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Using Analysis of Covariance with Unequal Slopes to Increase Efficiency and Information Obtained from Designed Experiments

Richard P. Marini[1] and Daniel Ward[2]

Abstract

Horticulturists often perform experiments involving both qualitative and quantitative factors. Sometimes the quantitative factor is a continuous variable (covariate) measured on each experimental unit and data can be analyzed by analysis of covariance (ANCOVA). ANCOVA is a powerful and flexible technique for extracting maximum information from a data set. Data from an experiment designed to compare the productivity of three peach (*Prunus persica* L. Batsch) genotypes were used to determine if genotype influenced average fruit weight, while accounting for the variation explained by the linear relationship between fruit weight and crop density. The data set was analyzed with SAS's MIXED procedure to demonstrate a strategy for analyzing experiments with a qualitative variable plus a covariate.

Agricultural researchers employ statistical techniques to help interpret experimental data. Analysis of variance (ANOVA) is commonly used to test the hypothesis that qualitative treatments have an equal effect on a response variable. Regression analysis is used to evaluate the relationship between a response variable and quantitative or continuous variables, sometimes known as regressor variables or covariates. Analysis of covariance (ANCOVA) is a special case of regression that combines features of ANOVA and regression and the linear model contains both qualitative and one or more continuous variables measured on each experimental unit. ANCOVA can be used in two ways: 1.) as an error-reduction technique, or 2.) multiple regression with a mixture of categorical and continuous regressors all of which are of interest for explanatory interpretation. Often ANCOVA is considered a modified ANOVA that uses information from an additional or uncontrolled variable that is linearly related to the response variable. Such a model containing a covariate may be expected to reduce residual variation and fit the observed values better than the original ANOVA (11). In such cases, a "typical" ANCOVA can be employed to compare treatment means while correcting for the covariate. Another use of ANCOVA is as a technique to compare a series of regression models (9). In addition to the assumptions underlying ANOVA (the error terms are independent, normally distributed and have constant variances); ANCOVA also requires that the covariate is not affected by the treatments, ranges of the covariate for each level of the treatment factors are similar, the response variable is linearly related to the covariate, and the regression lines of the response variable on the covariate are parallel (homogeneous slopes) for all levels of the qualitative factor(s).

In tree fruit research, ANCOVA has been used to evaluate the effect of qualitative treatments on fruit size. An example is a linear model that includes rootstock as the qualitative factor and crop density (CD; fruit·cm^{-2} trunk cross-sectional area) as a covariate. Average fruit weight (FW; g·fruit^{-1}) is the response variable. Previous reports show that FW is linearly related to CD (12) and ANCOVA has been used to compare rootstock means at the average value of CD (2, 8). More recently, Marini et al. (6, 7) found that the assumptions for a

[1] Professor, Department of Horticulture, The Pennsylvania State University, University Park, PA 16802
[2] Assistant Professor, Department of Plant Biology and Pathology, Rutgers Agricultural Research and Extension Center, 121 Northville Road, Bridgeton, NJ 08302

typical ANCOVA were not satisfied because the ranges of CD were not always similar for all rootstocks and the slopes were often not homogenous.

Many statistics text books describe the typical ANCOVA as an error reduction technique to compare treatment means while correcting for a covariate, but there are several approaches to analyzing data sets with a qualitative variable plus a covariate, depending on which factors in the model are significant. The purpose of this paper is to demonstrate how one may determine which approach is most appropriate and then demonstrate a strategy for analyzing experiments when a typical ANCOVA is not appropriate. For illustration purposes, unpublished data from an experiment comparing three peach (*Prunus persica* L. Batsch) genotypes were used to evaluate the effect of genotype on FW while correcting for CD.

