency (WUE) and carbon isotope discrimination (%, 0/00) for Kentucky bluegrass at the second harvest period. Individual observations (n=29) represent 11 cultivars.
Supplemental irrigation is one of the most important maintenance practices in the management of turfgrass for two reasons, i) turfgrass plants are dependent on available soil water essential for maintaining a functional turf, and ii) turfgrass consumptive water use often exceeds the amount provided by natural rainfall. In the future, as the competition for a finite, limited supply of potable water increases, the use of water for irrigating turfgrass will surely be restricted, or prohibited during drought conditions for more important needs (Dudek, 1987). As a result, current research efforts have emphasized management practices that reduce or improve the efficiency of irrigation water applied to turfgrass through cultural practices and species/genotype selections (Kneebone et al., 1992).

A substantial body of research on evapotranspiration (ET) rate in warm-season, C4 turfgrass (Kim and Beard, 1988a) and cool-season, C3 turfgrass (Bowman and Macaulay, 1991; Sherman, 1986; Sherman, 1989) demonstrate inter- and intra-variation in ET rate exists, and indicates the possibility of selecting and breeding for low water use. Turfgrass potential ET is routinely assessed under non-limiting soil moisture conditions using weighing lysimeters, e.g., the gravimetric water balance method (Beard, 1985). The identification of selections with a low water use pattern is difficult because turfgrass ET determined gravimetrically is relatively labor intense, and not well suited for mass screening. Thus, alternative methods that are more efficient need to be found.
Closely related to consumptive water use is water use efficiency (WOE, the ratio of carbon gain to water loss, or equivalently, the amount of dry matter produced per unit of water loss by the plant). In developing crop plants for maximum yield under limited water, improving water use efficiency has been the long term goal (Moss et al., 1974). In turfgrass breeding programs, however, combining superior turfgrass quality performance with conservative water use is the long term objective. In irrigated turfgrass, WUE is probably less important because superior productivity or yield does not necessarily equate to superior turf quality performance (Sherman, 1985; Youngner, 1985). Additionally, under non-limiting soil moisture conditions, transpirational water loss and associated resistances at the leaf level are not as important in controlling turfgrass ET as are canopy resistances external to the plant (Johns et al., 1983). However, this should not underscore the importance of WUE in turfgrass evaluations because improved WUE is the result of a combination of reduced transpiration and/or superior productivity, and therefore relates indirectly to turfgrass ET.

The objectives of this study are:
1. Development of a technique that would allow breeders to identify turfgrass selections with a low water use pattern. The identification would be based on relatively simple plant measurements that are consistent with the types of measurements routinely assessed by plant breeders.

Turfgrass ET and WUE are distinctly different phenomenon because improved WUE does not necessarily equate to low turfgrass ET if improved WUE is the result of superior productivity alone. It is hopeful, however, that improved WUE is the result of both superior productivity and reduced
transpiration. Because turfgrass ET and WUE are distinctly different, the screening procedures on which their identification are based are obviously different, representing morphological and physiological traits, respectively. The justification and theory behind the procedures will now be developed.

Screening For Water Conserving Kentucky Bluegrass Using Morphological Attributes And Discriminant Analysis

Plant characteristics and water use

Kentucky bluegrass (Poa pratensis L.) is one of the most widely used cool-season turfgrass species in the United States (Watson et al., 1992). The range in ET rate reported for this important turfgrass species is as broad as reported across all turfgrass species (Beard, 1973). Sherman (1986) evaluated the ET rate of 20 Kentucky bluegrass (KBG) cultivars under controlled environment conditions (25°C) and found cultivars differed by as much as 64% in daily ET rate. In warm-season turfgrass, Kim and Beard (1988a) have shown that plant characteristics associated with high canopy resistance to ET (high shoot and leaf density, and horizontal leaf orientation) combined with minimal leaf area components (slow vertical leaf extension rate and narrow leaf texture) are selection criteria important in breeding for low water use rate. They reasoned that canopy resistance components such as a substantial horizontal leaf orientation, and high leaf and shoot density would cause a greater resistance to the vertical movement of water vapor in the canopy, and at the same time decrease turbulence, with an increase in vapor density within the canopy. Leaf area components on the other hand contribute to total leaf areal evaporative surface.
In KBG, studies have been unable to specify the criteria to be used in selecting genotypes having reduced water use (Kneebone et al., 1992). Only a superficial treatment has been given to morphological characteristics and their relationship to water use in cool-season turfgrasses. Sherman (1986) found that the ET rate of 5 KBG cultivars measured at 35°C to be positively correlated to vertical leaf elongation rate, and negatively correlated to shoot density. However, the 5 cultivars were chosen from a 20 cultivar sample representing low, intermediate, and high water use cases based on cultivar ET measured at 25°C. No significant correlation between shoot density or elongation rate to ET measured at 25°C was detected in the 20 cultivars. Leaf density, leaf texture, and leaf orientation were not evaluated in the study. Further research is needed to identify individual components of canopy structure/leaf area important in water use in KBG. It is difficult to extrapolate the results from warm-season species to cool-season species because Kim and Beard (1988b) have demonstrated that the relative importance of each morphological characteristic and its affect on water use rate can vary significantly between species.

