USE OF SEEDLING SIZE AS A COVARIATE FOR ROOT GROWTH POTENTIAL STUDIES [1/]

David B. South, Harry S. Larsen, Hans M. Williams and James N. Boyer [2/]

Abstract. -- Measurements of root growth potential are often dependent on seedling morphology (i.e. size of the root system). When this occurs, covariate analysis can provide a means of removing the potential confounding effect of differences in morphology. Covariance analysis can be useful in reducing experimental error. Additional information from available data can also be achieved by use of covariance analysis. Several examples are presented to illustrate the potential effect of this type of analysis on the interpretation of treatment effects. Due to rapid fluctuations in RGP over time, covariance analysis (based on seedling size) may be less useful when RGP is sampled over time than when the measurements are from one point in time.

INTRODUCTION

Root growth potential (RGP) is a measure of a seedling's ability to initiate and elongate roots when placed into an environment favorable for root growth (Ritchie 1985). In the South, researchers are currently interested in measuring this attribute since outplanting survival can sometimes be correlated with RGP (Feret and others 1986; Hallgren and Tauer 1987; Larsen and others 1986). Since 1980, there have been over 35 papers that involve measuring the RGP of southern pine seedlings.

When outlining the basic procedures for measuring RGP, Ritchie (1985) stated that "since seedling size can also affect RGP, it may be desirable either to (1) select seedlings of relatively uniform size for the test (which will bias the results) or to (2) analyze the results using morphological properties as covariates." However, this type of analysis is seldom used for RGP studies. There have only been a few studies where RGP values have been adjusted for differences due to seedling morphology (Larsen and Boyer 1986; Williams et al. 1988).

Conclusions regarding the results of any experiment can depend on the type of analysis used. In some cases, commonly used statistics can result in incorrect conclusions regarding the data (Warren 1986). The objective of this paper was to compare the results of evaluating RGP data with either the traditional analysis of variance (ANOVA) or with covariate analysis. The authors have attempted to explain how the interpretation of the data can vary with the type of analysis used.

MATERIALS AND METHODS

Data reported here are from 3 container studies (studies 1, 2, and 3) and 9 bare-root studies (table 1). Except for four studies (1, 10, 11 and 12), treatments were laid out in the nursery in a randomized complete block design. Two studies (2 and 3) involved split-plots.

RGP was determined in each study by using either a hydroponic method (Ritchie 1985) or by potting the seedlings in sand (Larsen and Boyer 1986) and placing the containers on a heating bed. All studies were conducted in a greenhouse in order to provide high light intensity for production of photosynthate. Photoperiod was extended to either 15 or 16 hr. Root temperature was maintained at either 25 or 27°C.

Prior to placing seedlings in the test, all white root tips were removed and heights and diameters were recorded. After four weeks (three weeks for the bud type study), RGP was determined by measuring the number of new roots that were greater than 0.5 or 1.0 cm in length. After removal of the new roots, the weight of the remaining (original) root system was determined.

For each study, data were analyzed using both analysis of variance and covariate analysis. In all but two studies, seedling diameter was used as the covariate. Total seedling weight was used as the covariate in study 1 and original root weight was used in study 10.

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[2/] Associate Professors and Graduate Research Assistant, Auburn University Southern Forest Nursery Management Cooperative, School of Forestry, Auburn University, AL 36849-5418; and Post-doctorate, Clemson University, Forestry Sciences Laboratory, Charleston, SC 29414.

Table 1.--Information regarding the RGP studies

	Study	Seed source	RGP test	Lifting date	Treatments
1.	Bud type	half-sib	21-day water	Nov., Dec, Jan.	3 bud types; ± storage
2.	Fertilization	half-sib	30-day water	Nov., Jan.	3 levels of Diammonium phosphate (DAP)
3.	Fertilization	half-sib	30-day water	Dec., Jan.	DAP vs. ammonium nitrate vs. control
4.	Seed spacing	orchard mix	28-day sand	Dec.	1.9, 2.8, 4, 5, 6 cm spacing
5.	Seed spacing	orchard mix	28-day sand	Jan.	1, 2, 3, 4, 5, 6 cm spacing
6.	Seed spacing	Livingston P.	28-day sand	Dec.	1, 2, 4, 6 cm spacing
7.	Irrigation	orchard mix	30-day water	Dec.	irrigation vs. no fall irrigation
8.	Irrigation	orchard mix	30-day water	Jan.	irrigation vs. no fall irrigation
9.	Nursery	half-sib	28-day sand	Dec.	7 nurseries
10.	Nursery	Livingston P.	28-day sand	Dec.	20 nurseries
11.	Lift date	half-sib	28-day water	Sept.-March	3 sowing dates; 14 lift dates
12.	Lift date	half-sib	28-day water	Sept.-March	15 lift dates; with and without buds

