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| International Union for the Protection of New Varieties of Plants |  |

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| Technical Working Party on Automation and Computer Programs  Thirty-Seventh Session Hangzhou, China, October 14 to 16, 2019 | TWC/37/7  Original: English  Date: October 1, 2019 |

The Combined-Over-Years Uniformity Criterion (COYU)

Document prepared by an expert from the United Kingdom

Disclaimer: this document does not represent UPOV policies or guidance

# Background

The Combined Over Years Uniformity (COYU) criterion is a method used to assess uniformity on the basis of measured quantitative characteristics (TGP/8/3 “Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability”).

The Technical Working Party on Automation and Computer Programs (TWC), at its thirtieth session, held in Chisinau, Republic of Moldova, from June 26 to 29, 2012, agreed as follows (see document TWC/30/41 “Report”, paragraph 86):

“86. The TWC took note of the information contained in document TWC/30/10 and requested experts from Denmark and the United Kingdom to prepare a document on possible proposals for the improvement of COYU for consideration by the TWC at its next session.”

The TWC, at its thirty-first session, held in Seoul, Republic of Korea, from June 4 to 7, 2013, agreed as follows (see document TWC/31/32 “Report”, paragraphs 91 to 92):

“91. The TWC noted that the present method of calculation of COYU was overly strict due to the method of smoothing used and that very low probability levels were used in compensation (e.g.  p=0.1%). The TWC agreed that the bias in the present method of calculation of COYU could be addressed by a change of smoothing method from “moving average” to “cubic smoothing splines.

“92. The TWC welcomed the offer by the experts from the United Kingdom to write software for the proposed COYU method in FORTRAN for integration into the DUST software and to present a demonstration version of the DUST software using the proposed COYU method at the thirty-second session of the TWC.”

The TWC, at its thirty-fifth session, held in Buenos Aires, Argentina, from November 14 to 17, 2017, noted progress and concluded as follows (see document TWC/35/21 “Report”, paragraphs 81 to 84):

“81. The TWC considered documents TWP/1/13 and TWC/35/6 “Method of calculation of COYU: practical exercise, probability levels, extrapolation and software” and received a presentation by the United Kingdom, a copy of which is provided in document TWC/35/6 Add.

“82. The TWC considered the report on developments concerning the new method of calculation of COYU, provided by an expert from the United Kingdom and noted that the statistical development of the method had been completed.

“83. The TWC noted the results of the practical exercise and higher probability levels required by the new method to most closely match decisions using the current method for calculation of COYU

* probability levels 0.003 to match 0.001 for current COYU
* probability levels 0.02 to match 0.01 for current COYU

“84. The TWC noted the following areas identified for further improving the software using the new method of calculation of COYU and agreed to invite the expert from the United Kingdom to report on developments at its thirty-sixth session:

* Improve installation with DUST
* Improve error messages
* Ensure that problematic data sets can be dealt with appropriately
* Produce extrapolation flags according to approach agreed by TWC
* Ensure that the algorithm works well for unbalanced data (for cyclic planting).”

At the thirty-sixth session of the TWC, held in Hanover, Germany, from July 2 to 6, 2018, it was reported that work had concentrated on improving the functionality of the software (in R, which is a widely use programming language and free software environment for statistical computing and graphics). In particular:

* Improved the plots showing the relationship between uniformity and level of expression by adding points for candidates; and
* Added calculations of the level of extrapolation (method b using degree of inflation) for each candidate.

# PROGRESS SINCE THE THIRTY-SIXTH SESSION OF THE TWC

Since the thirty-sixth session of the TWC, work has concentrated on reviewing the usability of the COYU R package. In particular, the error messages have been made more informative. A through internal review of the code has been carried out and improvements made.

The revised COYU R package is now available online at <https://github.com/BiomathematicsAndStatisticsScotland/coyus> .

Mr. Christophe Chevalier (France) has been asked to review the new version of the software as he reviewed the previous version.

There are plans to meet the maintainers of the DUSTNT software to consider options for inclusion of the new procedure. It was learnt in the previous practical exercise that installation needs to be straightforward, especially considering the computing environments in which it will be used.

A draft text, proposed for inclusion in TGP/8 “Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability” is presented in the Annex to this document. The TWC may wish to consider whether such text should replace the text for the currently used procedure of COYU (using moving averages) or should it instead be alongside the current text.

# Next Steps and Proposals

It is proposed to request that TWC members who use R and COYU review the COYU package. It is important that the software be thoroughly validated before wider distribution.

We will work with the maintainers of the DUSTNT software to add the new procedure. Once this is available, we would like to ask TWC members who use both DUSTNT and COYU to review the new procedure.

Following discussion at the TWC session, we propose to refine the draft text intended for TGP/8.

*14. The TWC is invited to:*

*(a) consider whether to request that TWC members who use R and COYU review the COYU package, as set out in paragraph 11 of this document; and*

*(b) consider the proposed draft text for document TGP/8, Section 9, as presented in the Annex to this document.*

[Annex follows]

PROPOSED DRAFT TEXT FOR TGP/8

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| Note for Draft version  **~~Strikethrough~~ (highlighted in grey)** indicates deletion from the text of document TGP/8/3.  **Underlining (highlighted in grey)** indicates insertion to the text of document TGP/8/3. |

9. THE COMBINED-OVER-YEARS UNIFORMITY CRITERION (COYU)

9.1 Summary of requirements for application of method

* For quantitative characteristics.
* When observations are made on a plant basis over two or more years.
* When there are some differences between plants of a variety, representing quantitative variation rather than presence of off-types.
* It is recommended that there should be at least 20 degrees of freedom for the estimate of variance for the comparable varieties formed in the COYU analysis.

