COMBINING RESULTS FROM AUGMENTED DESIGNS OVER SITES

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Abstract: The class of augmented designs contains two kinds of treatments, standard or check and new of augmented. The latter are usually considered to be random effects while the check treatments are considered as fixed effects. New treatments are usually not replicated and the checks are replicated as points of reference. An augmented experiment design is a screening design. It is obtained by selecting any experiment design for the c check treatments and then enlarging the blocks or increasing the number of rows and/or columns to accommodate the n new treatments. The new treatments are randomly distributed among the blocks or among the rows and columns. An augmented design has many advantages over systematic check arrangements and is useful for shortening the selection cycle over standard methods. Statistical procedures are available for recovering inter-block or inter-row-column and inter-variety information at each site. Procedures are presented herein for combining the results from single experiments over sites. Inter-site information may be recovered for random site effects. The method recommended for combining results is invariant to experiment design changes, variance heterogeneity, changes in checks from site to site, and different response models for each site. An experiment involving 120 new wheat genotypes and checks was conducted at three sites. It is used to illustrate the statistical procedure for combining results over sites. The new wheat genotypes exhibited a relatively large genotype by site interaction, indicating their site and environmental specificity.

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by

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ABSTRACT

The class of augmented designs contains two kinds of treatments, standard or check and new or augmented. The latter are usually considered to be random effects while the check treatments are considered as fixed effects. New treatments are usually not replicated and the checks are replicated as points of reference. An augmented experiment design is a screening design. It is obtained by selecting *any* experiment design for the c check treatments and then enlarging the blocks or increasing the number of rows and/or columns to accommodate the n new treatments. The new treatments are randomly distributed among the blocks or among the rows and columns. An augmented design has many advantages over systematic check arrangements and is useful for shortening the selection cycle over standard methods. Statistical procedures are available for recovering inter-block or inter-row-column and inter-variety information at each site. Procedures are presented herein for combining the results from single experiments over sites. Inter-site information may be recovered for random site effects. The method recommended for combining results is invariant to experiment design changes, variance heterogeneity, changes in checks from site to site, and different response models for each site. An experiment involving 120 new wheat genotypes and checks was conducted at three sites. It is used to illustrate the statistical procedure for combining results over sites. The new wheat genotypes exhibited a relatively large genotype by site interaction, indicating their site and environmental specificity.

Key words or phrases: inter-regression information, inter-gradient information, inter-variety information, row-column design, incomplete block design, polynomial regression, computer programs, genotype by environment interaction, variance component

INTRODUCTION

The class of augmented experiment designs (Federer, 1956, 1961, 1991; Federer *et al.*, 1975a, 1975b) was introduced to replace the systematically spaced check arrangements for screening new genotypes in plant breeding research investigations. Usually material for the new treatments is limited, and it is necessary to include a new treatment only once. If the material is not limited but the number of new treatments is large, it is may be desirable to include new treatments only once. Federer (1998) and Federer *et al.* (1997, 1998) have provided a statistical procedure for analyzing such experiments at a site. The analysis takes account of

the random nature of the new treatments and of the blocking variables. This results in a more efficient analysis than if this information is ignored. Augmented designs have several advantages over the systematic check arrangement such as

(i) more than one check treatment may be included,
(ii) standard errors of differences between new treatments are available,
(iii) standard errors of differences between new and checks are available,
(iv) using the survivors of a previous screening stage as the checks is a device for testing the survivors at the same time that a new set of genotypes is being screened, and
(v) fewer cycles of selection are needed than for the standard method.

An augmented experiment design (AED) is useful for screening new treatments such as genotypes, insecticides, herbicides, drugs, etc. The number of new treatments, n, may be large, even in the hundreds and thousands. An AED is constructed by selecting an experiment design for the check treatments. The experiment design could be a randomized complete block (RCBD), an incomplete block (ICBD), a row-column design, or some other design. If the selected design for check treatments is an ICBD, then the rb blocks (b incomplete blocks per complete block or replicate) are enlarged to accommodate n/rb new treatments per incomplete block. If a row-column design is selected, the numbers of rows and columns are increased to include the n new treatments. If new treatments are included more than once, the analysis must take this into account. The augmented design analyses described herein are for one replicate of each new treatment. Responses may be obtained for all or for only a fraction of the new (augmented) treatments as only the replicated check treatment responses are used for obtaining solutions for blocking effects and an estimate of the error variance.

Using an appropriate response model for each site, analyses are presented for experiments at the different sites. The selected model needs to account for the spatial variation present in the experiment. Standard textbook models may be inappropriate, and a model needs to be selected from a class of plausible models (See, e.g., Federer, 1998). The standard procedure is to use a fixed effect analysis to select a response model and blocking parameters. Then, for the selected model, a mixed effect analysis of fixed checks and random blocking and new treatment effects is performed. The check means are adjusted for the information in the random blocking effects, i.e., recovery of inter-block, inter-regression (when regression is used as a blocking variable to explain trends in spatial variation), inter-row, or/and inter-column information. The new treatment means are adjusted by recovering inter-regression, inter-block, and/or inter-variety information. Recovery of random effect information results in adjusted means with smaller variances; it shrinks the size of fixed effect estimates by taking account of the random distribution of effects. Computer programs are available for both fixed and mixed effects analyses (Wolfinger *et al.*, 1997). For each site, the most appropriate response model and best estimate of the new treatment means should be utilized. Utilizing the adjusted means for each site, it is demonstrated how to combine the results over sites. Two methods for combining results over sites are presented.

The main objective of the experiment used for an illustrative example in this paper, was to measure genetic diversity for physiological performance within a family of spring wheat lines derived from a cross between a heat tolerant and a heat sensitive parent (Reynolds *et al.*, 1998). Quantitative traits such as those associated with heat tolerance may show considerable interaction with the environment. Therefore, the testing of new lines requires extensive evaluation in different locations (environments) to establish their genetic potential. Improved methods for identifying superior lines can result in significant savings. One approach is to reduce the number of replications used in a single field trial, assuming that performance can still be evaluated accurately. Augmented designs were developed for such situations.