Materials and Methods

Data for this study were obtained from an experiment initiated in 1999 at the Virginia Tech College of Agriculture and Life Sciences Kentland Research Farm to compare three peach genotypes (Standard, Pillar and Upright). Trees were trained to the open-vase form. The experiment was a randomized complete block design (RCBD) with five blocks. The experimental unit was a 10-tree-plot for each genotype, which was randomly assigned to each block. Some trees died during the first season, so there are unequal numbers of observations per plot. During harvest in 2002, the number of fruit and the weight of the fruit were recorded for each tree and FW and CD were estimated for each tree. ANCOVA was used to analyze the effect of genotype on FW while correcting for CD. The use of subsamples (10-tree-plots) added some complexity to this experiment. To simplify the analysis Hinkelmann and Kempthorne (4) suggest using the mean of the subsamples as the data for the ANCOVA. We chose not to use plot means because there were different numbers of observations per plot and plot means would provide only five observations for estimating regression lines. Before using means of subsamples, it is instructive to graphical explore the relationship between the response variable and the covariate using subsample means as well as with individual observations.

Like multiple-regression, ANCOVA is not a single-step analysis; the appropriate form of the covariate part of the model must be identified. This paper utilizes the strategy suggested by Littell et al. (5) and Milliken and Johnson (9) for determining the form of the linear model and the subsequent analysis. The experiment involved one qualitative factor (genotype) and one continuous variable (CD) in a RCBD with subsampling to determine if average FW was influenced by genotype, taking into account that FW is linearly related to CD. The data set, along with the SAS code associated with the data step, are presented in Table 1.

The strategy for identifying the form of the model and the resulting analyses begins by determining whether a straight line is an adequate model to describe the data for each level of the qualitative factor (genotype) and this can be done with linear regression. Before determining the covariance part of the model, homogeneity of variances should be tested to determine if an unequal variance model is required. Proc GLM was used to perform a Levene's test of equality of variances. Few statistics texts offer rules of thumb, based on α-levels, for data analysts to use when testing the assumptions underlying statistical techniques. Anderson and McLean (1) suggested considering remedial action when the homogeneity test has a P-value between 0.01 and 0.001. Since the P-value resulting from the Levene's test was 0.011, we assumed that an equal variance model was adequate for a valid ANCOVA. The strategy used to identify the covariance part of the model is explained in detail below. The slopes were not homogeneous, so a typical ANCOVA was not used and instead an unequal slopes model was fitted and slopes were compared using pair-wise comparisons. Finally treatment's least squared means (LSmeans) were compared at several values of the covariate.