Comparable varieties are varieties of the same type within the same or a closely related species that have been previously examined and considered to be sufficiently uniform (see document TGP/10, Section 5.2 “Determining acceptable level of variation”).

9.2 Summary

9.2.1 Document TGP/10 explains that when the off-type approach for the assessment of uniformity is not appropriate for the assessment of uniformity, the standard deviation approach can be used. It further states the following with respect to determination of the acceptable level of variation.

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| “5.2 Determining the acceptable level of variation  “5.2.1 The comparison between a candidate variety and comparable varieties is carried out on the basis of standard deviations, calculated from individual plant observations. UPOV has proposed several statistical methods for dealing with uniformity in measured quantitative characteristics. One method, which takes into account variations between years, is the Combined Over Years Uniformity (COYU) method. The comparison between a candidate variety and comparable varieties is carried out on the basis of standard deviations, calculated from individual plant observations. This COYU procedure calculates a tolerance limit on the basis of comparable varieties already known i.e. uniformity is assessed using a relative tolerance limit based on varieties within the same trial with comparable expression of characteristics.” |

9.2.2 Uniformity is often related to the expression of a characteristic. For example, in some species, varieties with larger plants tend to be less uniform in size than those with smaller plants. If the same standard is applied to all varieties then it is possible that some may have to meet very strict criteria while others face standards that are easy to satisfy. COYU addresses this problem by adjusting for any relationship that exists between uniformity, as measured by the plant-to-plant SD, and the expression of the characteristic, as measured by the variety mean, before setting a standard.

9.2.3 The method involves ranking comparable and candidate varieties by the mean value of the characteristic. Each variety’s SD is taken and the mean SD of the most similar varieties is subtracted. This procedure gives, for each variety, a measure of its uniformity expressed relative to that of similar varieties. The term comparable varieties here refers to established varieties which have been included in the growing trial and which have comparable expression of the characteristics under investigation.

9.2.4 The results for each year are combined in a variety-by-years table of adjusted SDs and analysis of variance is applied. The mean adjusted SD for the candidate is compared with the mean for the comparable varieties using a standard t‑test.

9.2.5 COYU, in effect, compares the uniformity of a candidate with that of the comparable varieties most similar in relation to the characteristic being assessed. The main advantages of COYU are that all varieties can be compared on the same basis and that information from several years of testing may be combined into a single criterion.

9.3 Introduction

9.3.1 Uniformity is sometimes assessed by measuring individual characteristics and calculating the standard deviation (SD) of the measurements on individual plants within a plot. The SDs are averaged over all replicates to provide a single measure of uniformity for each variety in a trial.

9.3.2 This section outlines a procedure known as the combined-over-years uniformity (COYU) criterion. COYU assesses the uniformity of a variety relative to comparable varieties based on SDs from trials over several years. A feature of the method is that it takes account of possible relationships between the expression of a characteristic and uniformity.

9.3.3 This section describes:

* The principles underlying the COYU method.
* UPOV recommendations on the application of COYU to individual species.
* Mathematical details of the method with an example of its application.
* The computer software that is available to apply the procedure.

## 9.4 The COYU Criterion

9.4.1 The application of the COYU criterion involves a number of steps as listed below. These are applied to each characteristic in turn. Details are given under Part II section 9.6.

* Calculation of within-plot SDs for each variety in each year.
* Transformation of SDs by adding 1 and converting to natural logarithms.
* Estimation of the relationship between the SD and mean in each year. The method used is based on moving averages of the log SDs of comparable varieties ordered by their means.
* Adjustments of log SDs of candidate and comparable varieties based on the estimated relationships between SD and mean in each year.
* Averaging of adjusted log SDs over years.
* Calculation of the maximum allowable SD (the uniformity criterion). This uses an estimate of the variability in the uniformity of comparable varieties derived from analysis of variance of the variety-by-year table of adjusted log SDs.
* Comparison of the adjusted log SDs of candidate varieties with the maximum allowable SD.

9.4.2 The advantages of the COYU criterion are:

* It provides a method for assessing uniformity that is largely independent of the varieties that are under test.
* The method combines information from several trials to form a single criterion for uniformity.
* Decisions based on the method are likely to be stable over time.
* The statistical model on which it is based reflects the main sources of variation that influence uniformity.
* Standards are based on the uniformity of comparable varieties.

9.5 Use of COYU

9.5.1 COYU is recommended for use in assessing the uniformity of varieties

* For quantitative characteristics.
* When observations are made on a plant basis over two or more years.
* When there are some differences between plants of a variety, representing quantitative variation rather than presence of off-types.

9.5.2 A variety is considered to be uniform for a characteristic if its mean adjusted log SD does not exceed the uniformity criterion.

9.5.3 The probability level “p” used to determine the uniformity criterion depends on the crop. Recommended probability levels are given in sections 9.~~11~~7 and 9.8.

9.5.4 The uniformity test may be made over two or three years. If the test is normally applied over three years, it is possible to choose to make an early acceptance or rejection of a variety using an appropriate selection of probability values.

