INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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to the
General Introduction to the Examination
of Distinctness, Uniformity and Stability and the
Development of Harmonized Descriptions of New Varieties of Plants (document TG/1/3)

DOCUMENT TGP/8

"USE OF STATISTICAL PROCEDURES IN

DISTINCTNESS, UNIFORMITY AND STABILITY TESTING"

Section TGP/8.5: Statistical Methods for DUS Examination

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SECTION 8.5

STATISTICAL METHODS FOR DUS EXAMINATION

8.5.1   ANALYSIS OF VARIANCE

1.   The analysis of variance (anova) of data from a designed experiment has two purposes. Firstly it subdivides the data's total variation into separate components with each component representing a different source of variation, so that the relative importance of the different sources can be assessed. Secondly it provides an estimate of the random variation in the data. This may be used as an estimate of precision when comparing means calculated from the data.

2.   Anova can take many forms. Just two forms will be considered in detail here. These are the two forms which arise as part of the statistical techniques recommended by UPOV. At their simplest, both operate on an $n \times m$ table of data. They are the :-

–   Two-way anova, e.g. as used in the analysis of variety-by-year means for v varieties grown in each of y years for some characteristic in the Combined Over Years Distinctness (COYD) criterion.

–   One-way anova, e.g. as used in the analysis of variety-by-year adjusted log(SD+1)'s (a measure of uniformity) for v reference varieties grown in each of y years for some characteristic in the Combined Over Years Uniformity (COYU) criterion.

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3.   The particular form an anova takes depends on the origins of the data. This determines the model for the data, i.e. what factors are likely to cause the data to vary, which in turn determines what components the total variation is divided into, and hence the form of the anova.

Two-way anova

The data model

4.   In two-way anova, the $n \times m$ table of data corresponds to nm data values classified by two factors: Factor 1 with m levels and Factor 2 with n levels. For example (Example A), each data value might be the mean over all plants in a plot for a characteristic from a trial with vb plots laid out in b blocks (Factor 1) of v varieties (Factor 2).

Example A: data from a trial with *v* varieties and *b* blocks

mean of all plants in the plot in block *b* with variety 2

	Block 1	Block 2	Block 3	…	Block *b*
Variety 1	-	-	-	…	-
Variety 2	-	-	-	…	-
Variety 3	-	-	-	…	-
…	…	…	…	…	…
Variety *v*	-	-	-	…	-

vb data values

mean of all plants in the plot in block 2 with variety 3

5.   Or, for the above COYD example (Example B), the data values might consist of the *vy* variety-by-year means for *v* varieties (Factor 2) grown in each of *y* years (Factor 1).

Example B: data for COYD example with *v* varieties grown in *y* years

variety-by-year mean for variety *v* in year 2

6.   If *x* represents one of the *nm* data values in the *n×m* table of data, the model explaining the variation in the data is as follows :-

$$x = \text{Factor 1 effect} + \text{Factor 2 effect} + \left(\begin{array}{c} \text{Factor 1 5 Factor 2} \\ \text{interaction effect} \end{array} + \text{random variation} \right)$$

Thus each of the *nm* data values is made up of a sum of effects. The "Factor 1 effect" and "Factor 2 effect" are due to the particular levels of Factors 1 and 2 influencing the data value. An interaction between two factors is when the effects of one factor differ, i.e. are inconsistent, from level to level of the other factor. So a Factor 1×Factor 2 interaction is when Factor 1 effects differ from level to level of Factor 2. In the above model the remainder, or residual, of the data value is the amount, additional to its Factor 1 effect and its Factor 2 effect, which appears to be due to its particular combination of Factor 1 and 2 levels. This amount might be partly due to a genuine interaction or it might be just due to random variation. As there is just a single data value for each combination of Factor 1 and 2 levels it is not possible to determine which.