Table 1. The SAS data step and data set for an experiment to evaluate the relationship between fruit weight (FW) and crop density (CD) as affected by three peach genotypes (type). The data set has five blocks, three genotypes (type), 10 trees per genotype nested in block and genotype, FW and CD.

```
option pagesize=80 linesize=80;
data peach_type;
input block tree type $ fw cd;
cards;
1 1  p 164 7.43
1 2  p 170 2.67
1 4  p 180 1.95
1 5  p 170 1.57
1 6  p 180 0.22
1 7  p 170 1.25
1 8  p 170 1.29
1 10 p 200 0.64
1 1  u 190 3.82
1 2  u 200 3.19
1 3  u 210 3.18
1 4  u 173 5.31
1 5  u 200 2.23
1 7  u 180 3.35
1 8  u 190 3.45
1 9  u 160 6.03
1 10 u 140 3.45
1 1  s 150 5.79
1 2  s 150 1.49
1 3  s 140 6.08
1 4  s 160 3.95
1 5  s 150 5.87
1 6  s 160 5.01
1 7  s 150 5.08
1 8  s 160 3.48
1 9  s 150 6.01
1 10 s 170 3.46
2 1  u 180 4.46
2 2  u 170 4.53
2 3  u 210 2.51
2 4  u 163 6.48
2 5  u 190 3.40
2 6  u 190 3.33
2 7  u 220 2.56
2 8  u 160 3.30
2 9  u 210 4.32
2 10 u 180 4.28
2 1  s 160 5.22
2 2  s 160 4.71
2 3  s 170 4.82
2 4  s 170 3.82
2 5  s 160 3.62
2 6  s 160 4.33
2 7  s 140 5.91
2 8  s 140 5.25
2 9  s 150 4.15
2 10 s 150 5.82
2 1  p 170 2.72
2 2  p 190 0.97
2 3  p 160 2.58
2 4  p 180 0.84
2 5  p 180 1.67
2 6  p 180 2.82
2 7  p 170 1.07
2 8  p 180 2.28
2 9  p 160 4.17
2 10 p 160 3.69
3 1  u 210 2.52
3 2  u 180 4.46
3 3  u 190 2.71
3 4  u 190 2.99
3 5  u 210 3.11
3 6  u 200 4.20
3 7  u 200 3.43
3 8  u 200 4.63
3 9  u 220 3.12
3 10 u 220 1.86
3 1  p 170 2.48
3 2  p 170 2.96
3 3  p 180 2.13
3 4  p 200 1.54
3 5  p 180 2.33
3 6  p 180 1.85
3 7  p 160 4.00
3 8  p 210 0.79
3 9  p 200 2.50
3 10 p 180 1.09
3 1  s 150 5.30
3 2  s 170 4.31
3 3  s 170 3.42
3 4  s 173 6.28
3 5  s 160 4.97
3 6  s 170 3.60
3 7  s 160 4.15
3 8  s 150 6.92
3 10 s 140 5.72
4 1  p 200 1.00
4 2  p 180 0.47
4 4  p 160 0.46
4 5  p 200 1.36
4 6  p 210 1.05
4 7  p 170 2.74
4 8  p 150 5.27
4 9  p 190 2.91
4 10 p 180 4.15
4 1  s 163 4.02
4 2  s 167 5.22
4 3  s 160 4.18
4 4  s 150 4.34
4 6  s 160 5.97
4 7  s 160 4.87
4 8  s 160 5.24
4 9  s 160 6.14
4 10 s 160 6.39
4 1  u 190 3.40
4 2  u 180 4.44
4 3  u 190 4.88
4 4  u 180 3.18
4 5  u 190 3.69
5 1  s 160 4.82
5 2  s 160 3.28
5 3  s 150 6.04
5 4  s 160 4.91
5 5  s 170 2.71
5 6  s 160 2.82
5 7  s 170 3.92
5 8  s 170 4.30
5 9  s 160 2.99
5 10 s 170 2.95
5 1  u 222 3.22
5 2  u 210 3.56
5 3  u 187 3.89
5 4  u 200 4.16
5 5  u 180 4.89
5 6  u 180 4.46
5 7  u 190 2.36
5 10 u 200 3.76
5 1  p 180 1.68
5 2  p 180 2.09
5 3  p 160 1.62
5 4  p 190 2.59
5 5  p 160 1.40
5 6  p 150 6.65
5 7  p 170 2.91
5 8  p 180 3.52
5 9  p 200 1.24
```

Results and Discussion

The data, along with the SAS code for the data step are presented in Table 1. The first step is to determine if the data for all genotypes can be described with lines. A plot of the data, generated with the GPLOT procedure suggests that a linear fit is reasonable (Fig. 1). To verify this, SAS's REG procedure was used to fit linear and quadratic models to the data. This is not really an ideal analysis because the experiment was a RCBD and the REG procedure doesn't allow inclusion of the random effects (block and the block x genotype for experimental error) in the model. Although the REG procedure generates incorrect standard errors, the parameter estimates are correct and can be used to determine the nature of the linear relationship between the response variable and the covariate. Results from the REG procedure indicated that the linear term, but not the quadratic term, was significant for all three genotypes (abbreviated as TYPE) indicating that straight lines fit the data adequately for ANCOVA.

To verify that the ranges of the covariate were similar for all three genotypes, the MEANS procedure was used to obtain the minimum, mean and maximum values of FW and CD for the three genotypes (Table 2). Based upon our graphical analyses and since the range of CD for the Pillar trees encompassed the range of the other tree types, we feel that the ranges of the covariate overlap adequately for a valid ANCOVA.