9.5.5 It is recommended that there should be at least 20 degrees of freedom for the estimate of variance for the comparable varieties formed in the COYU analysis. This corresponds to 11 comparable varieties for a COYU test based on two years of trials and 8 comparable varieties for three years. In some situations, there may not be enough comparable varieties to give the recommended minimum degrees of freedom. Advice is being developed for such cases.

9.6 Mathematical details

Step 1: Derivation of the within-plot standard deviation

9.6.1 Within-plot standard deviations for each variety in each year are calculated by averaging the plot between-plant standard deviations, SDj, over replicates:





where yij is the observation on the ith plant in the jth plot, **y**j is the mean of the observations from the jth plot, n is the number of plants measured in each plot and r is the number of replicates.

Step 2: Transformation of the SDs

9.6.2 Transformation of SDs by adding 1 and converting to natural logarithms. The purpose of this transformation is to make the SDs more amenable to statistical analysis.

Step 3: Estimation of the relationship between the SD and mean in each year

9.6.3 The revised version of COYU uses the method of splines rather the moving average approach used in the previous procedure.

9.6.4 For each year separately, the form of the average relationship between SD and characteristic mean is estimated for the comparable varieties. The method of estimation is a ~~9‑point moving average. The log SDs (the Y variate) and the means (the X variate) for each variety are first ranked according to the values of the mean. For each point (X~~~~i,~~ ~~Y~~~~i~~~~) take the trend value T~~~~i~~ ~~to be the mean of the values Y~~~~i-4~~~~, Y~~~~i-3~~~~, .... , Y~~~~i+4~~ ~~where i represents the rank of the X value and Y~~~~i~~ ~~is the corresponding Y value. For X values ranked 1~~~~st~~ ~~and 2~~~~nd~~ ~~the trend value is taken to be the mean of the first three values. In the case of the X value ranked 3~~~~rd~~ ~~the mean of the first five values are taken and for the X value ranked 4~~~~th~~ ~~the mean of the first seven values are used. A similar procedure operates for the four highest-ranked X values~~ cubic smoothing spline with four degrees of freedom. The log SDs (the Y variate) are fitted to the means (the X variate) for each variety using the spline.

9.6.5 A simple example in Figure 1 illustrates this procedure for 16 varieties. The points marked “~~0~~O” in Figure 1~~a~~ represent the log SDs and the corresponding means of 16 varieties. ~~The points marked “X” are the 9-point moving-averages, which are calculated by taking, for each variety, the average of the log SDs of the variety and the four varieties on either side. At the extremities the moving average is based on the mean of 3, 5, or 7 values.~~ The dashed line represents the fitted smoothing spline.

**~~Figure 1: Association between SD and mean – days to ear emergence in cocksfoot varieties~~** ~~(~~*~~symbol O is for observed SD, symbol X is for moving average SD~~*~~)~~

*[to delete this figure]*



**Figure 1: Association between SD and mean** (*symbol O is for observed SD, dashed line is fitted spline*)

*[to add this figure]*

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Step 4: Adjustment of transformed SD values based on estimated SD-mean relationship

9.6.6 Once the trend values for the comparable varieties have been determined, the trend values for candidates are estimated using ~~linear interpolation between the trend values of the nearest two comparable varieties as defined by their means for the characteristic. Thus if the trend values for the two comparable varieties on either side of the candidate are T~~~~i~~ ~~and T~~~~i+1~~ ~~and the observed value for the candidate is X~~~~c~~~~, where X~~~~i~~ ~~≤ X~~~~c~~ ~~≤ X~~~~i+1~~~~, then the trend value T~~~~c~~ ~~for the candidate is given by~~ predictions from the spline.

*[to delete this fomula]*

9.6.7 To adjust the SDs for their relationship with the characteristic mean the estimated trend values are subtracted from the transformed SDs and the grand mean is added back.

9.6.8 The results for the simple example with 16 varieties are illustrated in Figure 2.

**Figure 2: Adjusting for association between SD and mean ~~– days to ear emergence in cocksfoot varieties~~** (*symbol ~~A~~O is for adjusted SD, dashed line is the grand mean*)

*[to delete this figure]*



*[to add this figure]*



Step 5: Calculation of the uniformity criterion

~~9.6.8 An estimate of the variability in the uniformity of the comparable varieties is derived by applying a one-way analysis of variance to the adjusted log SDs, i.e. with years as the classifying factor. The variability (V) is estimated from the residual term in this analysis of variance.~~

9.6.9 The maximum allowable standard deviation (the uniformity criterion), based on k years of trials, is in the form

*[to delete this fomula]*



*[to add this fomula]*

where SD*r*is the mean of adjusted log SDs for the comparable varieties, *Vc* is ~~the~~ a variance specific to the candidate variety (related to the uncertainty of the ~~adjusted log SDs after removing year effects,~~ spline prediction), *tp* is the one-tailed t-value for probability level p with appropriate degrees of freedom taking into account the spline fit. For further information, see Roberts & Kristensen (2015).

9.6.10 ~~as for V,k~~ The uniformity criterion is specific to the ~~number of years~~ candidate and ~~R is the number of~~ depends on its level of expression relative to the comparable varieties.

9.7 Probablity levels

9.7.1 With the previous procedure, a probability level 0.1% was commonly used. For the current procedure, it is recommended that a probability level of 0.3% is used instead.

9.8 Early decisions for a three-year test

9.8.1 Decisions on uniformity may be made after two or three years depending on the crop. If COYU is normally applied over three years, it is possible to make an early acceptance or rejection of a candidate variety using an appropriate selection of probability values.