7.   For Example A, the model explaining the variation in the data is as follows :

$$x = \text{block effect} + \text{variety effect} + \left(\begin{array}{c} \text{variety 5 block} \\ \text{interaction effect} \end{array} + \text{random variation} \right)$$

In this example each of the *vb* data values (one from each of the *vb* plots) is made up of the sum of a "block effect", due to the block the plot is in, plus a "variety effect", due to the variety sown on the plot, plus a remainder. This remainder, or residual represents the amount that is additional to the variety and block effects. As there is just a single data value for each variety in each block, it is not possible to separate interaction effects and random variation. However, as the variety effects are not expected to differ from block to block, or in other words any variety×block interaction is expected to be negligible, the residual is likely to be due to random variation.

8.   For Example B (COYD), the model explaining the variation in the data is as follows :-

$$x = \text{year effect} + \text{variety effect} + \left(\begin{array}{l} \text{var}\textit{iety} \times \textit{year} \\ \text{int}\,\textit{eraction effect} \end{array} + \text{random variation} \right)$$

Here each of the *vy* variety-by-year means is made up of a sum of effects. The "year effect" is an amount due to the year the variety-by-year mean was recorded in. The year effects might or might not be the same for all years. The "variety effect" is an amount due to the variety of the variety-by-year mean, and might or might not be the same for all varieties. The remainder, or residual of the variety-by-year mean represents the amount that is additional to its variety effect and its year effect, which appears to be due to that particular variety in that particular year. This amount may be partly due to a genuine variety×year interaction effect or it may be just due to random variation caused by the means having been calculated from different plants grown on different plots, and possibly due to measurement error. As there is just a single variety-by-year mean for each variety in each year it is not possible to distinguish between interaction effects and random variation.

The two-way analysis of variance table

9.   Two-way anova produces a table as follows:-

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio
Factor 1	m - 1	-	-	-
Factor 2	n - 1	-	-	-
Residual	$(n - 1)(m - 1)$	-	-	
Total	nm - 1	-		

[As the anova computations are likely to be done by computer, details are not given here. The interested reader can find them in a good statistical book such as Mead and Curnow (1983).]

10.   For Example A, the two-way anova table is as follows:-

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio
Block	b - 1	-	-	-
Variety	v - 1	-	-	-
Residual	$(b - 1)(v - 1)$	-	-	
Total	vb - 1	-		

11.   For Example B (COYD), the two-way anova table is as follows:-

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio
Year	y - 1	-	-	-
Variety	v - 1	-	-	-
Residual	$(y - 1)(v - 1)$	-	-	
Total	vy - 1	-		

12.   The total variation in the data is measured by the Total Sum of Squares, which is the sum of the squared deviations of all the data from their mean, i.e. $\sum(x - \bar{x})^2$. It is subdivided into "sums of squares" representing the three component sources of variation included in the data model: variation due to Factor 1, variation due to Factor 2 and residual variation. These sums of squares are divided by their degrees of freedom (df) to give "mean squares", which can be directly compared in order to assess the relative magnitude of the different sources of variation. This is done in the final column where the F-ratio's are the ratios of each of the Factor 1 and Factor 2 mean squares to the residual mean square. Providing the assumptions discussed below about the data are valid, comparison of these F-ratio's with F tables on the df of the numerator and the denominator mean squares will provide "F-test's" of the significance of the variation due to each of Factors 1 and 2.

13.   The residual mean square is a variance. It estimates the combined variation due to any Factor 1×Factor 2 interaction and random variation. Hence, it is often referred to as the "Factor 1-by-Factor 2 mean square", e.g. the "variety-by-block mean square" in Example A or the "variety-by-year mean square" in the COYD example (Example B).

14.   Statistical theory shows that in two-way anova it is appropriate to use the residual mean square to estimate the variance or standard errors of means calculated from the data. This is the case whether the Factor 1×Factor 2 interaction is assumed to be negligible, such as in Example A, or not, such as in the COYD example (Example B).

15.   A worked example of two-way anova is given in Appendix A1.

One-way anova

The data model

16.   In one-way anova, the $n×m$ table of data corresponds to data classified into m groups by a single factor of interest such that there are n independent replicates within each group. For example (Example C), each data value might be the mean over all plants in a plot for a characteristic from a trial with tr plots laid out with r replicate plots of each of t treatments (the factor of interest).

