Statistical Consulting Report for

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Background and Objective:

Two groups of loblolly pine clones with different levels of water use efficiency (WUE) will be tested for drought tolerance in a greenhouse experiment. Two water treatments (control and drought) will be applied. There are 11 clones in each genetic group and 6 ramets per clone. The objective of the study is to compare genetic groups for their drought tolerance and test whether WUE is related to drought tolerance under a controlled environment.

The major hypotheses are;

1) The control and drought treatment means are not significantly different from each other for response variables (H0: $\mu c=\mu d$)

2) Two genetic groups are not significantly different for response variables (H0: $\mu 1=\mu 2$).

Let's say the drought treatment is factor A with 2 levels (Dry, Control) and genetic groups is factor B with two levels (High, Low). A classical randomized complete block split-plot design can be used to test the major hypotheses. Within each *r* blocks (replication), the *a* levels of the between factor A are randomized to the main units (whole or main plots). Because they are randomized to the main plots, the levels of *a* between A factor are called main treatments. Within each main plot, the *b* levels of between factor B are randomized to the *b* subunits (sub-plots). The levels of a within factor are called sub treatments. 11 clones within each genetic group (factor B) is another factor (C) with *c* levels. However, since each clone will have one ramet and clones are not the interest, they can be ignored.

Using the PLAN procedure of SAS, the following split plot design was generated.

The PLAN Procedure

Factor	Select	Levels	Order
block	3	3	Ordered
Treat (A)	2	2	Random
Group (B)	2	2	Random
Clones	11	11	Random

block	Treatment (A)	Group (B)	Clones										
1	2	2	3	8	2	10	9	11	7	5	6	4	1
		1	11	6	4	8	1	9	3	10	2	5	7
	1	1	1	10	11	5	9	3	6	7	2	8	4
		2	10	7	4	11	9	3	5	6	2	1	8
2	2	2	5	10	8	9	6	1	11	4	7	3	2
		1	11	4	1	9	2	3	7	8	6	10	5
	1	1	9	7	10	2	6	3	1	8	11	4	5
		2	11	10	1	8	9	5	2	7	3	6	4
3	2	1	7	3	9	10	2	4	8	11	1	6	5
		2	10	11	3	5	2	7	8	9	1	4	6
	1	1	1	3	7	4	5	8	2	9	11	10	6
		2	6	1	9	5	10	4	11	7	3	8	2

F tests for different factors should be constructed correctly. I simulated a data set and analyzed using the MIXED procedure of SAS to show appropriate F tests for different factors as follows.

```
proc mixed method=type3;
    class Treat Group Block;
    model Trait = Treat|Group;
    random Block Treat*Block;
 run;
```

```
             Split Plot Design          11:43 Monday, June 8, 2009   30

                               The Mixed Procedure

                                Model Information

                  Data Set                      WORK.M
                  Dependent Variable            Trait
                  Covariance Structure          Variance Components
                  Estimation Method             Type 3
                  Residual Variance Method      Factor
                  Fixed Effects SE Method       Model-Based
                  Degrees of Freedom Method     Containment
```

Class Level Information

Class	Levels	Values
Treat	2	1 2
Group	2	1 2
Block	3	1 2 3

Dimensions

Covariance Parameters	3
Columns in X	9
Columns in Z	9
Subjects	1
Max Obs Per Subject	132

Number of Observations

Number of Observations Read	132
Number of Observations Used	132
Number of Observations Not Used	0

Type 3 Analysis of Variance

Source	DF	Error Term	Error DF	F Value	Pr > F
Treat	1	MS(Treat*Block)	2	15.52	0.0588
Group	1	MS(Residual)	124	0.43	0.5146
Treat*Group	1	MS(Residual)	124	3.60	0.0601
Block	2	MS(Treat*Block)	2	4.27	0.1898
Treat*Block	2	MS(Residual)	124	0.21	0.8094
Residual	124	.	.	.	.

Covariance Parameter
Estimates

```
                    Cov Parm        Estimate

                    Block           0.001195
                    Treat*Block     -0.00272
                    Residual         0.07593


                        Fit Statistics

             -2 Res Log Likelihood          44.0
             AIC (smaller is better)        50.0
             AICC (smaller is better)       50.1
             BIC (smaller is better)        47.2


                 Type 3 Tests of Fixed Effects

                          Num      Den
         Effect            DF       DF     F Value    Pr > F

         Treat              1        2       15.52    0.0588
         Group              1      124        0.43    0.5146
         Treat*Group        1      124        3.60    0.0601
```

The analysis can be expanded by producing least square means, their standard errors and t-test probability values. The Estimate statements in MIXED procedure are handy to calculate any linear combinations of fixed and random effects.

APPENDIX

```
title 'Split Plot Design';
   proc plan seed=37277;
      factors block=3 ordered Treat=2 Group=2 clones=11;
   run;



   data wue;
   length Block Treat Group Clone 4. ;
   retain Seed_1 0 Trait;
   do Block=1 to 3 ;
     do treat=1 to 2;
        do group=1 to 2;
             do clone=1 to 11;
              call ranuni (Seed_1,Trait);
              output;
```

```
                end;
            end;
        end;
    end;
    drop seed_1 ;
    if treat=1 then trait=trait+0.5;
    if group=2 then trait=trait+0.75 ;
run;
```