MATERIALS AND METHODS

The example selected to illustrate the statistical procedures is a wheat variety trial conducted at three sites by the Physiology Unit of CIMMYT's Wheat Program. The experiment design at two of the sites was a 15 row by 12 column design. The treatment design was c = 2 checks and n = 120 new genotypes. The check treatments were replicated 30 times each and the new genotypes only once at each site. The two checks

appeared twice in each of the 15 rows. The field layouts, grain weights, and other responses at the two sites are available from the authors. The particular design used is not connected, i.e., not all row, column, and treatment effects have solutions, the rank being two less than needed for connectedness. Polynomial regressions for trend in rows and in columns were used for the analysis. A check treatment occurred on every third diagonal, indicating that the rows and columns of the design plan were not randomized for the field layout. This non-randomized arrangement may bias the residual mean square upward. The size of the experimental unit or plot was 5 m by 1.2 m. An appropriate randomization procedure for designs of the row-column type may be found in standard texts.

The experiment design at the third site was an incomplete block design of $v = 120$ treatments (the new treatments at the above two sites) in incomplete blocks of size $k = 8$. Check varieties were not included at this site. The 15 incomplete blocks in each of $r = 2$ replicates were laid out adjacent to each other effectively making an eight column by 15 row lay-out in each replicate. The spatial layout of the experiment indicates that the design of the experiment should be considered as a resolvable 15-row by 8-column design rather than as an incomplete block design.

At sites 1 and 2, polynomial functions of the rows and column effects were used to determine which functions of rows and columns describe the variation present in the experiment. Polynomial regressors use centered values of the powers of the independent variable. Hence, any of the regression coefficients may be large or small depending upon the nature of the spatial variation. The SAS/GLM procedure was used to obtain the analysis of variance (ANOVA). Polynomial functions for rows, R^j, and columns, C^i, were fitted, up to twelfth degree for rows ($j = 1, ..., 12$) and up to tenth degree ($i = 1, ..., 10$) for columns (See Federer and Wolfinger, 1998) as the rank of the normal equations was two less than needed for a row, column, treatment ANOVA. Fitting row effects is equivalent to using a fourteenth degree polynomial. Polynomial regression up to twelfth degree was used as the rank was two less than needed to estimate all row effects. Using the procedure described by Bozivich *et al.* (1956) and Federer *et al.* (1998), all terms in the polynomials with F-values less than the 25% level were relegated to the residual category. The remaining regressors were treated as random blocking effects for explaining the spatial variation present in the experiment. This rule results in a slight change in the Type I error probability levels (Bozivich *et al.*, 1956). Since the gradients in a field are not always in the same direction as the row-column layout, interactions of polynomial regressors such as row-linear by column-linear, row-linear by column-quadratic, etc. were fitted to account for this type of variation. The interactions C^i*R^j fitted were from $i = 1$ to 4 and $j = 1$ to 4. Again, the Bozivich *et al.* (1956) procedure was used to determine which of the 16 interactions should be pooled with the residual and which should be retained to account for the spatial variation present in the experimental area. This selection procedure provides a safeguard against over-parameterization. For the third site, differential linear gradients within the incomplete blocks accounted for the spatial variability present in the experiment.

The resulting fixed effect response models selected for the three sites were:

Site 1:
Yield = entry C1 C2 C3 C4 C6 C8 R1 R2 R4 R8 R10 C1*R1 C2*R1 C3*R1

Site 2:
Yield = entry C1 C4 C10 R2 C1*R1 C1*R3 C2*R2 C2*R4 C3*R2 C3*R4 C4*R3 C4*R4

Site 3:
Yield = replicate entry block(replicate) C1*block(replicate)

Ci and Rj represent the orthogonal polynomial regression coefficients of degree i for columns and degree j for rows, respectively. The models are written in the form required by the SAS/GLM procedure. All elements in the above response models for sites 1 and 2 except for the check treatments are considered to be random effects. Since the 120 new genotypes are untested, they are considered to be random effects (See Federer, 1998). The goal of model selection is to find the response model that explains the spatial variation present in the experiment. Higher degree polynomials and interactions are required to account for non-

uniform gradients as opposed to uniform ones. The ordering of regression degree is irrelevant; no explanation is needed beyond that it accounts for the spatial variation present in the experiment.

For site 3, only the 120 new treatments were included and it is still possible to consider the new treatment effects as random using the SAS/MIXED procedure. The following is the SAS code for the site 3 analysis:

```
PROC MIXED METHOD = REML;
CLASS REP BLOCK ENTRY;
MODEL YIELD = /SOLUTION;
RANDOM REP BLOCK(REP) C1*BLOCK(REP) ENTRY;
```

Notice that no effects are listed in the MODEL statement since only fixed effects can be included here. With the SOLUTION option in the MODEL statement and ENTRY listed in the RANDOM statement, one needs to add the intercept value to the solution for the entry effect to obtain the mean (called REML or BLUP mean). In the SAS/MIXED procedure, one may use the default option, restricted maximum likelihood (REML), or one of the other options such as, e.g., analysis of variance (ANOVA) solutions for the variance components. This is done using the statement

```
PARMS (A) (B) (C) /NOITER;
```

where X in (X) is the value for a variance component. "NOITER" denotes no iterations are to be used. This statement appears after the RANDOM statement. Also, the word NOPROFILE needs to be added in the PROC MIXED statement. This procedure is useful when the analyst wishes to use ANOVA instead of REML solutions for the variance components. ANOVA solutions are unbiased and do not depend upon the normality assumption.