Example C: data from a trial with *r* replicate plots of *t* treatments

	Treat' 1	Treat' 2	Treat' 3	…	Treat' *t*
Replicate 1	-	-	-	…	-
Replicate 2	-	-	-	…	-
Replicate 3	-	-	-	…	-
…	…	…	…	…	…
Replicate *r*	-	-	-	…	-

mean of all plants in replicate 2 plot of treatment *t*

tr data values

mean of all plants in replicate *r* plot of treatment 2

17.   Alternatively, as in the above COYU example (Example D), they might consist of variety-by-year adjusted log(SD+1)'s for *v* reference varieties (replicates) grown in each of *y* years (the factor of interest).

18.   It might surprise the reader to see the adjusted log(SD+1)'s of the *v* varieties within a year regarded just as replicates, and not as a second factor like in two-way anova. Year is included as a factor in the anova because the overall levels of uniformity, as measured by the adjusted log(SD+1)'s, can be expected to vary from year to year. However, regarding the uniformity values of the *v* varieties within a year as replicates allows the variation between them to be used as an estimate of the random variation in uniformity between the reference varieties, which are all considered to be uniform. It will be seen in the following that this estimate of the random variation in uniformity among the (uniform) reference varieties is used to compare the uniformity of a candidate variety with the mean uniformity of the (uniform) reference varieties. This is done in order to see whether the uniformity of the candidate variety is extreme relative to the uniformity of the reference varieties.

Example D: data for COYU example with *v* varieties grown in *y* years

	Year 1	Year 2	Year 3	…	Year *y*
Variety 1	-	-	-	…	-
Variety 2	-	-	-	…	-
Variety 3	-	-	-	…	-
…	…	…	…	…	…
Variety *v*	-	-	-	…	-

variety-by-year adjusted log(SD+1) for variety 2 in year 3

vy data values

variety-by-year adjusted log(SD+1) for variety *v* in year 2

19.   If *x* represents one of the *nm* data values in the *n×m* table of data, the model explaining the variation in the data is as follows :-

$$x = \text{Factor effect} + \text{random variation}$$

Thus each of the *nm* data values is made up of the sum of a "Factor effect", which is due to the particular level of the factor influencing the data value, plus a residual amount which is

random variation. This means that the variation between data values within a group is considered to be random variation.

20. For Example C, the model explaining the variation in the data is as follows :-
$$x = \text{treatment effect} + \text{random variation}$$

21. For Example D (COYU), the model explaining the variation in the data is as follows :-
$$x = \text{year effect} + \text{random variation}$$

The model can be interpreted as recognising that the vy variety-by-year adjusted log(SD+1)'s are likely to vary from year to year, and that uniformity is expected to vary at random from variety to variety within a year.

The one-way analysis of variance table

22. One-way anova produces a table as follows:-

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio
Factor	$m - 1$	-	-	-
Residual	$m(n - 1)$	-	-	
Total	$nm - 1$	-		

[Again, details of the anova computations are not given here but may be found in Mead and Curnow (1983).]

23. For Example C, the one-way anova table is as follows:-

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio
Treatment	$t - 1$	-	-	-
Residual	$t(r - 1)$	-	-	
Total	$tr - 1$	-		

24. For Example D (COYU), the one-way anova table is as follows:-

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio
Year	$y - 1$	-	-	-
Residual	$y(v - 1)$	-	-	
Total	$vy - 1$	-		

25. The total variation in the data is subdivided into "sums of squares" representing the two component sources of variation in the data model, i.e. variation due to the factor of interest

and residual or random variation. The sums of squares are divided by their degrees of freedom (df) to give directly comparable "mean squares" used to compare the two sources of variation. This is done in the final column where the F-ratio is the ratio of the factor mean square to the residual mean square. Providing the assumptions discussed below about the data are valid, comparison of the F-ratio with F tables on $m - 1$ and $m(n - 1)$ df provides an "F-test" of the significance of the variation due to the factor of interest.

26.   The residual mean square is a measure pooled over groups of the variation in the data from replicate to replicate within a group. Thus it is a variance and estimates the random variation in the $n{\times}m$ table of data that has been analysed. Consequently, it can be used to estimate the variance or standard errors of means calculated from the data.