9.8.2 The probability level for early rejection of a candidate variety after two years should be the same as that for the full three-year test. For example, if the three-year COYU test is applied using a probability level of 0.~~2~~3%, a candidate variety can be rejected after two years if its uniformity exceeds the COYU criterion with probability level 0. ~~2~~3%.

9.8.3 The probability level for early acceptance of a candidate variety after two years should be larger than that for the full three-year test. As an example, if the three-year COYU test is applied using a probability level of 0. ~~2~~3%, a candidate variety can be accepted after two years if its uniformity does not exceed the COYU criterion with probability level 2%.

9.8.4 Some varieties may fail to be rejected or accepted after two years. In the example set out in section 9.8, a variety might have a uniformity that exceeds the COYU criterion with probability level 2% but not the criterion with probability level 0. ~~2~~3%. In this case, such varieties should be re-assessed after three years.

9.8.5 If a probability level for early rejection of a candidate variety after two years of 1% was used with the previous procedure, it is recommended that a probability level of 2% is used.

~~9.8~~ *~~Example of COYU calculations~~*

~~9.8.1 An example of the application of COYU is given here to illustrate the calculations involved. The example consists of days to ear emergence scores for perennial ryegrass over three years for 11 comparable varieties (R1 to R11) and one candidate (C1). The data is tabulated in Table 1.~~

**~~Table 1: Example data-set – days to ear emergence in perennial ryegrass~~**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | ~~Character Means~~ | | | ~~Within Plot SD~~ | | | ~~Log (SD+1)~~ | | |
| ~~Variety~~ | ~~Year 1~~ | ~~Year 2~~ | ~~Year 3~~ | ~~Year 1~~ | ~~Year 2~~ | ~~Year 3~~ | ~~Year 1~~ | ~~Year 2~~ | ~~Year 3~~ |
| ~~R1~~ | ~~38~~ | ~~41~~ | ~~35~~ | ~~8.5~~ | ~~8.8~~ | ~~9.4~~ | ~~2.25~~ | ~~2.28~~ | ~~2.34~~ |
| ~~R2~~ | ~~63~~ | ~~68~~ | ~~61~~ | ~~8.1~~ | ~~7.6~~ | ~~6.7~~ | ~~2.21~~ | ~~2.15~~ | ~~2.04~~ |
| ~~R3~~ | ~~69~~ | ~~71~~ | ~~64~~ | ~~9.9~~ | ~~7.6~~ | ~~5.9~~ | ~~2.39~~ | ~~2.15~~ | ~~1.93~~ |
| ~~R4~~ | ~~71~~ | ~~75~~ | ~~67~~ | ~~10.2~~ | ~~6.6~~ | ~~6.5~~ | ~~2.42~~ | ~~2.03~~ | ~~2.01~~ |
| ~~R5~~ | ~~69~~ | ~~78~~ | ~~69~~ | ~~11.2~~ | ~~7.5~~ | ~~5.9~~ | ~~2.50~~ | ~~2.14~~ | ~~1.93~~ |
| ~~R6~~ | ~~74~~ | ~~77~~ | ~~71~~ | ~~9.8~~ | ~~5.4~~ | ~~7.4~~ | ~~2.38~~ | ~~1.86~~ | ~~2.13~~ |
| ~~R7~~ | ~~76~~ | ~~79~~ | ~~70~~ | ~~10.7~~ | ~~7.6~~ | ~~4.8~~ | ~~2.46~~ | ~~2.15~~ | ~~1.76~~ |
| ~~R8~~ | ~~75~~ | ~~80~~ | ~~73~~ | ~~10.9~~ | ~~4.1~~ | ~~5.7~~ | ~~2.48~~ | ~~1.63~~ | ~~1.90~~ |
| ~~R9~~ | ~~78~~ | ~~81~~ | ~~75~~ | ~~11.6~~ | ~~7.4~~ | ~~9.1~~ | ~~2.53~~ | ~~2.13~~ | ~~2.31~~ |
| ~~R10~~ | ~~79~~ | ~~80~~ | ~~75~~ | ~~9.4~~ | ~~7.6~~ | ~~8.5~~ | ~~2.34~~ | ~~2.15~~ | ~~2.25~~ |
| ~~R11~~ | ~~76~~ | ~~85~~ | ~~79~~ | ~~9.2~~ | ~~4.8~~ | ~~7.4~~ | ~~2.32~~ | ~~1.76~~ | ~~2.13~~ |
| ~~C1~~ | ~~52~~ | ~~56~~ | ~~48~~ | ~~8.2~~ | ~~8.4~~ | ~~8.1~~ | ~~2.22~~ | ~~2.24~~ | ~~2.21~~ |