Step 1: Test the hypothesis that the slopes are equal to zero. The SAS statements, along with the resulting output, are presented in Table 3. The model statement contains the terms TYPE and CD*TYPE plus the option for "no intercept" (NOINT). The term TYPE with the NOINT options produces estimates of the intercepts for each genotype if the SOLUTION option is included in the model statement. If the NOINT option is omitted, as in Step 2, the intercept estimates are obtained by setting the last genotype (Upright) to zero because when sorted alphabetically Upright is listed last. The estimates corresponding to the intercepts can still be calculated for each genotype, but they must be obtained by subtracting the estimates for Pillar and Standard types from the estimate for Upright. The term CD*TYPE generates the part of the design matrix corresponding to the slopes. By not including the CD term, the covariate part of the model is nonsingular and slopes are estimated for each genotype. If CD was included in the model statement, the slope for Upright would be set to zero and slopes for Pillar and Standard types could be obtained by subtraction.

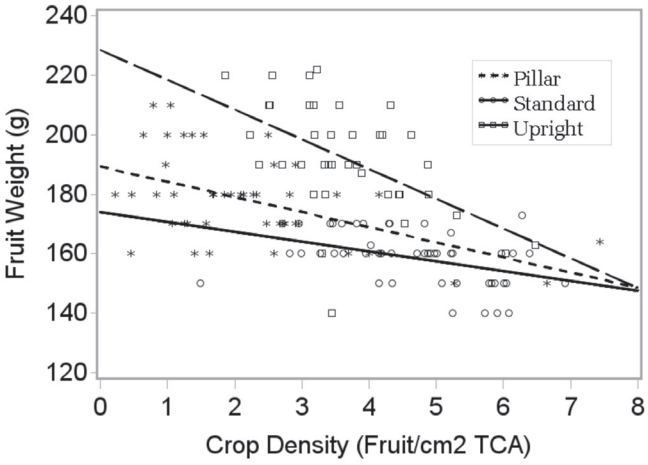

Fig. 1. Relationship between fruit weight and crop density for three peach genotypes. The figure was generated with SAS's GPLOT procedure. A scatterplot with regression lines for each genotype was requested with the interpolation=rl option in the symbol statement.

The use of linear mixed models (via SAS's MIXED procedure) for data sets containing random effects or repeated measures requires specification of appropriate denominator degrees-of-freedom for test statistics for fixed effects (genotype). This becomes complicated even in the case of balanced designs with simple covariance structures and becomes increasingly difficult for

Table 2. SAS code for proc means, along with resulting output for minimum, mean and maximum values for CD and FW for the three genotypes (TYPE).

```
proc means maxdec=2 min  mean max;
   class type;
   var cd fw;
   run;
```

The Means Procedure

type	N Obs	Variable	Minimum	Mean	Maximum
P	46	cd	0.22	2.27	7.43
		fw	150.00	177.70	210.00
S	48	cd	1.49	4.66	6.92
		fw	140.00	158.60	173.00
U	42	cd	1.86	3.72	6.48
		fw	140.00	191.31	222.00

Table 3. SAS statements and output for fitting a model to test the hypothesis that all slopes are equal to zero (step 1). The first section of output for Type 3 Tests of Fixed Effects contains the numerator and denominator degrees of freedom associated with the F-tests. The lower section is the Solution for Fixed Effects. For the effect labeled "type" the Estimate is the value for the intercept, and for the effect labeled "cd*type" the Estimate is the value for the slope. Standard errors of the estimates, degrees of freedom, t-value and probability of a greater t are for testing the hypotheses that the estimates are equal to zero.

```
proc mixed;
    class block type tree;
     model fw = type cd*type/noint solution ddfm=kr;
     random block block*type;
run;
```

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
type	3	77.8	1245.25	<.0001
cd*type	3	128	16.00	<.0001

Solution for Fixed Effects

Effect	type	Estimate	Standard Error	DF	t Value	Pr > |t|
type	p	189.21	3.5732	43.5	52.95	<.0001
type	s	174.39	7.5318	121	23.15	<.0001
type	u	226.78	7.6036	124	29.83	<.0001
cd*type	p	-5.1090	1.1808	126	-4.33	<.0001
cd*type	s	-3.3676	1.5354	129	-2.19	0.0301
cd*type	u	-9.5107	1.9353	127	-4.91	<.0001