Pertinent references for combining results from a series of experiments in a different context are Cochran (1939), Yates and Cochran (1938), Federer (1951), Cochran and Cox (1957), references on analysis of crop rotations, and references on meta-analysis. Combined analyses using means per site is simple and straightforward. As suggested by Cullis *et al.* (1996) and Frensham *et al.* (1997) for the case of replicated experiments, heterogeneity of within site error variance can be considered by using the reciprocal of the standard errors of the means. Crossa and Cornelius (1997) used this transformation in the multiplicative site regression model with the purpose of identifying subsets of sites without genotypic crossover genotype by environment interaction. Piepho (1999) suggested a weighted two-stage analysis across sites where, in the first stage, the treatment-site means and their associated error variances are obtained considering sites as fixed effects. The second stage considers a mixed model with sites and genotypes by site interaction as random effects and where weights of the reciprocal of the standard error of a mean are used.

Several procedures are available for combining results over sites. Some of these are

(i) a fixed effects analysis of rows, columns, sites, and entries,
(ii) a mixed model analysis of random effects sites, rows, columns, and new genotypes with checks as fixed effects and perhaps adding row by column interaction as random,
(iii) a fixed effects analysis on fixed effects means,
(iv) a mixed model analysis with site and new as random using the fixed effects means,
(v) a regression-site-new random effects analysis over sites with checks as fixed, or
(vi) a bootstrap analysis of the yields in Tables 1 and 2 or of those in Table 3.

The above methods are flawed in one respect or another with regard to the type of data considered here. Analysis (i) is easily accomplished using a software procedure like SAS/GLM but it does not make use of inter-regression and inter-variety information, and hence is inefficient. Analysis (ii) could be used using SAS/MIXED, say, as the REML solutions obtained depend upon the normal distribution and not on the over-parameterization or the connectedness of the design. Standard errors for over-parameterized models with REML will be inflated. Analysis (iii) is straightforward but does not make use of the random effects information. Analysis (iv) performs a fixed effect analysis at the individual sites and then considers sites

and new entries as random effects using only the least squares fixed effect means for the new genotypes. Analysis (v) requires the blocking (trend) effects to be the same at all sites, and hence is not usable here.

Several possibilities exist to test for genotype by site interaction. Analysis (i) could be used in some situations. Also, an average effective error of the means or solutions could be obtained and used as an error term to test for site by genotype interaction in analyses (iii), (iv), and (v). In many situations, a test for genotype by site interaction may be of minor interest. Instead, the plant breeder selects those entries having the highest yields over all sites and environments. It may be that selections will be made for specific environments. Since it is known that a genotype by environment interaction more than likely exists for the entries in this study, there is little interest in testing for it. For random sites, the genotype by site interaction mean square is the appropriate error mean square for testing genotype effects over sites. The new by site mean square has a different expectation than the check by site mean square. A comparison of new with check is the Behrens (also called the Behrens-Fisher) situation of unequal variances.

To overcome the flaws listed above, we shall use the following two methods for combining results from experiments over sites:

Method 1: Best estimates of new treatment means are obtained for each site. Then a two-way analysis of random site effects and fixed entry effects is performed. An estimate of the error variance over sites is obtained by computing the average effective error variance at each site and then averaging these over sites. This is a method suggested by Cochran and Cox (1957), Chapter 14. Their procedure is modified here to treat the new treatments as random effects.

Method 2: Best estimates of new treatment means are obtained for each site. These means are divided by their standard errors. These are called the standardized means and will have an expected error variance of unity. This means that estimates of variance components from a site by entry analysis will be ratios of the variance component to the error variance component.

These methods *do not* require the same experiment design at each site, *do not* require the same check treatments at each site, *do not* require the same response model at each site, and *do not* require homogeneity of error variances from site to site. These two methods are applied to the data from the three sites for means and for standardized means (smean). Also, since the two replicate means at site three were quite different, they were also treated as two sites. There was a heavy disease infection in one of the replicates. The unadjusted data from individual plots were used. A randomized complete block design variance was used to obtain the standard error for the data from the two replicates at site 3. Owing to the large residual (error) variance, this resulted in a lower weight being given to the data from site 3. This is counter-balanced to some extent by using the replicates as two sites. The new treatments are considered as random effects in this case. Four sites were used for the third (means) and fourth (smeans) analyses of the data.

RESULTS

The 120 entry REML means obtained from the SAS/MIXED procedure for sites 1, 2, and 3 are presented in the second, third and fourth columns of Table 1. The plot (eu) data from replicates 1 and 2 of the site 3 experiment are given in columns 5 and 6 of Table 1 as sites 4 and 5, respectively. Since none of the 120 entries were replicated in replicate 1 or 2, the unadjusted plot data were used.

The means in Table 1 were divided by their standard errors to obtain the standardized means in Table 2. The standard error for site 1 was taken to be an average of the REML solution standard errors divided by the square root of two. The site 1 means were divided by 30.06, site 2 means by 49.50, and site 3 means by 154.00 to obtain the standardized means in columns 2, 3, and 4 of Table 2. Since the data for sites denoted as 4 and 5 were unadjusted values, they were considered as coming from a randomized complete block design. The standard error for a single plot was 546.63. Dividing the data in columns 5 and 6 of Table 1 by 546.63 produced the standardized means in columns 5 and 6 of Table 2. When data are standardized in this manner, the expected population error variance is unity, as in the unit normal distribution. This theoretical variance is the parameter and has infinite degrees of freedom.