27.   A worked example of one-way anova is given in Appendix A2.

Assumptions about the data

28.   Two assumptions are necessary for one-way and two-way anova. They are:-

–   That the variability of the data in the $n{\times}m$ table of data is the same for the different levels of the classifying factors. Thus it is assumed that the variability of the vy variety-by-year means is the same for all varieties and for all years in the two-way anova COYD example (Example B). In the one-way anova COYU example (Example D) it is assumed that the variation between the adjusted log(SD+1)'s of the different varieties in a year is the same from year to year and is the same for all varieties.

–   That the model describes the data adequately in that the effects of the classifying factors are additive. For example (Example A), it is assumed that the expected difference in the data values for two varieties is the same in one block as it is in any other block.

Failure of this assumption will lead to large residuals, as the residual is the part of a data value which is not explained by the additive factor effects. This in turn will lead to a large residual mean square, which will give large standard errors of means, and so large differences will be required between factor means in order for them to be declared significant.

An example of such a failure would when a variety×year interaction occurs, i.e. when the variety effects are inconsistent over years, in the two-way anova COYD example (Example B). Here only large differences between varieties would be declared significant.

29.   For F-tests of ratios of anova mean squares and t-tests based on anova mean squares (or the equivalent use of LSD's based on anova mean squares) it is also necessary to assume that the data values are independent and that the random variation in the data has an approximately Normal distribution.

The precision of means and the differences between means

30.   Let $\bar{x}_1$ and $\bar{x}_2$ be factor means of r_1 and r_2 data values from the $n{\times}m$ table of data that has been analysed by anova (or from equivalent data). The precision of a mean $\bar{x}_i$, where $i =$

1 or 2, is measured by its standard error ($\text{SE}(\bar{x}_i)$), which is estimated by

$$\text{SE}(\bar{x}_i) = \sqrt{\frac{RMS}{r_i}}$$

Where *RMS* is the residual mean square from the anova of the $n \times m$ table of data. The precision of the difference in two means, $\bar{x}_1 - \bar{x}_2$ is measured by its standard error, ($\text{SE}(\bar{x}_1 - \bar{x}_2)$), which is estimated by

$$\text{SE}(\bar{x}_1 - \bar{x}_2) = \sqrt{RMS\left(\frac{1}{r_1} + \frac{1}{r_2}\right)}$$

Comparisons of means

31.   The significance of the difference between $\bar{x}_1$ and $\bar{x}_2$ can be tested by either:-

–   Comparing the t-statistic $t = \dfrac{\bar{x}_1 - \bar{x}_2}{\text{SE}(\bar{x}_1 - \bar{x}_2)}$ with Student's t-tables on the df of the

RMS in either a one-tailed test, if it is known *apriori* which mean will be larger, or a two-tailed test otherwise.

–   Comparing the absolute difference between the means, $\left|\bar{x}_1 - \bar{x}_2\right|$, with the $100 \times p\%$

least significant difference (LSD), i.e. comparing
$$\left|\bar{x}_1 - \bar{x}_2\right| \text{ with } t \times \text{SE}(\bar{x}_1 - \bar{x}_2)$$

where *t* is the $100 \times p\%$ critical value from Student's t-tables on the df of the *RMS*. The critical value should be the one-tailed value if it is known *apriori* which mean will be larger, and two-tailed otherwise.

100 × p%

0

one-tailed critical value

100 × p/2 %   **100 × p/2 %**

0

two-tailed critical value
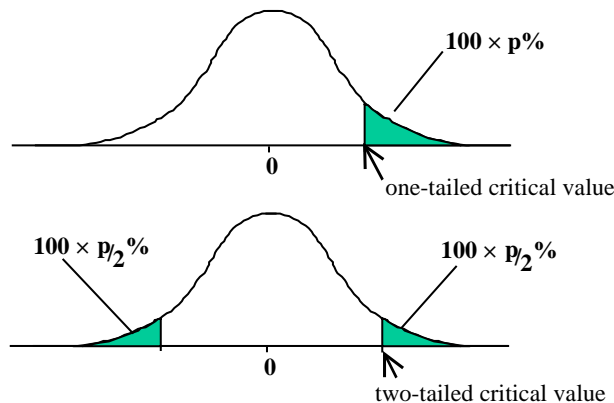
Higher-order anova's