Table 4. SAS statements and output for fitting a model to test the hypothesis that all slopes are equal (step 2). The Type 3 Tests of Fixed Effects contains the numerator and denominator degrees of freedom associated with the F-tests.

```
proc mixed;
    class block type tree;
    model fw = type cd cd*type / ddfm=kr;
    random block block*type;
  run;
```

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
type	2	128	14.14	<.0001
cd	1	127	43.63	<.0001
cd*type	2	128	3.15	0.0461

complex designs involving complicated covariance structures, unbalanced data or small sample sizes. The MIXED procedure offers several denominator degrees-of-freedom options and the "containment method" is the default when another method is not requested in the model statement. The Kenward-Roger method for estimating denominator degrees-of-freedom for fixed effects was requested with the option "ddfm=kr". The Kenward-Roger approximation (3) reportedly performs well with more complicated covariance structures when sample sizes are moderate to small and the design is reasonably balanced (10).

Blocks, plots (combinations of blocks and genotypes) and trees (tree-to-tree is the residual variance) were random effects, and block and block x type were included in the Random statement. Unlike the GLM procedure, the MIXED procedure does not generate an ANOVA table with sums of squares because the GLM and MIXED procedures use different estimation methods. The GLM procedure uses the ordinary least squares estimation, whereas the MIXED procedure uses maximum likelihood. The Type 3 F-statistic corresponding to genotype in the Type 3 tests of fixed effects is for the hypothesis that all genotypes are equal. This is equivalent to comparing the regression models at CD=0, which is not of interest and cannot be easily interpreted because there is no FW at CD=0. The F-statistic corresponding to CD*TYPE in the Type 3 tests of fixed effects is for the hypothesis that all slopes are equal to zero. The P-value of <0.0001 indicates that the slopes are not all equal to zero, so for these data a typical ANOVA is not appropriate for comparing genotypes.

Step 2: Determine if a common slopes model is adequate to describe the data. Now that we know that the slopes are not all equal to zero, we want to know if the slopes are all parallel to each other. The SAS statements plus the resulting output are presented in Table 4. The model statement includes type (fixed effect), CD (the covariate) and CD*TYPE (the interaction), and the model becomes singular because the NOINT option is not included. The Type 3 F-statistic for the CD*TYPE effect tests the hypothesis that all slopes are equal. The P-value of 0.0461 indicates that there is adequate evidence to reject the hypothesis that slopes are equal, so an equal slopes model (a typical ANCOVA) will not be adequate for these data. The next step is to compare genotypes by comparing the regression lines or characteristics of the regression lines.

Step 3: Fit an unequal slopes model. The SAS statements plus the output are presented in Table 5. The model statement is the same as in step 1, but the SOLUTION option is included in the model statement to generate estimates of intercepts and slopes for each genotype. Three LSmeans statements were

Table 5. SAS statements for fitting an unequal slopes model to obtain estimates for intercepts and slopes for each genotype (step 3). LSmeans statements are used to obtain estimates of fruit weight (FW) and to compare genotypes at three levels of crop density (CD). Estimate statements are used to perform pair-wise comparisons on the slopes. The first section of output for Type 3 Tests of Fixed Effects contains the numerator and denominator degrees of freedom associated with the F-tests. The middle section is the Solution for Fixed Effects. For the effect labeled "type" the Estimate is the value for the intercept, and for the effect labeled "cd*type" the Estimate is the value for the slope. Standard errors of the estimates, degrees of freedom, t-value and probability of a greater t are for testing the hypotheses that the estimates are equal to zero. The third section of output, Least Squares Means, is FW estimated at three levels of CD, along with associated t-tests to test the hypothesis that the estimate mean is equal to zero. The forth section of the output contains all possible pair-wise comparisons of the LSmeans for tree type, along with the difference between the LSmeans and the t-value used to test the hypothesis that the differences are equal to zero.

```
proc mixed;
    class block type;
    model fw = type cd*type / noint solution ddfm=kr;
    random block block*type;
     lsmeans type / at cd=1 diff;
     lsmeans type / at cd=3 diff;
     lsmeans type / at cd=5 diff;
* - - - - - - - - - - - - - - - -      P     S    U;
     estimate 'P vs. S' cd*type      1    -1    0;
     estimate 'P vs. U' cd*type      1     0   -1;
     estimate 'S vs. U' cd*type      0     1   -1;
  run;
```