RESULTS AND DISCUSSION

In ten of the studies, seedling morphology accounted for a significant amount of variation in RGP (the covariate in most cases was significant at the 0.0001 level of probability; table 2). In general, treatments that increased seedling size (i.e. additional fertilization, additional irrigation, lowering seedbed density) also increased RGP. This agrees with previous research that demonstrates that larger loblolly pine seedlings tend have higher RGP than smaller seedlings (Barden 1987; Brissette and Roberts 1984; Carlson 1986).

The use of covariance analysis can often aid the experimenter in understanding the principles underlying the results of an investigation. This type of analysis can help to explain if an increase in RGP is confounded with an increase in seedling size. The following are some examples of how the interpretation of RGP data might be improved by using covariance analysis.

Study 1

Without covariance analysis, the conclusions from this study would be that storage, bud type and seedling age (lifting date) affected RGP (table 2). However, the covariance analysis suggests that bud type and seedling age are confounded with seedling size. The affect of these variables on RGP could simply be explained by older seedlings being larger and seedling with buds being larger than seedlings without well formed buds.

Study 2

Without covariance analysis, the conclusions would be that increased nitrogen fertilization increased RGP. However, the covariance analysis suggests that rate of fertilization may have an effect on RGP which is over and above that of increasing seedling size.

Study 3

Regardless of method of analysis, the conclusion is that source of nitrogen had no effect on RGP. However, the covariance analysis also indicates that regardless of the lack of treatment effect, there still exists a positive relationship between seedling size and RGP.

Studies 4 and 5

Without covariance analysis, the conclusion is that RGP increases with lower seedbed densities. However, with the covariance analysis, the conclusion is that the increase in RGP can be accounted for simply by the effect of seedbed density on seedling size.

Table 2.--Comparison between ANOVA and Analysis of Covariance for various RGP studies

Study	ANOVA				Analysis of covariance			
	Source	df	F value	P > F	Source	df	F value	P > F
1. Bud type					covariate	1	134.3	0.0001
	bud type	1	51.7	0.0001	bud type	1	1.0	0.3850
	lift date	2	528.4	0.0001	lift date	2	2.1	0.1332
	storage	2	1227.2	0.0001	storage	2	11.2	0.0014
	error	54			error	53		
2. Fertilization					covariate	1	31.0	0.0001
	block	3	0.4	0.7413	block	3	9.0	0.0123
	treatment	2	65.1	0.0001	treatment	2	32.3	0.0006
	error A	6			error A	6		
3. Fertilization					covariate	1	24.5	0.0001
	block	3	0.7	0.5849	block	3	0.9	0.5043
	treatment	2	3.6	0.0933	treatment	2	3.0	0.1259
	error A	6			error A	6		
4. Seed spacing					covariate	1	35.6	0.0001
	block	4	0.7	0.5872	block	4	2.1	0.1157
	treatment	4	5.1	0.0074	treatment	4	1.1	0.3754
	error	16			error	15		
5. Seed spacing					covariate	1	78.1	0.0001
	block	4	4.2	0.0128	block	4	4.2	0.0097
	treatment	5	11.5	0.0001	treatment	5	0.3	0.8868
	error	20			error	19		
6. Seed spacing					covariate	1	26.1	0.0001
	block	4	2.8	0.0769	block	4	1.9	0.1471
	treatment	3	3.2	0.0616	treatment	3	6.0	0.0073
	error	12			error	11		
7. Irrigation					covariate	1	82.6	0.0008
	block	2	3.3	0.2308	block	2	.4	0.5700
	treatment	1	427.6	0.0111	treatment	1	0.0	0.8585
	error	2			error	1		
8. Irrigation					covariate	1	10.6	0.0056
	block	7	2.5	0.1249	block	2	1.5	0.2395
	treatment	1	1.9	0.2105	treatment	1	1.8	0.1960
	error	7			error	6		
9. Nursery					covariate	1	33.5	0.0001
	nursery	6	5.3	0.0001	nursery	6	5.5	0.0001
	error	189			error	188		
10. Nursery					covariate	1	192.5	0.0001
	nursery	19	10.3	0.0001	nursery	19	8.0	0.0001
	error	369			error	368		
11. Lift date					covariate	1	0.3	0.5707
	block	3	1.8	0.1487	block	3	1.9	0.1343
	lift date	13	25.4	0.0001	lift date	13	24.1	0.0001
	sowdate	2	42.4	0.0001	sowdate	2	41.1	0.0001
	error	123			error	122		
12. Lift date					covariate	1	0.2	0.6520
	sample	14	3.2	0.0009	sample	14	3.2	0.0008
	bud	1	14.2	0.0004	bud	1	13.9	0.0004
	error	60			error	59		