In a recent review of turfgrass water requirements, Kneebone et al. (1992) concluded that more research is needed to identify the interaction of canopy structure with atmospheric demand as it relates to water use. Turfgrass ET at both the inter- and intra-specific levels can respond differently as atmospheric conditions change (Aronson et al., 1987; Sheffer, 1979; Sherman, 1986). As a result, the relative rankings of species and cultivars in ET rate can shift significantly between years, seasons, locations or environments. Sherman (1986) reported that the ET rate for 5 KBG cultivars increased from 1.1- to 1.7-fold depending on the cultivar when temperature increased from 25 to 35°C. No formal statistical tests were performed to determine if cultivars differed significantly in the rate of ET loss. Further research is needed to quantify the
extent of which cultivars differ in ET response with atmospheric/evaporative demand.

In developing cultivars for turf usage, plant breeders typically evaluate plant characteristics obtained from both spaced plant nurseries and dense-mowed swords (Bourgoin and Mansat, 1977). Plant measurements from mowed turfs can be cumbersome and obstructed by diminutive tillers and high stand densities (Brede and Duich, 1982). Physical measurements from unmown space plants are not necessarily the most reliable criteria for predicting the performance of mowed turfgrass (Kramer, 1947; Bourgoin, and Mansat, 1977). However, occasionally a relationship between turf performance and single plant morphology can be found (van Wijk, 1989). Because KBG is apomictic, plant characteristics evaluated on single plants of the same genotype which are vegetatively identical, may be applicable to a turfgrass-plant (genotype) in a competitive population or sword. Water use studies comparing single plant morphology and turfgrass morphology have yet to be investigated.

*Discriminant analysis*

Morphological criteria have been used successfully in warm-season turfgrass in identifying selections with low water use rate (Sifer et al., 1986). The success in warm-season turfgrass has been based on the premise that those turfs with a high canopy resistance to ET and low leaf area components have lower ET (Kim and Beard, 1988a). These studies demonstrate that comparative water use in turfgrass measured under non-limiting soil moisture conditions is a multivariate problem because comparative water use is affected by several morphological and growth characteristics associated with components of canopy resistance to ET and leaf area. Efficiency in water use prediction can be improved by considering simultaneously several plant attributes that are
correlated with water use. The multivariate technique of discriminant analysis developed by Fisher (1936) provides an effective method for this purpose when groups are of interest, e.g., comparative water use groups.

The interest in discriminant analysis in this study is to predict group membership for the purpose of screening for water conserving types. Discriminant analysis has been used successfully in horticulture in classifying plant material. For example, Lapins and Nash (1957) used discriminant analysis to identify peach cultivars, and Eaton and Lapins (1970) used it to distinguish between standard and compact types of apple trees. The method has seen little application because computations are complex and time-consuming. However, the use of discriminant analysis in classifying plant material has become more convenient than it has previously been the case because of the increasing speed of the personal computer and the availability of statistical software that performs discriminant analysis.

