Examination of the Importance of Root Hairs in Plant Water-Use Studies by Repeated Measures Analysis

Carolyn K. Yoder, Department of Environmental and Resource Sciences, University of Nevada-Reno, Reno, NV 89557-0107

ABSTRACT

Root hairs are cylindrical elongations from root epidermal cells that increase the absorbing area of roots greatly. Due to the destructive nature of root sampling the role of root hairs in relation to plant water-use is seldom investigated. In this study a repeated measures analysis was performed in the SAS® System for personal computers (SAS Institute, 1988) to analyze root hair data collected for three species growing in a mixed-grass prairie: *Bouteloua curtipendula* (sideoats grama), *Hilaria Belangeri* (curlymesquite), and *Amphiachyris dracunculoides* (annual broomweed). The results indicate that species is highly significant while depth and the species by depth interaction are not. CONTRAST statements in the general linear model (GLM) procedure were used to show that sideoats grama has significantly more root hairs per mm of root than either curlymesquite or annual broomweed. These results may explain why sideoats grama is a drought resistant species whereas curlymesquite becomes dormant early under drought conditions.

INTRODUCTION

Root distribution data are commonly reported on a mass, length, or number basis, with little or no attention given to root hairs. Root hairs, however, increase the absorbing area of roots by a factor of 5 to 18 (Dittmer, 1937) and actively absorb water as a function of metabolism (Cailloux, 1972). Therefore, knowledge of root hair distribution (number of root hairs per mm of root) can provide insight into plant water-use patterns. In this study, the number of root hairs per mm of root was measured for three species: curlymesquite (*Hilaria belangeri* (Steud.) Nash), a C_4 shortgrass with a stoloniferous growth form; sideoats grama (*Bouteloua curtipendula* (Michx.) Torr), a C_4 midgrass with a rhizomatous or bunchgrass growth form; and annual broomweed (*Amphiachyris dracunculoides* (DC.) Nutt), a single-stemmed annual forb. All three of these species are dominant in plant communities of the Texas Rolling Plains in the southern Great Plains of North America.

PROCEDURES

The data was collected at the end of a study designed to examine patterns of water utilization by species growing in a mixed-grass prairie (Yoder, 1993). The study was conducted at the Wagon Creek Spade Experimental Ranch in Throckmorton County approximately 22 km north of Throckmorton, Texas (33°20'N; 99°14'W). This area is part of the Rolling Plains of Texas. Climate is semi-arid continental. Annual precipitation (1950-1988 median = 646 mm) is bimodal with peaks occurring in May and September. The soils at the study site are of the Nuvalde series (fine-silty, mixed thermic family of Typic Calciustolls, 1-3% slopes). Range site classification is Clay Loam. Nine weighing lysimeters (of circular cross-section) filled with undisturbed, vegetated soil monoliths were installed on the Wagon Creek Spade Research Ranch in 1985. The lysimeters were divided randomly into sets of three monocultures so that three lysimeters contained sideoats grama, three contained curlymesquite, and three contained annual broomweed. Root distribution data were collected for the three species by taking soil cores from each of the 9 lysimeters in 76 mm increments to a depth of 684 mm. Roots were separated from the soil by washing in a 60 μm sieve. The number of root hairs per mm of root was determined on random samples from each depth by counting the number of root hairs or root hair abscission points along the length of root segments under a microscope (Caesar, 1991).

STATISTICAL ANALYSIS

Because data were collected at several points in space on the same experimental units (lysimeters) a repeated measures analysis was performed in the SAS® System for personal computers, general linear model (GLM) procedure (SAS Institute, 1988). PROC GLM was used rather than the analysis of variance procedure (PROC ANOVA) so that contrast statements could be used to test for differences between species. The contrast transformation specified in the REPEATED statement was PROFILE. The PRINTE option was used with the REPEATED statement in order to perform a test for sphericity. However, because the

number of depths examined equalled the number of experimental units, there were insufficient error degrees of freedom to perform the sphericity test. Therefore, the partial correlation coefficients defined by the PROFILE transformation were examined in order to determine if the errors were correlated. The partial correlation coefficients were not close to 0 and were not approximately equal which indicates that the Huynh-Feldt (H-F) condition (Huynh and Feldt, 1970) was not met and that the errors were correlated. Therefore, the numerator and denominator degrees of freedom were adjusted (deflated) before determining the significant levels for the univariate F-tests. The two adjustments available in the REPEATED statement in the SAS System for personal computers are the Greenhouse and Geisser (1959) (G-G) adjustment and the Huynh and Feldt (1976) (H-F) adjustment. Both of these adjustments estimate an epsilon and multiply the numerator and the denominator degrees of freedom by the epsilon before determining the significant level of the F test (Fernandez, 1991). The G-G adjustment is more conservative than the H-F adjustment, especially for small samples (Huynh and Feldt, 1976). The results of the analysis and the F tests for both adjustments are shown in Table 1.

Table 1. Results of repeated measures analysis of variance on root hair data.