Sites were considered as random effects in computing the REML means over sites. The 120 entry means and standardized means for sites 1, 2, and 3 and for sites 1, 2, 4, and 5 are given in Table 3. Each of the four sets of means averaged over sites has been ranked in descending order. The highest 15 means for sites 1, 2, and 3 were the following entries (Table 3)

90, 108, 29, 94, 93, 11, 91, 4, 96, 31, 8, 43, 89, 62, 33

For the smeans of sites 1, 2, and 3 in column four, eight of the above 15 appeared in the top 15 smeans. For the means averaged over sites 1, 2, 4, and 5, ten of the top 15 were in agreement. For the standardized means over sites 1, 2, 4, and 5, entries 108, 11, 90, 120, 93, 95, 99, 114, 13, 60, 2, and 53 appeared in the top 15 smeans for sites 1, 2, and 3. The large error variance for the randomized complete block design analysis at site 3 resulted in small smeans and a shorter range of means at sites 4 and 5. Thus, more weight is given to the data from sites 1 and 2. Similar comparisons may be made for the lowest yielding group of entries. For example, entries 44, 69, and 74 appeared at the bottom for means. The same entries appeared at the bottom or near the bottom for the other three methods of combining results.

Analyses of variance (ANOVAs) were obtained for the four sets of means and are presented in Table 4. The site or environment means squares are relatively large compared to the site by entry (genotype by environment) interaction. This is probably explained by the fact that the three sites differed in planting date as well as location. For the experiment at Tlaltizapan, site 1 was planted in November and site 2 in February and both were controlled for disease. For the third site, Obregon, the experiment was sown in February and had a heavy stem rust infection. The later the sowing date the greater the decrease in grain yield in these environments. Environmental factors influenced by the location and sowing date included temperature, photo-period, and soil physical and chemical properties. Yields generally decline at the higher temperatures, mainly because the growing cycle is truncated. All of the factors are likely to interact with genotype. Since such environmental differences exist, there is the question as to whether some real explainable effect is present and should be utilized in the interpretation of the results.

F-tests could be performed but their relevance in this context is in question. Instead some multiple comparisons procedure like sub-set selection appears more appropriate. If tables of Dunnett's comparison with a control were available for more than 20 entries (See Bechhofer and Dunnett, 1988), one could use this procedure. To illustrate, let us suppose that the tabled value is 3.00 for 120 entries with 238 degrees of freedom for the entry error variance and that a 90% coverage is desired. Then, for the entry with the highest mean over sites 1, 2, and 3, number 90, the interval is computed as

$$1576 - 3.00\,[2(33{,}663)/3]^{1/2} = 1576 - 449.4 = 1126.6$$

Thus all means lower than 1126.6 are considered to be significantly different from entry 90 and all those higher than 1126.6 are not. The site by entry mean square, 33,663, was used as the error term since the sites are considered to be a random sample of sites. Instead of the above, a multiple range test could be utilized for comparing means. For the means from sites 1, 2, and 3, a 95% studentized multiple range test is computed as

$$q_{v=120,\ df=238,\ a=.05}\ (\text{standard error of a mean}) = 6.15\ (33{,}663/3)^{1/2} = 651.5.$$

Owing to the large genotype by environment interaction, a study needs to be made of the results at each of the three sites. The ANOVAs for the three sites are given in Table 5. The F-values indicate large entry differences at each site. The fifteen top yielding entries at each of the three sites are

Site 1: 60, 21, 11, 99, 2, 35, 118, 58, 111, 46, 120, 61, 38, 82, 90
Site 2: 18, 103, 37, 72, 85, 65, 26, 11, 79, 94, 110, 15, 25, 20, 95
Site 3: 90, 29, 31, 4, 93, 94, 108, 62, 96, 91, 8, 43, 32, 33, 11

The fifteen lowest yielding entries at each of the three sites are

Site 1: 66, 5, 107, 55, 42, 56, 28, 17, 6, 51, 52, 43, 44, 81, 50
Site 2: 29, 69, 36, 83, 31, 107, 73, 19, 117, 35, 74, 118, 56, 34, 32
Site 3: 66, 10, 102, 18, 110, 111, 49, 113, 109, 98, 44, 71, 69, 100, 74

There is considerable disagreement in the ranking at the three sites: e.g., genotype 90 ranked fifteenth in site 1 and first in site 3. Entry 29 ranked second in site 3 and 106th in site 2. Entry 110 ranked eleventh in site 2 but 106th in site 3. Many of these genotypes appear to be site-specific for yield.

Variance component estimates for genotype by environment interaction as well as site are often desired. To do this, it is necessary to obtain an estimate of the pooled error mean square. One procedure is to average the error variances at the three sites as

$$(3,449 + 6,088 + 41,604/(r=2))/3 = 10,113.$$

(Note that there were r = 2 replicates of the 120 entries at site 3.) This gives equal weight to each site. Another procedure is to weight the error variances by their degrees of freedom as follows

$$(151,762 + 280,070 + 2,537,822/2)/(44 + 46 + 61) = 11, 263.$$

Owing to the relatively large spatial variations at site 3, and to some extent at site 2, there is some genotype by environment interaction in the residual mean square. The site by entry interaction has an expected value of error variance component plus site by entry variance component. The site by entry variance component for means at sites 1, 2, and 3 would be 33,663 - 11,263 = 22,400. The ratio of the site by entry variance component to the error variance component would be 22,400/11,263 = 1.99. Note that 10,113 may be used in place of 11,263 if equal site weights are desired.

For the ANOVA on smeans for sites 1, 2, and 3, the expected value of the site by entry mean square is 1 + the site by entry variance component divided by the error variance component. Thus only a ratio of the two variance components can be obtained from the ANOVA on smeans. Ratios of variance components to the error variance component are used in measures of genetic advance (See, e.g., Sprague and Federer, 1951). For this example for smeans for sites 1, 2, and 3, this ratio is 2.63 - 1 = 1.63, or roughly equal to that obtained above.