The use of discriminant analysis for identification of KBG cultivars having low water use patterns based on the cultivar's morphological properties is a reasonable procedure for identification because of the relationship that exists between turfgrass morphology and comparative water use (Kim and Beard, 1988a; Sherman, 1986). The success of discriminant analysis in this present study is based on the premise that cultivar groups that are dissimilar in their water use properties are likely to differ in their morphological properties and therefore classification functions can be developed that will recognize the differences in pattern between water use groups. We are interested in the effectiveness of physical measurements based on unmown space plant morphology and how they compared to morphology from mowed turfgrass in discriminating between water use groups.
A brief description of discriminant analysis will be given here. For a detailed discussion, see Johnson and Wichern (1992) and McLachlan (1992). Discriminant functions are used in deciding to which group (comparative water use) a cultivar belongs based on the measurements of its characteristics. Observations can be classified effectively into the groups using linear combinations of the original variables if their mean values change considerably from group to group. A discriminant function is defined as

\[ d_i = C_i + k_1X_1 + k_2X_2 + \ldots + k_pX_p \]  \hspace{1cm} [1]

where \( d_i \) is the discriminant score for an observation, \( i \) is 1,2,..., \( g \) number of groups, \( C_i \) is a constant, \( X_1, \ldots, X_p \) are mean values for \( p \) plant characters measured on an observation, and \( k_1, \ldots, k_p \) are the weights of the individual characters. The coefficients are chosen in such a way that the values of \( d \) are used as rules for sorting samples into groups of interest. Observations are allocated to group \( i \) for which the discriminant score \( (d_i) \) is the largest.

Equivalently, an observation \( x \) is classified into group \( i \), if the generalized squared distance of \( x \) to group \( i \) is the smallest. The generalized squared distance of observation \( x \) to the center (mean) of group \( i \) is given by:

\[ D_i^2(x) = D_{i\text{general}} = -2[X'm_iSp^{-1}x - \frac{1}{2}m_i'Sp^{-1}m_i + \ln p_i] + x'Sp^{-1}x \]  \hspace{1cm} [2]

where \( x \) is a column vector of length \( p \) containing the values of the predictors for an observation, \( x' \) is the corresponding row vector of length \( p \), \( m_i \) is a column vector of length \( p \) containing the means of the predictors calculated from the data in group \( i \), \( m_i' \) is the corresponding row vector of length \( p \), \( Sp \) is the pooled covariance matrix, and \( \ln p_i \) is the prior probability \( (P_i) \) that an observation is in group \( i \) transformed by its natural logarithm \( (\ln) \).
The term in the bracket is a linear function of \( x \) and is called the linear discriminant function for group \( i \) (see Eq. 1). For a given \( x \), the group with the smallest generalized squared distance has equivalently the corresponding largest discriminant score. Hence an observation is allocated into the group generating the largest discriminant score (Eq. 1) or the corresponding smallest generalized squared distance (Eq. 2). Note that the generalized squared distance is penalized the least by \( \ln p \), for those observations whose prior probabilities are largest. If the prior probabilities are unknown then \( p_1=p_2=\ldots=p_g=1/g \), and equal priors are assumed.

Linear discriminant analysis assumes multivariate normality and equal group covariance structure. For the unequal group covariance case the pooled covariance matrix, \( S_p \), is substituted with the within group covariance matrix, \( S_i \), and the term within the bracket no longer reduces to linear functions of \( x \), hence the term quadratic discriminant analysis is used.

When a large number of predictor variables are being considered in discriminant analysis, the analysis can be exploratory because variable selection procedures are used to find a subset of predictors/discriminators making a significant contribution in the variability of the categorical-classification variable (dependent variable). A categorical classification variable can be derived using hierarchical agglomerative cluster analysis (Jolliffe et al., 1989) by categorizing cultivars into comparative water use groups on the basis of cultivar ET rate measured tinder non-limiting soil moisture conditions. All the limitations for variable selection procedures in regression analysis apply to discriminant analysis. Therefore, a subset of models of various sizes are often reported rather than a single model.

In assessing the effectiveness of discriminant functions in predicting group membership, an optimistic or apparent error rate (APER) results when
the same data set that was used to derive the classification function is then used to validate the function. Cross-validation employs the holdout procedure described by Lachenbruch and Mickey (1968) to compensate for the optimistic APER. The holdout procedure or leave-one-out method (LOER) is one of several methods of cross-validation used to reduce the optimistic bias in estimating actual error rate. This procedure omits the first case (cultivar) from the analysis, develops a classification function using the (n-1) remaining cases, then classifies the omitted observation. The omitted case is then returned to the data set and the holdout procedure is repeated with every case omitted and then classified. The holdout procedure is an alternative to splitting a data set into training samples and validation samples in estimating actual error rate (Johnson and Wichern, 1992).