~~9.8.2 The calculations for adjusting the SDs in year 1 are given in Table 2. The trend value for candidate C1 is obtained by interpolation between values for varieties R1 and R2, since the characteristic mean for C1 (i.e. 52) lies between the means for R1 and R2 (i.e. 38 and 63). That is~~

~~~~

**~~Table 2: Example data-set – calculating adjusted log(SD+1) for year 1~~**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ~~Variety~~ | ~~Ranked mean~~  ~~(X)~~ | ~~Log (SD+1)~~  ~~(Y)~~ | ~~Trend Value~~  ~~T~~ | ~~Adj. Log (SD+1)~~ |
| ~~R1~~ | ~~38~~ | ~~2.25~~ | ~~(2.25 + 2.21 + 2.39)/3 = 2.28~~ | ~~2.25 - 2.28 + 2.39 = 2.36~~ |
| ~~R2~~ | ~~63~~ | ~~2.21~~ | ~~(2.25 + 2.21 + 2.39)/3 = 2.28~~ | ~~2.21 - 2.28 + 2.39 = 2.32~~ |
| ~~R3~~ | ~~69~~ | ~~2.39~~ | ~~(2.25 + . . . + 2.42)/5 = 2.35~~ | ~~2.39 - 2.35 + 2.39 = 2.42~~ |
| ~~R5~~ | ~~69~~ | ~~2.50~~ | ~~(2.25 + . . . + 2.48)/7 = 2.38~~ | ~~2.50 - 2.38 + 2.39 = 2.52~~ |
| ~~R4~~ | ~~71~~ | ~~2.42~~ | ~~(2.25 + . . . + 2.32)/9 = 2.38~~ | ~~2.42 - 2.38 + 2.39 = 2.43~~ |
| ~~R6~~ | ~~74~~ | ~~2.38~~ | ~~(2.21 + . . . + 2.53)/9 = 2.41~~ | ~~2.38 - 2.41 + 2.39 = 2.36~~ |
| ~~R8~~ | ~~75~~ | ~~2.48~~ | ~~(2.39 + . . . + 2.34)/9 = 2.42~~ | ~~2.48 - 2.42 + 2.39 = 2.44~~ |
| ~~R7~~ | ~~76~~ | ~~2.46~~ | ~~(2.42 + . . . + 2.34)/7 = 2.42~~ | ~~2.46 - 2.42 + 2.39 = 2.43~~ |
| ~~R11~~ | ~~76~~ | ~~2.32~~ | ~~(2.48 + . . . + 2.34)/5 = 2.43~~ | ~~2.32 - 2.43 + 2.39 = 2.28~~ |
| ~~R9~~ | ~~78~~ | ~~2.53~~ | ~~(2.32 + 2.53 + 2.34)/3 = 2.40~~ | ~~2.53 - 2.40 + 2.39 = 2.52~~ |
| ~~R10~~ | ~~79~~ | ~~2.34~~ | ~~(2.32 + 2.53 + 2.34)/3 = 2.40~~ | ~~2.34 - 2.40 + 2.39 = 2.33~~ |
| ~~Mean~~ | ~~70~~ | ~~2.39~~ |  |  |
| ~~C1~~ | ~~52~~ | ~~2.22~~ | ~~2.28~~ | ~~2.22 – 2.28 + 2.39 = 2.32~~ |

~~9.8.3 The results of adjusting for all three years are shown in Table 3.~~

**~~Table 3: Example data-set – adjusted log(SD+1) for all three years with over-year means~~**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | ~~Over-Year Means~~ | | ~~Adj. Log (SD+1)~~ | | |
| ~~Variety~~ | ~~Char. mean~~ | ~~Adj. Log (SD+1)~~ | ~~Year 1~~ | ~~Year 2~~ | ~~Year 3~~ |
| ~~R1~~ | ~~38~~ | ~~2.26~~ | ~~2.36~~ | ~~2.13~~ | ~~2.30~~ |
| ~~R2~~ | ~~64~~ | ~~2.10~~ | ~~2.32~~ | ~~2.00~~ | ~~2.00~~ |
| ~~R3~~ | ~~68~~ | ~~2.16~~ | ~~2.42~~ | ~~2.10~~ | ~~1.95~~ |
| ~~R4~~ | ~~71~~ | ~~2.15~~ | ~~2.43~~ | ~~1.96~~ | ~~2.06~~ |
| ~~R5~~ | ~~72~~ | ~~2.20~~ | ~~2.52~~ | ~~2.14~~ | ~~1.96~~ |
| ~~R6~~ | ~~74~~ | ~~2.12~~ | ~~2.36~~ | ~~1.84~~ | ~~2.16~~ |
| ~~R7~~ | ~~75~~ | ~~2.14~~ | ~~2.43~~ | ~~2.19~~ | ~~1.80~~ |
| ~~R8~~ | ~~76~~ | ~~2.02~~ | ~~2.44~~ | ~~1.70~~ | ~~1.91~~ |
| ~~R9~~ | ~~78~~ | ~~2.30~~ | ~~2.52~~ | ~~2.16~~ | ~~2.24~~ |
| ~~R10~~ | ~~78~~ | ~~2.22~~ | ~~2.33~~ | ~~2.23~~ | ~~2.09~~ |
| ~~R11~~ | ~~80~~ | ~~2.01~~ | ~~2.28~~ | ~~1.78~~ | ~~1.96~~ |
| ~~Mean~~ | ~~70~~ | ~~2.15~~ | ~~2.40~~ | ~~2.02~~ | ~~2.04~~ |
| ~~C1~~ | ~~52~~ | ~~2.19~~ | ~~2.32~~ | ~~2.08~~ | ~~2.17~~ |

~~9.8.4 The analysis of variance table for the adjusted log SDs is given in Table 4 (based on comparable varieties only). The variability in the uniformity of comparable varieties is estimated from this (V=0.0202).~~

**~~Table 4: Example data set – analysis of variance table for adjusted log (SD+1)~~**

|  |  |  |  |
| --- | --- | --- | --- |
| ~~Source~~ | ~~Degrees of~~  ~~freedom~~ | ~~Sums of~~  ~~squares~~ | ~~Mean~~  ~~squares~~ |
| ~~Year~~ | ~~2~~ | ~~1.0196~~ | ~~0.5098~~ |
| ~~Varieties within years (=residual)~~ | ~~30~~ | ~~0.6060~~ | **~~0.0202~~** |
| ~~Total~~ | ~~32~~ | ~~1.6256~~ |  |

~~9.8.5 The uniformity criterion for a probability level of 0.2% is calculated thus:~~