CONCLUSIONS

Statistical difficulties encountered in combining results from experiments conducted at a number of sites may be overcome using either of the two methods described herein. Since the two methods used do not depend upon

(i) homogeneity of error variance,
(ii) using the same checks at every site,
(iii) having the same response model at every site, and/or
(iv) having the same experiment design at every site,

they are recommended for use by analysts when combining groups of experiments with the same entries at every site. If it is desired to give equal weight to each site, then Method 1 should be used. In order to weight responses in relation to their error variance at each site, it is recommended that standardized means, Method 2, be used. Site means with high error variances are given small weights. Method 2 has the property that it is unit free. For example, the grain weights may be measured in grams at one site and pounds at another site. Using standardized means, the need for conversion of measurements to the same scale is unnecessary. Standardized means are unit-free in the same manner as a correlation coefficient, a t statistic, or an F statistic. Standardization in this form may be used to compare measurements on a wet-weight basis with ones on a dry-weight basis, for example.

A large genotype by environment interaction for yield of these 120 wheat genotypes was found. It should be noted that each variable may have its own response model at a given site as they may be affected by different spatial variation patterns during the course of the experiment. A response model should be selected for each characteristic measured in an experiment. For yield, the large difference in variation at each site would indicate that some genotype by environment interaction was occurring at some sites. For site 3, a large difference in replicate means occurred. For site 2, there was considerable variation in yields from one part of the row-column design to another. Since this represents a change in environment, an interaction is possible and suspected.

Apart from the savings on land and management costs, reducing the number of repetitions at a site and increasing the number of sites, improves the efficiency with which certain traits are evaluated by reducing the time it takes to characterize a family of lines. An example would be where trait evaluation is sensitive to changes in environmental conditions such as temperature and radiation intensity, as is the case of canopy temperature measurement (Reynolds *et al.*, 1998).

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Table 1. REML means by sites.

	Site				
Entry1	1	2	3	4	5
1	857	915	2071	2132	2693
2	942	946	2394	2448	2331
3	906	878	2414	2252	2717
4	891	807	2735	3115	2520
5	851	919	1896	1503	1233
6	836	881	2372	3103	2540
7	877	917	2308	2293	2332
8	891	924	2579	2522	2496
9	904	983	1852	1956	1265
10	881	985	1756	2139	1205
11	947	1036	2497	2472	2578
12	920	1051	2247	2653	1658
13	913	1031	2127	2599	1890
14	896	861	1918	2191	1999
15	883	1032	1836	1912	1134
16	865	1032	1988	2311	1578
17	839	1041	2254	2503	1624
18	921	1128	1739	1867	1262
19	875	842	2295	2108	2427
20	882	1030	2207	2814	1612
21	951	882	2118	2606	1800
22	872	959	2363	3011	1639
23	851	905	2375	2877	2506
24	902	1021	1878	1832	1983
25	887	1030	2116	2255	1889
26	875	1070	2022	1932	2057
27	883	938	2166	2237	2411
28	846	854	1841	1863	2620
29	863	845	2849	3053	3156
30	886	925	2316	2349	1928
31	871	801	2744	2988	2999
32	896	728	2508	2920	2631
33	920	873	2502	2694	2639
34	868	697	2311	2760	2465
35	937	800	2188	1790	2006
36	921	811	1970	2435	1236
37	909	1017	1871	1878	1432
38	927	930	1898	2103	1099
39	924	914	2071	2575	1429
40	872	808	2158	2994	1785
41	883	923	2016	2442	1948
42	847	1029	2138	2070	1433
43	808	965	2564	3718	2458
44	808	824	1603	1670	895
45	920	919	2132	2280	2266
46	932	973	2071	2262	1905
47	881	997	1854	1724	1919
48	922	901	1778	1955	1880
49	904	989	1707	1977	1099
50	796	888	1841	1901	1068

1	51	830	978	1930	2082	1746
2	52	813	939	1980	2231	1800
3	53	913	961	2351	2028	3028
4	54	878	957	2378	3246	2424
5	55	847	959	1847	2074	1591
6	56	846	717	2195	2386	2920
7	57	902	958	1894	2323	1679
8	58	937	916	2320	3009	1621
9	59	910	953	2116	2437	1744
10	60	974	1011	1997	1669	1843
11	61	931	855	2139	2498	2093
12	62	860	825	2637	3433	2194
13	63	879	1006	1976	2265	2058
14	64	872	881	1992	2380	1192
15	65	858	1019	1778	1456	1572
16	66	852	856	1774	1668	1126
17	67	908	953	2000	2552	1189
18	68	908	905	2188	2555	1527
19	69	889	859	1562	1386	1115
20	70	860	895	1946	2508	1271
21	71	853	970	1590	1892	1174
22	72	907	1017	1834	1976	1545
23	73	859	785	2039	2151	1909
24	74	866	688	1509	1640	1344
25	75	880	779	1995	2296	1844
26	76	899	975	1852	1903	1417
27	77	876	915	2073	2037	1523
28	78	877	962	2125	2243	1819
29	79	863	1026	2113	2651	1496
30	80	859	974	1967	2337	1744
31	81	802	978	2218	2122	1934
32	82	927	959	1981	2268	2076
33	83	896	800	1919	2139	2571
34	84	909	947	1891	1849	1049
35	85	899	1033	1949	1762	1866
36	86	907	987	1921	1771	1885
37	87	887	988	2285	2384	2359
38	88	886	919	1736	1938	1488
39	89	894	936	2493	2426	2051
40	90	926	901	2901	3158	3301
41	91	921	937	2596	2443	2232
42	92	918	992	2106	2718	1283
43	93	903	986	2663	2722	2670
44	94	872	1025	2656	2427	2297
45	95	910	1061	2301	1868	2526
46	96	880	948	2602	2569	2763
47	97	873	971	1791	2390	744
48	98	902	952	1612	1817	799
49	99	944	994	2117	2661	1712
50	100	876	956	1553	1999	1417
51	101	919	977	1955	2320	1558
52	102	891	1075	1745	2220	1615
53	103	876	1099	2014	2372	1447
54	104	867	985	1963	2761	1863
55	105	869	922	2283	2771	2314

1	106	893	963	2396	2393	2260
2	107	848	853	1789	2302	1268
3	108	921	1016	2643	2578	3504
4	109	912	961	1613	2158	1558
5	110	891	1038	1724	1992	1021
6	111	934	922	1711	1797	938
7	112	857	971	1832	1688	1373
8	113	869	919	1628	2127	1525
9	114	906	989	2268	2506	2619
10	115	873	960	2287	2384	2592
11	116	890	922	2068	1938	1962
12	117	890	828	2052	2730	2123
13	118	937	762	2124	2416	1724
14	119	896	975	2005	2198	1370
15	120	932	988	2318	2687	2517

Table 2. REML means divided by site means standard deviation.