Type 3 Tests of Fixed Effects [z]

Effect	Num DF	Den DF	F Value	Pr > F
type	3	125	1364.83	<.0001
cd*type	3	128	16.85	<.0001

Solution for Fixed Effects

Effect	type	Estimate	Standard Error	DF	t Value	Pr > |t|
type	p	189.80	3.4597	118	54.86	<.0001
type	s	175.30	7.4862	130	23.42	<.0001
type	u	226.26	7.4367	130	30.43	<.0001
cd*type	p	-5.3874	1.1782	128	-4.57	<.0001
cd*type	s	-3.5499	1.5450	130	-2.30	0.0232
cd*type	u	-9.3227	1.8978	127	-4.91	<.0001

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
P vs. S	-1.8375	1.9449	129	-0.94	0.3465
P vs. U	3.9353	2.2318	127	1.76	0.0803
S vs. U	5.7728	2.4608	129	2.35	0.0205

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
P vs. S	-1.8375	1.9449	129	-0.94	0.3465
P vs. U	3.9353	2.2318	127	1.76	0.0803
S vs. U	5.7728	2.4608	129	2.35	0.0205

Least Squares Means

Effect	type	cd	tree	Estimate	Standard Error	DF	t Value	Pr > |t|
type	p	1.00	5.32	184.41	2.6793	87	68.83	<.0001
type	s	1.00	5.32	171.75	6.0288	129	28.49	<.0001
type	u	1.00	5.32	216.94	5.6589	130	38.34	<.0001
type	p	3.00	5.32	173.64	2.4343	61.1	71.33	<.0001
type	s	3.00	5.32	164.65	3.3577	111	49.04	<.0001
type	u	3.00	5.32	198.30	2.6588	89.7	74.58	<.0001
type	p	5.00	5.32	162.86	3.9721	116	41.00	<.0001
type	s	5.00	5.32	157.55	2.3020	53.3	68.44	<.0001
type	u	5.00	5.32	179.65	3.3056	118	54.35	<.0001

Differences of Least Squares Means

Effect	type	_type	cd	tree	Estimate	Standard Error	DF	t Value	Pr > |t|
type	p	s	1.00	5.32	12.6571	6.3640	130	1.99	0.0488
type	p	u	1.00	5.32	-32.5301	5.9261	127	-5.49	<.0001
type	s	u	1.00	5.32	-45.1872	8.0909	129	-5.58	<.0001
type	p	s	3.00	5.32	8.9821	3.6570	129	2.46	0.0154
type	p	u	3.00	5.32	-24.6595	3.0149	128	-8.18	<.0001
type	s	u	3.00	5.32	-33.6416	3.8392	129	-8.76	<.0001
type	p	s	5.00	5.32	5.3072	4.0628	127	1.31	0.1938
type	p	u	5.00	5.32	-16.7889	4.7863	128	-3.51	0.0006
type	s	u	5.00	5.32	-22.0960	3.5301	127	-6.26	<.0001

[z]The P-value of <0.0001 for the cd*type interaction in the Type 3 tests for Fixed Effects indicates that the slopes are not all equal to zero, so a comparison of slopes is more informative that performing a typical ANCOVA.

included to perform pair-wise comparisons of predicted FW estimated at three levels of CD (1, 3, and 5 fruit•cm^{-2} TCA). Three estimate statements were also included to perform pair-wise comparisons on the three slopes.