~~~~

~~where t~~~~p~~ ~~is taken from Student’s t table with p=0.002 (one-tailed) and 30 degrees of freedom.~~

~~9.8.6 Varieties with mean adjusted log (SD + 1) less than, or equal to, 2.42 can be regarded as uniform for this characteristic. The candidate variety C1 satisfies this criterion.~~

9.9 Extrapolation

9.9.1 If a candidate has a level of expression in a characteristic outside that seen in other seen in other varieties, we call this “extrapolation”.

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

* *“6.4.2.2.1 For measured characteristics, the acceptable level of variation for the variety should not significantly exceed the level of variation found in comparable varieties already known.”*

9.9.3 If the level of expression is very different from other varieties in test, it should be considered whether these varieties are actually comparable.

9.9.4 The COYU procedure has tools to evaluate whether there is extrapolation and the degree of extrapolation. The information produced by COYU can also aid the crop expert in making a decision on uniformity when there is extrapolation.

9.9.5 Firstly, the procedure indicates whether the mean for the candidate is outside the range of means seen in other varieties under test in any of the years.

9.9.6 The degree of extrapolation is based on the inflation of the COYU criterion for the candidate compared to that of the nearest comparable variety (see TWC/35/6 “Method of calculation of COYU, practical exercise, probability levels, extrapolation & software”). In the case of extrapolation, the degree of extrapolation will be more than 1. The greater the number, the greater is the severity of the extrapolation. It is suggested that all cases of extrapolation be reviewed using the output from COYU (see examples below) but that special care be taken when the degree is more than 2.

9.9.7 In cases where the degree of extrapolation is sufficiently high to cause concern, the crop expert may consider output from the COYU procedure to assist the decision-making. These include plots of the log(SD+1) against mean values, along with tables of results. Examples are given below.

9.10 Implementing COYU

9.10.1 The COYU criterion can be applied using COYUS9 module of the DUST software package for the statistical analysis of DUS data. This is available from Dr. Sally Watson, (Email: info@afbini.gov.uk) or from http://www.afbini.gov.uk/dustnt.htm. There is also an R package. This can be found at <https://github.com/BiomathematicsAndStatisticsScotland/coyus/>.

9.11 Example of the use of COYU software

*9.11.1 DUST computer program*

~~9.10.1.1 The main output from the DUST COYU program is illustrated in Table A1. This summarises the results of analyses of within-plot SDs for 49 perennial ryegrass varieties assessed over a three-year period. Supplementary output is given in Table A2 where details of the analysis of a single characteristic, date of ear emergence, are presented. Note that the analysis of variance table given has an additional source of variation; the variance, V, of the adjusted log SDs is calculated by combining the variation for the variety and residual sources.~~

~~9.10.1.2 In Table A1, the adjusted SD for each variety is expressed as a percent of the mean SD for all comparable varieties. A figure of 100 indicates a variety of average uniformity; a variety with a value less than 100 shows good uniformity; a variety with a value much greater than 100 suggests poor uniformity in that characteristic. Lack of uniformity in one characteristic is often supported by evidence of poor uniformity in related characteristics.~~

~~9.10.1.3 The symbols “\*” and “+” to the right of percentages identify varieties whose SDs exceed the COYU criterion after 3 and 2 years respectively. The symbol “:” indicates that after two years uniformity is not yet acceptable and the variety should be considered for testing for a further year. Note that for this example a probability level of 0.2% is used for the three-year test. For early decisions at two years, probability levels of 2% and 0.2% are used to accept and reject varieties respectively. All of the candidates had acceptable uniformity for the 8 characters using the COYU criterion.~~

~~9.10.1.4 The numbers to the right of percentages refer to the number of years that a within‑year uniformity criterion is exceeded. This criterion has now been superseded by COYU.~~

~~9.10.1.5 The program will operate with a complete set of data or will accept some missing values, e.g. when a variety is not present in a year.~~

9.11.1.1 Results are produced in detailed for each characteristic and then a summary over characteristics is given.

9.11.1.2 Table A1 shows an example of the detailed results for a characteristic (ear emergence). This was for a two-year test. In this case, neither candidate exceeds the COYU criterion (with probablity level at 0.003). However, candidate C1 show signs of a high degree of extrapolation. Figure A1 shows the log(SD) values plotted against the means for this characteristic. This shows the candidate being much earlier than the comparable varieties. Such plots may be used by the crop expert to help evaluate the uniformity of a candidate which has a level of expression different from that of the comparable varieties.