	Site				
Entry	1	2	3	4	5
1	28.50	18.49	13.45	3.90	4.93
2	31.34	19.11	15.55	4.48	4.26
3	30.13	17.74	15.68	4.12	4.97
4	29.65	16.30	17.76	5.70	4.61
5	28.32	18.57	12.31	2.75	2.26
6	27.80	17.80	15.40	5.68	4.65
7	29.19	18.53	14.99	4.19	4.27
8	29.63	18.66	16.75	4.61	4.57
9	30.06	19.85	12.03	3.58	2.31
10	29.30	19.89	11.40	3.91	2.20
11	31.49	20.93	16.21	4.52	4.72
12	30.59	21.24	14.59	4.85	3.03
13	30.37	20.83	13.81	4.75	3.46
14	29.81	17.39	12.45	4.01	3.66
15	29.38	20.84	11.92	3.50	2.07
16	28.76	20.85	12.91	4.23	2.89
17	27.90	21.02	14.64	4.58	2.97
18	30.65	22.78	11.29	3.42	2.31
19	29.10	17.00	14.90	3.86	4.44
20	29.35	20.82	14.33	5.15	2.95
21	31.62	17.82	13.76	4.77	3.29
22	29.01	19.38	15.34	5.51	3.00
23	28.31	18.28	15.42	5.26	4.58
24	30.01	20.62	12.19	3.35	3.63
25	29.52	20.81	13.74	4.13	3.46
26	29.09	21.61	13.13	3.53	3.76
27	29.36	18.95	14.06	4.09	4.41
28	28.15	17.25	11.95	3.41	4.79
29	28.70	17.06	18.50	5.59	5.77
30	29.47	18.69	15.04	4.30	3.53
31	28.98	16.19	17.82	5.47	5.49
32	29.81	14.70	16.29	5.34	4.81
33	30.59	17.64	16.25	4.93	4.83
34	28.89	14.07	15.003	5.05	4.51

1	35	31.18	16.16	14.21	3.27	3.67
2	36	30.63	16.39	12.79	4.45	2.26
3	37	30.24	20.55	12.15	3.44	2.62
4	38	30.84	18.78	12.33	3.85	2.01
5	39	30.73	18.46	13.45	4.71	2.61
6	40	29.01	16.32	14.01	5.48	3.27
7	41	29.38	18.65	13.09	4.47	3.56
8	42	28.18	20.79	13.88	3.79	2.62
9	43	26.87	19.49	16.65	6.80	4.50
10	44	26.87	16.65	10.41	3.06	1.64
11	45	30.59	18.56	13.84	4.17	4.15
12	46	31.01	19.66	13.44	4.14	3.48
13	47	29.32	20.15	12.04	3.15	3.51
14	48	30.67	18.19	11.55	3.58	3.44
15	49	30.06	19.99	11.08	3.62	2.01
16	50	26.49	17.94	11.96	3.48	1.95
17	51	27.61	19.75	12.53	3.81	3.19
18	52	27.04	18.97	12.86	4.08	3.29
19	53	30.36	19.42	15.26	3.71	5.54
20	54	29.20	19.34	15.44	5.94	4.43
21	55	28.18	19.38	11.99	3.79	2.91
22	56	28.16	14.49	14.25	4.36	5.34
23	57	30.00	19.34	12.30	4.25	3.07
24	58	31.16	18.50	15.07	5.50	2.97
25	59	30.27	19.24	13.74	4.46	3.19
26	60	32.41	20.42	12.97	3.05	3.37
27	61	30.96	17.26	13.89	4.57	3.83
28	62	28.62	16.67	17.12	6.28	4.01
29	63	29.26	20.33	12.83	4.14	3.76
30	64	29.02	17.80	12.94	4.35	2.18
31	65	28.53	20.59	11.55	2.66	2.88
32	66	28.33	17.30	11.52	3.05	2.06
33	67	30.19	19.26	12.99	4.67	2.18
34	68	30.21	18.27	14.21	4.67	2.79
35	69	29.57	17.34	10.14	2.54	2.04
36	70	28.61	18.08	12.64	4.59	2.33
37	71	28.39	19.59	10.32	3.46	2.15
38	72	30.17	20.55	11.91	3.61	2.83
39	73	28.57	15.85	13.24	3.94	3.49
40	74	28.82	13.91	9.80	3.00	2.46
41	75	29.27	15.74	12.95	4.20	3.37
42	76	29.90	19.70	12.02	3.48	2.59
43	77	29.16	18.49	13.46	3.73	2.79
44	78	29.16	19.43	13.80	4.10	3.33
45	79	28.72	20.73	13.72	4.85	2.74
46	80	28.59	19.69	12.77	4.28	3.19
47	81	26.68	19.76	14.40	3.88	3.54
48	82	30.83	19.38	12.86	4.15	3.80
49	83	29.81	16.15	12.46	3.91	4.70
50	84	30.23	19.13	12.28	3.38	1.92
51	85	29.89	20.87	12.65	3.22	3.41
52	86	30.18	19.93	12.47	3.24	3.45
53	87	29.52	19.96	14.84	4.36	4.32
54	88	29.47	18.57	11.28	3.55	2.72
55	89	29.75	18.91	16.19	4.44	3.75