32.   Anova has been introduced as the subdivision of the total variation among the data values in an $n \times m$ table of data such that it :-

–   allows a comparison of the different sources of variation

–   provides an estimate of the random variation affecting the *nm* data values

The $n \times m$ table of data may consist of means calculated from a higher order table of data such as an $l \times n \times m$ table of data or a $k \times l \times n \times m$ table of data. For example, in the above two-way anova COYD example (Example B) the $v \times y$ table of data are variety-by-year means for *v* varieties in each of *y* years and these can be viewed as having been calculated from an $l \times v \times y$

table of plot means from trials with *l* blocks and *v* varieties in each of *y* years. Alternatively they can be viewed as having been calculated from a *k×l×v×y* table of measurements on *k* plants in each plot of trials with *l* blocks and *v* varieties in each of *y* years.

33.   If the *n×m* table of data takes the form of means calculated from a higher order table, the experimenter can analyse the data using a multi-way anova that is a logical extension of the two-way anova. In this the total variation is subdivided into components for each of the factors classifying the data table plus components for two-way, three-way and all higher order interactions between the factors. As with the two-way anova, the components of variation can be compared using ratios of mean squares. Also the residual mean square is a variance which estimates the random variation at the level of the data values in the table of data that has been analysed.

34.   Given data values in a more-than-two-way table of data, the experimenter has the choice of analysing it by multi-way anova or by calculating an *n×m* table of means and using two-way anova. If the data values in the *n×m* table are the means of *r* of the original data values, then the mean squares in the multi-way anova are *r* times the size of the equivalent mean squares in the two-way anova. Thus whichever approach is used, the relative sizes of the mean squares are the same and the variances estimated by the residual mean square in the two-way anova can be derived from the mean squares in the higher order anova. However, it is important for the purposes of COYD that the variety means are compared using variances or standard errors based on the variety-by-year mean square as an estimate of random variation, such as is provided by the residual mean square in two-way anova.

Unbalanced data and the method of Fitting Constants

35.   An *n×m* table of data that has a data value present in each of the *nm* table cells is balanced. If data values are missing from one or more cells it is unbalanced or incomplete.

36.   Although the data for one-way anova was introduced for simplicity as having equal (*n*) replication in each of the *m* groups, i.e. balanced, this is not a necessary requirement for one-way anova. If, instead of being balanced, the data consist of a total of *w* data values unequally replicated within the *m* groups, the computations are straight forward, and the anova table looks similar to that for one-way anova except that the df differ. The total df is *w*-1, the factor df is *m*-1, and the residual df is calculated by subtracting the factor df from the total df, i.e. (*w*-1) - (*m*-1).

37.   By contrast, the data for two-way anova must be balanced. If the data is unbalanced, i.e. some of the *n×m* table's cells have no data, two-way anova cannot be used. Instead, some other method of analysing two-way data such as the method of Fitting Constants or Fitcon (Yates (1933)) or restricted maximum likelihood (REML) (Patterson and Thompson (1971)) must be used. Unbalanced data would arise in the above two-way anova COYD example (Example B) if one or more of the *v* varieties either was not present or failed to grow in one or more of the *y* years. It also arises in the calculation of Long Term LSD for use in Long Term COYD. In this a table of variety-by-year means that extends over more years and varieties than are present in the test years is to be analysed. As not all varieties are present in all years, this table is unbalanced. Like two-way anova, Fitcon subdivides the total variation in the data into different components for the different sources of variation, and the residual mean square provides an estimate of the random error variation. The df are as for the two-way anova except that the total df is *w*-1 where *w* is the total number of data values, and the residual df is calculated by subtracting the df for each of Factor 1 and Factor 2 from the total df, i.e. (*w*-1) -

(m-1) - (n-1).