**TABLE A1:** **Example of ~~summary~~ detailed output for a character from the COYUS program**

[*to delete this table]*

8 - DATE EE

\*\*\*\* UNIFORMITY ANALYSIS OF BETWEEN-PLANT STANDARD DEVIATIONS (SD) \*\*\*\*

*[to add this table]*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| AFP | VARIETY | Extrapolation | Char\_Mean | Adj\_LogSD | Unadj\_Log\_SD | Mean\_y1 | Mean\_y2 | Log(SD+1)\_y1 | Log(SD+1)\_y2 |
|  |  |  |  |  |  |  |  |  |  |
| CANDIDATE | |  |  |  |  |  |  |  |  |
| 101 | C1 | 6.0 | 75.0! | 1.45 | 1.92 | 75.3 | 74.7 | 1.85 | 2.00 |
| 102 | C2 | - | 83.6 | 1.69 | 1.67 | 81.9 | 85.3 | 1.63 | 1.71 |
|  |  |  |  |  |  |  |  |  |  |
| REFERENCE | MEANS |  |  | 82.9 | 1.73 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| REFERENCE | |  |  |  |  |  |  |  |  |
| 1 | R1 |  | 81.9 | 1.76 | 1.77 | 84.4 | 88.7 | 1.38 | 1.76 |
| 2 | R2 |  | 82.9 | 1.83 | 1.83 | 82.7 | 84.9 | 1.46 | 1.78 |
| 3 | R3 |  | 84.5 | 1.63 | 1.58 | 81.7 | 83.8 | 1.57 | 1.96 |
| 4 | R4 |  | 83.7 | 1.55 | 1.54 | 81.5 | 83.5 | 1.51 | 2.02 |
| 5 | R5 |  | 79.5 | 1.74 | 1.85 | 80.3 | 81.9 | 1.69 | 1.96 |
| 6 | R6 |  | 82.5 | 1.75 | 1.77 | 82.3 | 85.1 | 1.37 | 1.71 |
| 7 | R7 |  | 81.1 | 1.75 | 1.83 | 81.2 | 81.2 | 1.59 | 1.92 |
| 8 | R8 |  | 82.5 | 1.78 | 1.84 | 81.2 | 81.7 | 1.48 | 1.74 |
| 9 | R9 |  | 81.2 | 1.74 | 1.76 | 81.4 | 84.5 | 1.61 | 2.06 |
| 10 | R10 |  | 82.7 | 1.76 | 1.76 | 80.1 | 78.9 | 1.71 | 1.99 |
| 11 | R11 |  | 86.5 | 1.72 | 1.57 | 81.9 | 81.9 | 1.54 | 2.00 |
| 12 | R12 |  | 83.8 | 1.64 | 1.62 | 80.3 | 84.6 | 1.66 | 2.02 |
| 13 | R13 |  | 82.4 | 1.56 | 1.57 | 83.3 | 85.7 | 1.44 | 1.72 |
| 14 | R14 |  | 84.7 | 1.78 | 1.74 | 81.5 | 83.4 | 1.39 | 1.74 |
| 15 | R15 |  | 81.8 | 1.81 | 1.84 | 82.6 | 86.8 | 1.63 | 1.85 |
| 16 | R16 |  | 83.6 | 1.90 | 1.90 | 81.2 | 82.5 | 1.59 | 2.08 |
| 17 | R17 |  | 85.2 | 1.79 | 1.70 | 82.5 | 84.6 | 1.73 | 2.06 |
| 18 | R18 |  | 81.4 | 1.59 | 1.61 | 83.8 | 86.6 | 1.39 | 2.00 |

SYMBOLS

+ SD EXCEEDS OVER-YEARS UNIFORMITY CRITERION AFTER 2 YEARS WITH PROBABILITY 0.0030

\_ NO VERDICT.

! EXTRAPOLATION DETECTED.

**FIGURE A1:** **Example plot of log SD vs mean from the COYUS program**

*[to add these figures]*



9.11.1.3 The program also gives a summary over characteristics. See Table A2 for an example. It can be seen that neither candidate fails the COYU uniformity criterion in any characteristics. However, C2 exhibits signs of extrapolation in several characteristics. So the expert would be advised to look at this candidate with care.

**TABLE A2:** **Example of ~~supplementary DUST output for date of ear emergency (char.8)~~ summary output from the COYUS program**

*[to delete this table]*

****

CANDIDATE SUMMARY

*[to add this table]*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| AFP | VARIETY | 4 | 9 | 5 | 60 | 70 | 8 | 10 | 11 | 14 | 15 | 17 | 24 | 31 | 33 | 34 | 35 | 41 |
| 101 | C1 | \_ | \_ | \_ | \_ | \_ | ! | ! | \_ | \_ | ! | \_ | ! | \_ | \_ | \_ | ! | ! |
| 102 | C2 | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |

SYMBOLS

+ SD EXCEEDS OVER-YEARS UNIFORMITY CRITERION AFTER 2 YEARS WITH PROBABILITY 0.0030

! EXTRAPOLATION DETECTED.

CANDIDATE UNIFORMITY CRITERIA

*[to add this table]*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 4 | 9 | 5 | 60 | 70 | 8 | 10 | 11 | 14 | 15 | 17 | 24 | 31 | 33 | 34 | 35 | 41 |
| 2 YEAR REJECT | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 101 | C1 | 2.57 | 2.55 | 2.51 | 2.49 | 2.49 | 3.05 | 2.89 | 2.7 | 1.95 | 1.2 | 2.8 | 1.94 | 1.77 | 1.75 | 1.24 | 1.74 | 0.196 |
| 102 | C2 | 2.57 | 2.55 | 2.51 | 2.49 | 2.49 | 1.99 | 2.75 | 2.69 | 1.94 | 1.19 | 2.8 | 1.88 | 1.77 | 1.74 | 1.23 | 1.68 | 0.187 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 YEAR ACCEPT | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 101 | C1 | 2.57 | 2.55 | 2.51 | 2.49 | 2.49 | 3.05 | 2.89 | 2.7 | 1.95 | 1.2 | 2.8 | 1.94 | 1.77 | 1.75 | 1.24 | 1.74 | 0.196 |
| 102 | C2 | 2.57 | 2.55 | 2.51 | 2.49 | 2.49 | 1.99 | 2.75 | 2.69 | 1.94 | 1.19 | 2.8 | 1.88 | 1.77 | 1.74 | 1.23 | 1.68 | 0.187 |

9.11.1.4 The COYUS program also outputs a comma-separated value formatted file of results to allow easy transfer to Excel.