1	90	30.80	18.20	18.84	5.78	6.04
2	91	30.63	18.92	16.86	4.47	4.08
3	92	30.53	20.04	13.67	4.97	2.35
4	93	30.05	19.91	17.29	4.98	4.88
5	94	29.00	20.70	17.25	4.44	4.20
6	95	30.28	21.43	14.94	3.42	4.62
7	96	29.27	19.15	16.90	4.70	5.05
8	97	29.03	19.61	11.63	4.37	1.36
9	98	30.00	19.23	10.47	3.32	1.46
10	99	31.42	20.08	13.75	4.87	3.13
11	100	29.14	19.32	10.08	3.66	2.59
12	101	30.58	19.73	12.69	4.24	2.85
13	102	29.65	21.71	11.33	4.06	2.95
14	103	29.14	22.19	13.08	4.34	2.65
15	104	28.84	19.89	12.74	5.05	3.41
16	105	28.90	18.64	14.82	5.07	4.23
17	106	29.71	19.46	15.56	4.38	4.13
18	107	28.20	17.23	11.62	4.21	2.32
19	108	30.65	20.53	17.16	4.72	6.41
20	109	30.34	19.42	10.47	3.95	2.85
21	110	29.65	20.98	11.19	3.64	1.87
22	111	31.07	18.62	11.11	3.29	1.72
23	112	28.51	19.62	11.89	3.09	2.51
24	113	28.92	18.56	10.57	3.89	2.79
25	114	30.15	19.97	14.73	4.58	4.79
26	115	29.04	19.40	14.85	4.36	4.74
27	116	29.61	18.63	13.43	3.55	3.59
28	117	29.60	16.74	13.33	4.99	3.88
29	118	31.17	15.39	13.80	4.42	3.15
30	119	29.81	19.70	13.02	4.02	2.51
31	120	31.00	19.96	15.05	4.92	4.60
32						
33						
34						

35 Table 3. Ranked REML means (mean) and standardized means (smean) over sites.

36

37	Sites 1, 2, 3				Sites 1, 2, 4, 5			
38	Entry	mean	Entry	smean	Entry	mean	Entry	smean
39	90	1576	11	22.87	90	2072	108	15.58
40	108	1526	108	22.78	108	2005	11	15.42
41	29	1519	90	22.61	43	1987	90	15.21
42	94	1518	93	22.42	29	1979	120	15.12
43	93	1517	94	22.32	31	1915	93	14.96
44	11	1493	95	22.22	54	1876	95	14.94
45	91	1484	12	22.14	6	1840	12	14.93
46	4	1478	91	22.14	4	1833	99	14.88
47	96	1477	120	22.00	62	1828	114	14.87
48	31	1472	2	22.00	93	1820	13	14.85
49	8	1465	60	21.93	32	1794	60	14.81
50	43	1446	96	21.77	96	1790	2	14.80
51	89	1441	99	21.75	23	1785	18	14.79
52	62	1441	53	21.68	33	1782	53	14.76
53	33	1432	8	21.68	120	1781	54	14.73
54	2	1427	13	21.67	11	1758	102	14.59
55	95	1424	89	21.62	114	1755	94	14.59

1	106	1417	114	21.62	53	1733	103	14.58
2	120	1413	106	21.58	105	1719	46	14.57
3	53	1408	58	21.58	56	1717	20	14.57
4	12	1406	18	21.57	8	1708	96	14.54
5	54	1404	20	21.50	115	1702	87	14.54
6	3	1399	33	21.49	34	1698	82	14.54
7	22	1398	103	21.47	3	1688	58	14.53
8	58	1391	87	21.44	2	1667	91	14.53
9	114	1388	29	21.42	94	1655	26	14.50
10	87	1387	92	21.41	87	1655	33	14.50
11	17	1378	46	21.37	1	1650	25	14.48
12	32	1377	25	21.36	117	1643	92	14.47
13	23	1377	54	21.33	91	1633	106	14.42
14	30	1376	26	21.28	106	1627	43	14.42
15	115	1373	22	21.24	58	1621	24	14.40
16	20	1373	4	21.24	22	1620	115	14.39
17	7	1367	17	21.19	104	1619	21	14.38
18	6	1363	3	21.18	27	1617	63	14.37
19	105	1358	85	21.14	40	1615	8	14.37
20	13	1357	115	21.10	13	1608	45	14.37
21	99	1352	59	21.08	7	1605	101	14.35
22	25	1344	21	21.07	83	1602	85	14.35
23	92	1339	30	21.07	45	1596	104	14.30
24	42	1338	79	21.06	61	1594	59	14.29
25	19	1337	82	21.02	95	1591	72	14.29
26	79	1334	43	21.00	20	1585	29	14.28
27	68	1334	101	21.00	99	1578	79	14.26
28	81	1333	31	21.00	89	1577	3	14.24
29	103	1330	45	21.00	12	1571	22	14.23
30	27	1329	37	20.98	19	1563	37	14.21
31	60	1328	42	20.95	21	1560	89	14.21
32	59	1326	24	20.94	82	1558	105	14.21
33	46	1325	7	20.90	63	1552	27	14.20
34	45	1324	68	20.90	41	1549	86	14.20
35	26	1322	102	20.90	28	1546	16	14.18
36	78	1321	39	20.88	30	1522	57	14.17
37	21	1317	72	20.88	46	1518	61	14.16
38	35	1309	86	20.86	25	1515	109	14.14
39	61	1308	119	20.84	59	1511	39	14.13
40	39	1303	16	20.84	79	1509	17	14.12
41	16	1295	67	20.81	17	1502	23	14.11
42	85	1294	63	20.81	14	1487	67	14.08
43	116	1293	62	20.80	26	1484	4	14.07
44	34	1292	78	20.80	80	1479	7	14.05
45	119	1292	27	20.79	92	1478	110	14.04
46	82	1289	105	20.79	78	1475	31	14.03
47	77	1288	15	20.71	68	1474	47	14.03
48	63	1287	61	20.70	57	1466	41	14.02
49	67	1287	23	20.67	39	1461	119	14.01
50	101	1284	38	20.65	118	1460	78	14.01
51	1	1281	9	20.64	81	1459	30	14.00
52	40	1279	110	20.61	102	1450	68	13.99
53	118	1275	116	20.56	75	1450	6	13.98
54	41	1274	57	20.55	103	1449	48	13.97
55	86	1272	84	20.55	16	1447	1	13.96