Source	DF	F-value	P-value	G-G Adj	H-F Adj
species	2	629	0.0001		
depth	8	0.6	0.725	0.5392	0.6331
depth*species	16	1.24	0.2724	0.3440	0.3160

These results show that species is highly significant while depth and the species by depth interaction are not. Therefore, the number of root hairs per mm of root is significantly different for at least one of the species and the number of root hairs per mm of root is not significantly affected by depth. CONTRAST statements in PROC GLM were used to determine which species had significantly different numbers of root hairs per mm of root. These results are shown in table 2.

Table 2. Results of CONTRAST statements in PROC GLM. Sideoats = so, curlymesquite = cm, annual broomweed = bw.

CONTRAST	DF	CONTRAST SS	F-value	P-value
so vs cm	1	4.59958519	88.66	0.0001
cm vs bw	1	0.01275741	0.25	0.6376
so vs bw	1	5.09681667	98.25	0.0001

These results show that sideoats grama has significantly more root hairs per mm of root than either curlymesquite or annual broomweed and that there is not a significant difference in the number of root hairs per mm of root between curlymesquite and annual broomweed. These results are also seen clearly in the species by depth interaction plot (Fig. 1).

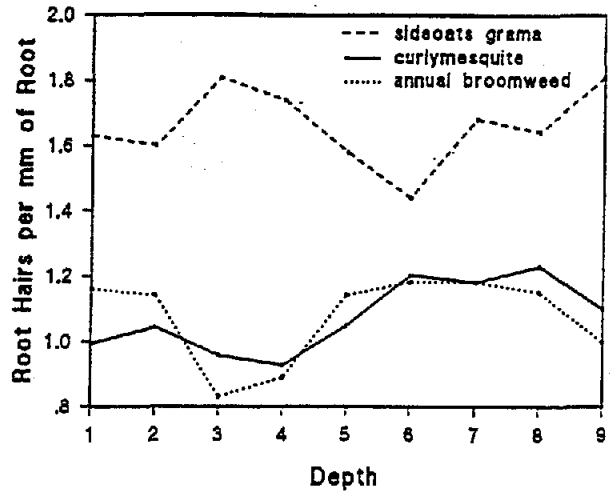

Fig. 1. Species by depth interaction plot for root hair data.

After completion of the repeated measures analysis the UNIVARIATE procedure was used for residual analysis (Fernandez, 1992) and to test for violations of the assumptions of ANOVA. The Shapiro-Wilkes test for normality yielded a P-value of 0.30 which indicated that the residuals were normally distributed. However, a plot of the residuals showed that the variances were larger for larger values and hence heteroscedasticity existed (Fig. 2a). To determine whether a transformation was needed the method of Box et al. (1978) as reported by Fernandez (1992) was used to estimate the appropriate transformation for this data set. This method indicated that an inverse cubed root transformation was appropriate. This transformation was run and the residuals were replotted. As can be seen from figure 2b, the transformation resulted in equal variances for all data. The transformed data was then reanalyzed with the REPEATED statement in PROC GLM. The results of this analysis are shown in table 3.

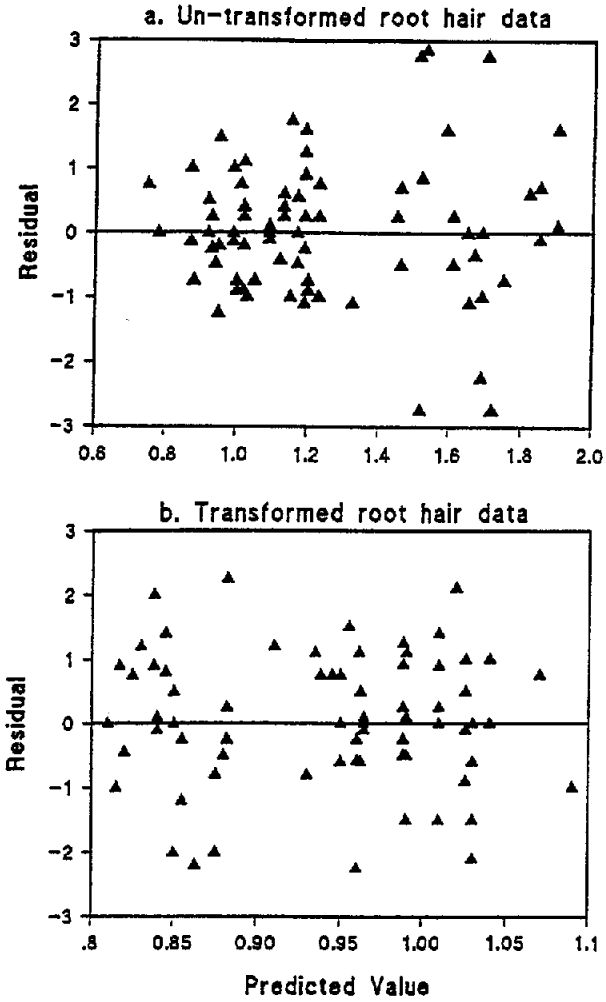

Fig. 2. Residual plots of the original and inverse-cubed root transformed root hair data.