9.12 Schemes used for the application of COYU

The following four cases are those which, in general, represent the different situations which may arise where COYU is used in DUS testing:

Scheme A: Test is conducted over 2 independent growing cycles and decisions made after 2 growing cycles (a growing cycle could be a year and is further on denoted by cycle)

Scheme B: Test is conducted over 3 independent growing cycles and decisions made after 3 cycles

Scheme C: Test is conducted over 3 independent growing cycles and decisions made after 3 cycles, but a variety may be accepted after 2 cycles

Scheme D: Test is conducted over 3 independent growing cycles and decisions made after 3 cycles, but a variety may be accepted or rejected after 2 cycles

The stages at which the decisions are made in Cases A to D are illustrated in figures 1 to 4 respectively. These also illustrate the various standard probability levels (pu2, pnu2 and pu3) which are needed to calculate the COYU criteria depending on the case. These are defined as follows:

|  |  |
| --- | --- |
| **Probability Level** | **Used to decide whether a variety is :-** |
| pu2 | uniform in a characteristic after 2 cycles |
| pnu2 | non-uniform after 2 cycles |
| pu3 | uniform in a characteristic after 3 cycles |

In Figures 1 to 4 the COYU criterion calculated using say the probability level pu2 is denoted by UCpu2 etc. The term “U” represents the mean adjusted log(SD+1) of a variety for a characteristic.

Table 1 summarizes the various standard probability levels needed to calculate the COYD and COYU criteria in each of Cases A to D. For example, in Case B only one probability level is needed (pu3), whereas Case C requires two (pu2 and pu3).

|  |  |  |  |
| --- | --- | --- | --- |
| Table 1 | COYU | | |
| CASE | pu2 | pnu2 | pu3 |
| A |  |  |  |
| B |  |  |  |
| C |  |  |  |
| D |  |  |  |

Figure 1. COYU decisions and standard probability levels (pi ) in Case A

COYU Decision after 2nd cycle

|  |  |  |
| --- | --- | --- |
| CANDIDATE  VARIETY  NON  UNIFORM  variety  UNIFORM  for the  characteristic  U < UCpu2  (e.g.pu2 = 0.00~~2~~3)  U > UCpu2  (e.g.pu2 = 0.00~~2~~3) |  |  |

Figure 2. COYD and COYU decisions and standard probability levels (pi ) in Case B

COYU Decision after 3rd cycle

|  |  |  |
| --- | --- | --- |
| U > UCpu3  (e.g. pu3 = 0.00~~2~~3)  U < UCpu3  (e.g. pu3 = 0.00~~2~~3)  UNIFORM  for the  characteristic  NON  UNIFORM  variety  CANDIDATE  VARIETY |  |  |

NOTE:-

“U” is the mean adjusted log(SD+1) of the candidate variety for the characteristic.

UCp is the COYU criterion calculated at probability level p.

Figure 3. COYU decisions and standard probability levels (pi ) in Case C

COYU Decision after 2nd cycle Decision after 3rd cycle

|  |  |  |
| --- | --- | --- |
| CANDIDATE  VARIETY  U > UCpu2  (e.g. pu2 = 0.00~~2~~3)  Go to 3rd  cycle  UNIFORM  for the  characteristic  NON  UNIFORM  variety  U < UCpu3  (e.g. pu3 = 0.002)  U > UCpu3  (e.g. pu3 = 0.002)  U < UCpu2  (e.g.pu2 = 0.00~~2~~3)  UNIFORM  for the  characteristic |  |  |

Figure 4. COYD and COYU decisions and standard probability levels (pi ) in Case D

COYU Decision after 2nd cycle Decision after 3rd cycle

|  |  |  |
| --- | --- | --- |
| CANDIDATE  VARIETY  NON  UNIFORM  variety  Go to 3rd  cycle  UNIFORM  for the  characteristic  NON  UNIFORM  variety  U < UCpu3  (e.g. pu3 = 0.00~~2~~3)  U > UCpu3  (e.g. pu3 = 0.00~~2~~3)  U < UCpu2  (e.g. pu2 = 0.02)  U > UCpnu2  (e.g. pnu2 = 0.00~~2~~3)  UNIFORM  for the  characteristic  pnu2=0.00~~2~~3)  UCpu2 < U< UCpnu2  (e.g. pu2 = 0.02, |  |  |

NOTE:-

“U” is the mean adjusted log(SD+1) of the candidate variety for the characteristic

UCp is the COYU criterion calculated at probability level p

9.13 References

Roberts A.M.I., Kristensen K (2015) An improved Combined-Over-Year Uniformity Criterion for assessing uniformity based on quantitative characteristics. Biuletyn Oceny Odmian 34, 49-57.

[End of Annex and of document]