1	104	1272	76	20.54	52	1446	9	13.95
2	24	1267	35	20.52	101	1444	15	13.95
3	80	1267	47	20.50	24	1435	80	13.94
4	37	1266	104	20.49	116	1428	49	13.92
5	18	1263	49	20.38	73	1426	76	13.92
6	117	1257	41	20.37	48	1415	62	13.90
7	56	1253	77	20.37	51	1409	38	13.87
8	72	1253	80	20.35	67	1401	42	13.85
9	38	1252	6	20.33	109	1397	116	13.85
10	57	1251	19	20.33	85	1390	10	13.83
11	15	1250	81	20.28	86	1388	117	13.80
12	84	1249	32	20.27	70	1384	14	13.72
13	64	1248	111	20.27	35	1383	100	13.68
14	9	1246	65	20.22	47	1380	111	13.68
15	51	1246	10	20.20	60	1374	65	13.67
16	47	1244	1	20.15	55	1368	84	13.67
17	52	1244	48	20.14	72	1361	32	13.67
18	76	1242	118	20.12	113	1360	83	13.64
19	102	1237	97	20.09	119	1360	19	13.60
20	36	1234	109	20.08	36	1351	97	13.59
21	70	1234	112	20.01	42	1345	51	13.59
22	73	1228	51	19.96	77	1338	88	13.58
23	14	1225	36	19.94	64	1331	35	13.57
24	5	1222	64	19.92	107	1318	55	13.57
25	112	1220	98	19.90	100	1312	77	13.54
26	65	1218	117	19.89	37	1309	113	13.54
27	75	1218	14	19.88	88	1308	118	13.53
28	55	1218	55	19.85	10	1303	40	13.52
29	110	1218	40	19.78	76	1299	98	13.50
30	97	1212	70	19.78	18	1295	81	13.47
31	10	1207	88	19.77	9	1277	36	13.43
32	83	1205	5	19.73	38	1265	112	13.43
33	48	1200	52	19.62	97	1245	70	13.40
34	49	1200	100	19.51	49	1242	28	13.40
35	111	1189	83	19.47	15	1240	71	13.40
36	28	1180	71	19.43	110	1236	52	13.35
37	88	1180	113	19.35	65	1226	64	13.34
38	50	1175	34	19.32	112	1222	75	13.15
39	107	1163	75	19.32	71	1222	34	13.13
40	109	1162	73	19.22	84	1189	56	13.09
41	66	1161	28	19.12	50	1163	107	12.99
42	98	1155	66	19.05	111	1148	5	12.98
43	113	1139	69	19.02	74	1135	73	12.96
44	71	1138	107	19.02	5	1127	69	12.87
45	100	1128	56	18.97	66	1126	66	12.69
46	69	1103	50	18.80	98	1118	50	12.47
47	44	1078	44	17.98	69	1062	44	12.06
48	74	1021	74	17.51	44	1049	74	12.05

Table 4. ANOVAs for means and standardized means of sites 1, 2, and 3 and for means and standardized means of sites 1, 2, 4, and 5.

Sites 1, 2, 3 means

Source of variation	Degrees of freedom	Mean square
Site	2	55,935,666
Entry	119	32,063
Site by entry	238	33,663

Sites 1, 2, 3 standardized means

Source of variation	Degrees of freedom	Mean squares
Site	2	7,905
Entry	119	2.71
Site by entry	238	2.63
Error	infinity	1

Sites 1, 2, 4, and 5 means

Source of variation	Degrees of freedom	Mean square
Site	3	59,627,911
Entry	119	183,680
Site by entry	357	114,771

Sites 1, 2, 4, and 5 standardized means

Source of variation	Degrees of freedom	Mean square
Site	3	18,907
Entry	119	1.54
Site by entry	357	1.44
Error	infinity	1

Table 5. Individual ANOVAs for sites 1, 2, and 3.

Site 1

Source of variation	Degrees of freedom	Mean square
Entry	121	8,411
C1	1	12,953
C2	1	48,712
C3	1	42,867
C4	1	22,613
C6	1	31,220
C8	1	77,300
R1	1	28,678
R2	1	12,832
R4	1	4,993
R8	1	20,170
R10	1	15,068
C1*R1	1	52,885
C2*R1	1	24,977
C3*R1	1	7,998
Residual (error)	44	3,449

Site2

Source of variation	Degrees of freedom	Mean square
Entry	121	20,306
C1	1	37,044
C4	1	56,069
C10	1	24,484
R2	1	108,367
C1*R1	1	218,001
C1*R3	1	30,779
C2*R2	1	17,944
C2*R4	1	46,852
C3*R2	1	27,722
C3*R4	1	58,389
C4*R3	1	19,184
C4*R4	1	33,627
Residual (error)	46	6,088

Site 3

Source of variation	Degrees of freedom	Mean square
Replicate	1	11,149,108
Entry	119	212,224
Block(replicate)	28	427,339
C1*block(replicate)	30	74,336
Residual (error)	61	41,604