The F-statistics in the "type 3 tests of fixed effects" table test the hypotheses that all in-

tercepts are equal to zero (type) and that all slopes are equal to zero (CD*TYPE). The P-value indicates that the CD*TYPE interaction is significant (P <0.0001), so slopes are not all equal. The "Solution for Fixed Effects" table provides estimates for intercepts and slopes for each model. For example, for Pillar trees the intercept is 189.8 and the slope is -5.3874. The P-value in the far right column is for the hypothesis that the coefficient is equal to zero. The "Estimates" table shows the difference (Estimate) between each pair of slopes and the t-statistic tests the hypothesis that the difference is equal to zero. The difference between slopes for Pillar vs. Standard is -5.3874 – (-3.5499) = -1.8375, and the standard error of the difference is 1.9449. The associated t-value is -0.94 and the P-value is 0.3465, indicating that the slopes for Pillar and Standard types are not different at the 5% level of significance. Slopes for Pillar and Upright are not different (P < 0.0803), and slopes for Standard and Upright are different (P < 0.0205).

The "Least Squares Means" table provides estimates of FW at three levels of CD (1, 3, and 5 fruit•cm^{-2} TCA) for the three genotypes, along with the standard errors of the estimate. The t-statistic is for the hypothesis that the estimate is equal to zero. For example, the LSmeans for FW for Pillar trees with CD=1.0, 3.0 and 5.0 are 184.41 g, 173.64 g and 162.86 g, respectively. The "Differences of Least Squares Means"

Table 6. SAS statements for fitting a "typical" ANCOVA, along with the resulting output, where all slopes are assumed to be equal, so the LSmeans are corrected for the mean value of the covariate, CD. The bottom section labeled Differences of Least Squares Means performs all pairwise comparisons of the means with the DIFF test.

```
proc mixed;
  class block type ;
  model fw= type cd / ddfm=kr;
  random block block*type;
  lsmeans type/adjust=diff;
run;
```

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
type	2	129	61.97	<.0001
cd	1	129	40.45	<.0001

Least Squares Means

Effect	type	Estimate	Standard Error	DF	t Value	Pr > |t|
type	p	170.62	2.6993	13.4	63.21	<.0001
type	s	164.65	2.6122	11.8	63.03	<.0001
type	u	192.27	2.5428	10.5	75.61	<.0001

Differences of Least Squares Means

Effect	type	_type	Estimate	Standard Error	DF	t Value	Pr > |t|
type	p	s	5.9666	3.2271	128	1.85	0.0668
type	p	u	-21.6517	2.8824	129	-7.51	<.0001
type	s	u	-27.6183	2.6957	128	-10.25	<.0001

table provides all possible pair-wise comparisons for the genotypes at three levels of CD (1.0, 3.0 and 5.0) and the t-statistic is for the hypothesis that the difference is equal to zero. As an example, at CD=1.0, the difference between Pillar and Standard is 184.41-171.75 = 12.6571, and these means are different at the 0.0488 level of significance. It is apparent that the estimated differences between genotype become more similar as CD increases and at CD=5.0 the difference between Pillar and Standard (162.86-157.55=5.3072) is not significant (P = 0.1938). This is verified by noticing the convergence of the three lines at CD values greater than 7 in Fig. 1.

For comparative purposes a "typical" ANCOVA was performed and the means for the three tree types were compared with the DIFF option. The output in Table 6 indicates that both tree type and the covariate CD are significant (P<0.0001). The second section of the table contains the LSmeans for the three tree types corrected for the mean value of CD (3.55 g). The bottom section of Table 6 shows results for the pair-wise comparisons. The values for the Estimates are the differences of the means. The interpretation of this analysis is that FW for Upright trees was greater than for the other two types and FW for Standard and Pillar trees were not different. This typical ANCOVA would lead the researcher to conclude that Upright trees produce larger fruit than the other types, but the appropriate analysis shows that at higher CDs the effect of tree type diminishes.

The analysis presented in this paper provides an introduction to ANCOVA used to compare a series of regression lines, but the experiment was more complicated than many experiments involving tree fruit because there was subsampling. Since the slopes for the Standard and Pillar trees are not different, another estimate statement could be constructed to compare slopes and LSmeans for Upright trees vs. the average of Standard and Pillar trees. Although the analysis will become more complicated, this analysis can also be extended to split-plot designs, to experiments with more than one qualitative variable or more than one covariate, and to factorial treatment structures. Before analyzing data from more complicated experiments, graphical techniques should be used to identify potential interactions and violation of the underlying assumptions.

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