38.   If the data is unbalanced in either one-way anova or two-way analysis such as Fitcon or REML, the standard errors needed for LSD's or t-tests for comparing factor means are more complicated because they differ depending on which factor mean is being compared with which. In one-way anova this simply affects the replication of the factor means (r_1 and r_2) and the standard errors are calculated as given above. In two-way analysis the standard errors are further complicated because they depend on the pattern of missing values for the two factor means being compared. However they are easily calculated by a computer programme.

POSSIBLE OTHER TOPICS FOR TGP 8.5:-

1. Maybe mention of a paragraph on the analysis of incomplete block designs?

2. Maybe a paragraph on paired comparison tests, such as the paired t-test, as a specific case of two-way anova

3. Maybe a paragraph/section on REML and where it may be of use in DUS testing

4. Maybe a section on binomial based tests for assessing uniformity by counting off-types

5. Maybe a section on Chi squared tests as used for testing distinctness in flower colour in France.

6. NOT Multivariate methods. This was suggested initially, but, according to responses from TWC experts, it seems as though it is not used on a regular basis. Consequently, if it is to be covered, it should go in a separate section on ad-hoc statistical methods.

REFERENCES

Mead, R. and Curnow, R. N. (1983). *Statistical Methods in Agriculture and Experimental Biology*. Chapman & Hall, London.

Patterson, H. D. and Thompson, R. (1971). Recovery of interblock information when block sizes are unequal. *Biometrika*, 58, 545-554.

Yates, F. (1933). The principles of orthogonality and confounding in replicated experiments. *Journal of Agricultural Science, Cambridge*, 23, 108-45.

APPENDICES

Appendix A1

Example of two-way anova

1.   This example illustrates the calculation of the COYD criterion. The data are the variety-by-year means for 11 varieties of italian ryegrass in three years for the "plant width at ear emergence" characteristic.

Variety	Year 1	Year 2	Year 3
VAR L	60.66	61.47	55.18
VAR N	58.91	62.28	55.66
VAR O	54.46	56.68	51.32
VAR P	57.69	54.75	54.94
VAR Q	56.57	57.62	51.46
VAR R	51.33	53.40	49.18
VAR S	58.59	59.08	51.67
VAR T	63.47	58.94	54.97
VAR V	66.14	65.49	60.15
VAR W	62.63	63.90	58.84
VAR AC	60.36	58.42	58.51

2.   Two-way anova of the data produces the following table:-

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio
Year	2	148.821	74.4106	26.843
Variety	10	383.679	38.3679	13.841
Residual	20	55.443	2.7721	
Total	31	587.944		

3.   From F-tables the 5%, 1% and 0.1% critical F-values on 10 and 20 df are 2.348, 3.368 and 5.075 respectively. Comparison of the Variety F-ratio with these shows that there is a very highly significant variety effect ($P<0.001$). The residual mean square or variety-by-year mean square is an estimate of the random variability of the 33 means in the above data table. It may be used to estimate the variance or standard errors of the variety means calculated from the data.

4.   The variety means are the means of 3 data values and are as follows:-

Variety	Mean
VAR L	59.103
VAR N	58.950
VAR O	54.153
VAR P	55.793
VAR Q	55.217
VAR R	51.303
VAR S	56.447
VAR T	59.127
VAR V	63.927
VAR W	61.790
VAR AC	59.097

5.   Their standard error $\mathrm{SE}(\bar{x})$ is estimated by

$$\mathrm{SE}(\bar{x}) = \sqrt{\frac{RMS}{r}} = \sqrt{\frac{2.7721}{3}} = 0.9613$$

6.   The standard error of the difference in two means, $(\mathrm{SE}(\bar{x}_1 - \bar{x}_2))$ is estimated by

$$\mathrm{SE}(\bar{x}_1 - \bar{x}_2) = \sqrt{RMS\left(\frac{1}{r_1} + \frac{1}{r_2}\right)} = \sqrt{2.7721\left(\frac{1}{3} + \frac{1}{3}\right)} = 1.3594$$

7.   The significance of the difference between pairs of variety means can be tested by comparing the absolute difference between pairs of means with the 1% LSD where

$$1\%\ \mathrm{LSD} = t \times \mathrm{SE}(\bar{x}_1 - \bar{x}_2) = 2.845 \times 1.3594 = 3.868$$

and t is the 1% two-tailed critical value from Student's t-tables on 20 df.   Thus varieties L and N are not significantly different at the 1% level, whereas varieties L and O and varieties L and Q are significantly different at the 1% level etc.