Carbon Isotope Discrimination And Water Use Efficiency In Kentucky Bluegrass Turfgrass

Stable C isotopic composition (δ) may be a potentially valuable method for evaluating turfgrass selections for water conserving patterns and improved WUE because i) C analysis is rapid, has high resolution, and requires very small plant samples (Ehleringer, 1991), ii) C isotope analysis integrates performance over the life of the particular plant tissue being analyzed and therefore has obvious advantages over instantaneous gas exchange measurements, and iii) in pot experiments, C isotope discrimination (Δ, derived from δ measurements) and WUE have been correlated in several cool-season, C3 grasses (Johnson and Bassett, 1991; Johnson et. al., 1990; Farquhar and Richards, 1984), thus Δ may provide an indirect assessment of water loss. This research has emphasized C3 forage grasses such as crested wheat grass (A.
desertorum), Altai wildrye (L. angustus), orchardgrass (D. glomerata), tall fescue (F. arundinacea), and perennial ryegrass (L. perenne) under unmown conditions, and not as turf. The relationship between ~ and WUE in KBG maintained as turfgrass has not been studied.

There are two naturally occurring stable isotopes of C, 12C and 13C, with the heavier isotope contributing =1.1% of the C to CO2 in the atmosphere with the balance as 12C (= 98.9%). During fixation of C by photosynthesis the heavier isotope 13C is discriminated against, resulting in a smaller ratio of 13C to 12C in plant tissues compared to the source air. The ratio of 13/12C (R) in plants vary because of the differential diffusion rate through stomata and due to the enzymatic processes by which C is initially fixed by the primary carboxylating enzyme (Rubisco) in C3 plants. Both processes discriminate against the heavier isotope. Early studies have reported values in terms of the isotopic composition (δ13C) which is the difference in the carbon ratio between a sample and the PDB (Peedee belemnite carbonate formation) standard measured on a mass spectrometer and calculated as:

\[ \delta^{13}C = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \]  

expressed in units of per mil basis (‰). δ13C for plant and air sample yield negative values indicating that samples are depleted in 13C compared to the PDB standard.

Farquhar and Richards (1984) offered ~ (or biological discrimination) values as an alternative to δ13C values calculated from the C isotopic composition of the plant sample (Op) and the source air (oa) as:

\[ \Delta = \delta_a - \delta_p / 1 + \delta_p. \]
13C isotope discrimination, $\delta_1$, are positive values since the proportion of $^{13}C/^{12}C$ fixed by photosynthesis and incorporated into plant tissues is less than the proportion of $^{13}C/^{12}C$ in the source air.

WUE and $\delta_1$ are related in C3 plants in part through stomatal conductance and the ratio of the leaf intercellular CO$_2$ concentration ($c_i$) to that in the atmosphere ($c_a$), hence the $c_i/c_a$ ratio, (Farquhar et. al., 1989). Discrimination in Cg leaves according to Farquhar et. al.(1989) can be expressed in relationship to the $c_i/c_a$ ratio in its simplest form as:

$$\delta_1 = a + (b - a) \frac{c_i}{c_a} \quad [3]$$

where $a$ is the fractionation caused by diffusion in air (4.4%0) and $b$ is the fractionation caused by carboxylation (27%0). From this expression, when stomata are relatively closed then $c_i/c_a$ approaches zero and $\delta_1$ values approximate 4.4%0. Conversely, if stomatal limitations are minimal then $c_i/c_a$ approaches unity and $\delta_1$ values approximate 27%0.

Carbon isotope discrimination in C3 plants is correlated with WUE because both processes are related to $c_i$. This follows because WUE can be expressed as the molar ratio of photosynthesis ($A$) to transpiration ($E$) as:

$$\frac{A}{E} = \frac{(c_a - c_i)}{1.6 \times v} \quad [4]$$

where $v$ is the leaf-to-air water vapor gradient and 1.6 is the ratio of the diffusivities of water and CO$_2$ in air. Thus $C_a$ and $v$ are constants (assuming evaporative demand $v$, is equal across all plants and environments), and WUE in the above expression varies with $c_i$. It can be seen from expressions 3 and 4 that $\delta_1$ values are inversely related to WUE ($A/E$) and that a high WUE in C3
leaves is associated with low A. This allows for intraspecific comparisons to be made in WUE if $\delta^{13}C_{\text{air}}$ is known and if leaf temperatures (and hence $v$) can assume to be the same across genotypes (Farquhar and Richards, 1984).
REFERENCES