Study 6

For this density study, covariance analysis, greatly increased the precision of the test (table 3). In addition, for this test, there may exist an effect of seedbed density on RGP which is independent of seedling size.

Study 7

With ANOVA, the conclusion is that RGP was increased by fall irrigation. However, the covariance analysis indicates that this effect may simply be due to the treatment causing an increase in seedling size.

Study 8

Regardless of method of analysis, the conclusion is that RGP was not increased by fall irrigation. However, the covariance analysis also indicates that regardless of the lack of treatment effect, there still exists a positive relationship between seedling size and RGP.

Studies 9 and 10

With ANOVA, the conclusion is that RGP varies by nursery. With covariance analysis, the conclusion is that differences among nurseries exists even after taking into account differences in seedling size.

Study 11 and 12

For these two studies, the type of analysis made no difference to the conclusions because the covariate did not account for a significant proportion of the variation. This can be explained by the fact that RGP can change rapidly (either up or down) during a 2 week period while seedling diameter normally increases only slightly during the same period. In otherwords, the relationship between seedling size and RGP was masked by the rapid fluctuation in RGP over time.

In all of the studies, covariance analysis provided more information than the regular ANOVA. In 8 cases, the use of covariate analysis helped to interpret the nature of the treatment effects. In 4 of the studies, the effect of various treatments on RGP could be completely explained by the changes in seedling morphology.

In 10 studies, the error mean square was reduced by 13 to 65% (table 3). For these cases, the precision of the test was increased by the use of covariate analysis. Covariance analysis did not improve the precision of studies that involved sampling RGP over a long period of time (September to March).

The improvement in precision will depend on which morphological trait is used as the covariate. For example, use of seedling height as a covariate will not be as consistently reliable as root-collar diameter, root volume, root weight, or weight of lateral roots.

Table 3.--Error mean square reduction by use of covariance analysis

Study	ANOVA	Covarince	% decrease
	-- error mean square --		
Study 1	255	160	37%
Study 2	194	129	33%
Study 3	646	473	27%
Study 4	315	177	44%
Study 5	528	349	34%
Study 6	1314	480	63%
Study 7	35	12	65%
Study 8	108	89	18%
Study 9	2289	1984	13%
Study 10	308	244	21%
Study 11	88	91	-
Study 12	34	32	5%

In some cases, the use of multiple covariance analysis may result in further improvement in precision (Woollons and Whyte 1988). Multiple covariance has been used to adjust RGP values for differences in morphology among seedling samples (Larsen and Boyer 1986). Since RGP is dependent on current photosynthesis, it might be possible to improve the precision of tests by including foliar nitrogen content along with morphological covariates such as diameter or lateral root weight.

CONCLUSIONS

Covariance analysis of RGP tests can be useful in increasing the precision of the test. In addition, this type of analysis can be helpful in determining if the treatment response can be simply related to a change in seedling size. The trait used as the covariate should be related to the size of the root system rather than the height of the shoot. Covarinace analysis based on morphology will be useful when (1) RGP is measured in one point in time; and (2) RGP is affected by some nursery treatment. This type of analysis may be less effective when the RGP data are from a date of lifting study.

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