Appendix A2

Example of one-way anova

1.   This example illustrates a stage in the calculation of the COYU criterion. The data are the variety-by-year adjusted log(SD+1) of the "days to ear emergence" characteristic for 11 reference varieties of perennial ryegrass in three years. The data have been adjusted for any relationships between log(SD+1) and mean values for the characteristic. The data are as follows:-

Variety	Year 1	Year 2	Year 3
R1	2.36	2.13	2.30
R2	2.32	2.00	2.00
R3	2.42	2.10	1.95
R4	2.43	1.96	2.06
R5	2.52	2.14	1.96
R6	2.36	1.84	2.16
R7	2.43	2.19	1.80
R8	2.44	1.70	1.91
R9	2.52	2.16	2.24
R10	2.33	2.23	2.09
R11	2.28	1.78	1.96

2.   One-way anova of the data produces the following table:-

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio
Year	2	1.011	0.5053	25.06
Residual	30	0.605	0.0202	
Total	32	1.616		

3.   From F-tables the 5%, 1% and 0.1% critical F-values on 2 and 30 df are 3.316, 5.390 and 8.773 respectively. Comparison of the Year F-ratio with these shows that there is a very highly significant year effect (P<0.001) on uniformity. However, this F-test is of minor importance in calculating the COYU criterion. Of real importance is the overall mean adjusted log(SD+1) for all the reference varieties, and the residual mean square. The residual mean square provides an estimate of the random variability in the data, i.e. the variation between reference varieties within years, and allows the overall mean of the reference varieties to be compared with a candidate variety's mean adjusted log(SD+1).

4.   The overall mean adjusted log(SD+1) is 2.154. It is the mean of the reference varieties' 33 data values. Its standard error $SE(\bar{x})$ is estimated by

$$SE(\bar{x}) = \sqrt{\frac{RMS}{r}} = \sqrt{\frac{0.0202}{33}} = 0.0247$$

5.   If $\bar{x}_1$ represents the overall mean adjusted log(SD+1) and $\bar{x}_2$ a candidate variety's mean adjusted log(SD+1) which is the mean of the equivalent of 3 data values, then the standard error of the difference in the two means, ($SE(\bar{x}_1 - \bar{x}_2)$) is estimated by

$$SE(\bar{x}_1 - \bar{x}_2) = \sqrt{RMS\left(\frac{1}{r_1} + \frac{1}{r_2}\right)} = \sqrt{0.0202\left(\frac{1}{33} + \frac{1}{3}\right)} = 0.0857$$

6.   The significance of the difference between $\bar{x}_1$, the overall mean adjusted log(SD+1) and

$\bar{x}_2$, a candidate variety's mean adjusted log(SD+1) can be tested by comparing

$$\left|\bar{x}_1 - \bar{x}_2\right| = \bar{x}_2 - \bar{x}_1 \text{ with } t \times \text{SE}(\bar{x}_1 - \bar{x}_2) = 3.118 \times 0.0857 = 0.2672$$

Where t is the 0.2% one-tailed critical value (one-tailed because the candidate's mean is only rejected if it is larger than the overall mean) from Student's t-tables on 30 df. This is equivalent to comparing

$$\bar{x}_2 \text{ with } 0.2672 + \bar{x}_1 = 2.4212$$

7.   Thus if a candidate variety has a mean adjusted log(SD+1) greater than 2.42, its mean is significantly greater than the mean of the reference varieties at the 0.2% level, and hence the candidate variety is considered to be significantly less uniform than the reference varieties. If the candidate variety's mean adjusted log(SD+1) is less than 2.42, the mean will be considered to be not significantly different from the mean of the reference varieties at the 0.2% level, and hence the candidate variety not significantly different from the reference varieties in uniformity.

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