Table 3. Results of repeated measures analysis on transformed data.

Source	DF	F-value	P-value	G-G Adj	H-F Adj
species	2	52.61	0.0002		
depth	8	1.65	0.1360	0.2193	0.1515
depth*species	16	1.52	0.1305	0.2353	0.1484

Hence, transforming the values to obtain equal variances, which is a critical assumption of ANOVA, did not change the results of the study.

SIGNIFICANCE

The results of this analysis are particularly relevant in relation to documented differences in water-use patterns of the two grass species, sideoats grama and curlymesquite. Yoder (1993) reports that root biomass does not vary significantly for these two species throughout the soil profile and that there are few significant differences in root length throughout the soil profile. However, several studies have shown that sideoats grama lysimeters have lower soil moisture contents and higher evapotranspirational losses than curlymesquite lysimeters (Franklin, 1987; Caesar, 1991; Norman, 1991; Yoder, 1993) while maintaining similar amounts of above-ground biomass. Hence, sideoats grama may have a competitive advantage over curlymesquite due to a greater number of root hairs per mm of root that enable sideoats grama to access soil water more effectively than curlymesquite. Water use patterns of the forb, annual broomweed, are reported in Yoder (1993) and are not discussed here because it would be inappropriate to compare the long central tap root of the forb to the fibrous root systems of the grasses.

CONCLUSIONS

Repeated measures analysis in the SAS System for personal computers was used to analyze root hair data that was collected at the end of a study designed to examine water use patterns of three mixed-grass prairie species. The results of the analysis show that sideoats grama has significantly more root hairs per mm of root than curlymesquite. This may explain why sideoats grama is able to utilize soil water more effectively and is more drought resistant than curlymesquite despite a lack of difference in root biomass or root length between species. This indicates the importance of root hairs in plant water-use studies and emphasizes that comparisons of root biomass and or root length between species may not be indicative of differences in root absorptive capabilities.

ACKNOWLEDGEMENTS

This research was supported by Texas A&M University and the Texas Agricultural and Experiment Station, Expanded Research Grant No. 15570-2005-6965. Appreciation is extended to G.C.J. Fernandez for providing advice on statistical analyses and to T.L. Thurow for providing guidance throughout the study. K.B. Hays and D.L. Hays helped with field data collection and lab analyses.

SAS is a registered trademark or trademark of SAS Institute Inc. in the USA and other countries.
® indicates USA registration.

REFERENCES

Box, G.E.P., W.G. Hunter, and I.S. Hunter. (1978), *Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building.* New York: Wiley, Inc.

Caesar, B. L. (1991), *Hydrologic Aspects of a Midgrass Dominated and Shortgrass Dominated Sites on the Edwards Plateau of Texas.* M.S. Thesis. Texas A&M University, College Station, Texas, 84 pp.

Cailloux, M. (1972), "Metabolism and the absorption of water by root hairs," *Canadian. J. Bot.,* 50, 557-573.

Dittmer, H. J. (1937), "A quantitative study of the roots and root hairs of a Winter rye plant (*Secale cereale*)," *Amer. J. Bot.*, 24, 417-420.

Fernandez, G.C.J. (1991), "Repeated measure analysis of line-source sprinkler experiments," *HortScience*, 26(4), 339-342.

Fernandez, G.C.J. (1992), "Residual analysis and data transformations: important tools in statistical analysis," *HortScience*, 27(4), 297-300.

Franklin, J. D. (1987), *Hydrologic Aspects of Grass Dominated and Honey Mesquite Dominated Areas on the Rolling Plains of Texas.* M.S. Thesis. Texas A&M University, College Station, Texas, 133 pp.

Greenhouse, S.W. and S. Geisser. (1959), "On methods in the analysis of profile data," *Psychometrika* 32, 95-112.

Huynh, H. and L.S. Feldt. (1970), "Conditions under which mean and square ratios in repeated measurement designs have exact F-Distribution," *J. Amer. Stat. Assn.* 65, 1582-1589.

Huynh, H. and L.S. Feldt. (1976), "Estimation of the Box correction for degrees of freedom from sample data in randomized block and split-plot design," *J. Educ. Stat.* 1, 69-82.

Norman A. J. (1991), *Comparison of Water Use Patterns by Bouteloua curtipendula and Hilaria belangeri in Response to Small Rainfall Events.* M.S. Thesis. Texas A&M University, College Station, Texas, 81 pp.

SAS Institute Inc. (1988), *SAS/STAT User's Guide. Version 6.05 Edition,* Cary, NC: SAS Institute Inc.

Yoder, C. K. (1993), *Soil Water Utilization by Herbaceous Species of the Southern Great Plains: Evidence from Isotopically Labeled Water.* M.S. Thesis. Texas A&M University, College Station, Texas, 212 pp.