

Technical Working Party on Automation and Computer Programs TWC/38/6

**Thirty-Eighth Session
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THE COMBINED-OVER-YEARS UNIFORMITY CRITERION (COYU)

Document prepared by an expert from the United Kingdom

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EXECUTIVE SUMMARY

1. The purpose of this document is to present a draft replacement section on the method of calculation of the Combined Over Years Uniformity Criterion (COYU) for document TGP/8 “Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability”. It also gives an update on software development.
2. The TWC is invited to:
 - (a) consider the proposed draft text for document TGP/8, Sections 9 and 10, as presented in the Annexes to this document, and
 - (b) note progress on software development for COYU and that a timetable for evaluation of the software will be proposed to the TWC, at its thirty-eight session.

3. The structure of this document is as follows:

EXECUTIVE SUMMARY	1
BACKGROUND	1
PROGRESS SINCE THE THIRTY-SEVENTH SESSION OF THE TWC	3

- ANNEX I Draft replacement section on the method of calculation of the current COYU method for document TGP/8
 ANNEX II Draft new section on the improved COYU method for document TGP/8

4. The following abbreviations are used in this document:

- TC: Technical Committee
- TC-EDC: Enlarged Editorial Committee
- TWA: Technical Working Party for Agricultural Crops
- TWC: Technical Working Party on Automation and Computer Programs
- TWF: Technical Working Party for Fruit Crops
- TWO: Technical Working Party for Ornamental Plants and Forest Trees
- TWPs: Technical Working Parties
- TWV: Technical Working Party for Vegetables

BACKGROUND

5. 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”). This document concerns the implementation of improvements to the method.

6. 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."

7. 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."

8. 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)."

9. 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:

- the plots showing the relationship between uniformity and level of expression has been improved by adding points for candidates; and
- calculations had been added to show the level of extrapolation (method b using degree of inflation) for each candidate.

10. Also at the thirty-sixth session, the TWC agreed to invite the expert from the United Kingdom to draft a replacement section for document TGP/8 on the method of calculation of the Combined-Over-Years Uniformity Criterion.

11. At the thirty-seventh session of the TWC, held in Hangzhou, China, from October 14 to 16, 2019, the TWC considered a proposal for revision of document TGP/8, Section 9 "The Combined Over Years Uniformity Criterion (COYU)" in document TWC/37/7. The TWC agreed to invite the expert from the United Kingdom to prepare a revised version of the draft guidance at its thirty eighth session. Following an update on progress, the TWC agreed to invite members who use "R" or "DUST" Software to review the new COYU package to

identify possible improvement points. The TWC noted the expression of interest by experts from China, Finland, France and the United Kingdom to review the new COYU package.

PROGRESS SINCE THE THIRTY-SEVENTH SESSION OF THE TWC

12. Following the thirty-seventh session of the TWC, editorial suggestions were received from TWC experts for the draft guidance on the improved COYU method. Based on these suggestions, it is proposed that there should be two sections covering COYU in document TGP/8.

13. The first of these two sections would describe the current method, now described as the COYU pre-2020 version, and be a light revision of the existing text in TGP/8/4 “Trial design and techniques used in the examination of distinctness, uniformity and stability” Part II Section 9. The introduction of this section is revised to refer to the new version of COYU, described as the COYU improved version, and to recommend that this new version be preferred. There is also a new subsection on the issue of extrapolation. The revised text for the current COYU method is given in Annex I of this document. The reference to 2020 can be updated once there is agreement to “launch” the new version.

14. The second section covers the improved method, described as the COYU improved version. It has a similar structure to the section on the current method. The proposed draft text for the improved COYU method is given in Annex II of this document.

15. Software for the improved COYU method is in development. It is planned that there will be an “R” package and a module in “DUSTNT”. Actually, the module in DUST incorporates the R code.

16. The R package for COYU is available for evaluation by experts. To date, an expert from France has expressed interest in reviewing this software. The COYU R package is available online at <https://github.com/BiomathematicsAndStatisticsScotland/coyus>

17. This year, development work has focussed on integrating the R code into DUSTNT. Learning from a previous practical exercise, the aim is ensure that experts find the installation of the new version of DUSTNT incorporating the new COYU quick and easy. This has meant background development work on DUSTNT itself. Unfortunately, demands on the IT teams involved during the COVID-19 pandemic has delayed progress. There will be a further update on progress given at the thirty-eighth session of the TWC, when a timetable for the evaluation process for the software will also be proposed.

18. *The TWC is invited to:*

(a) consider the proposed draft text for document TGP/8, Sections 9 and 10, as presented in the Annexes to this document, and

(b) note progress on software development for COYU and that a timetable for evaluation of the software will be proposed to the TWC, at its thirty-eight session.

[Annexes follows]

PROPOSED DRAFT TEXT FOR DOCUMENT TGP/8, SECTIONS 9

Note for Draft version

Strikethrough (highlighted in grey) indicates deletion from the text of document TGP/8/4.

Underlining (highlighted in grey) indicates insertion to the text of document TGP/8/4.

9. THE COMBINED-OVER-YEARS UNIFORMITY CRITERION (COYU) – PRE-2020 VERSION9.1 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”).

This document describes the previous version of COYU, which since 2020 has been superseded by an improved version. It is recommended that the improved version be used. Please see TGP/8, section 10 “The Combined-over-years uniformity criterion (COYU) – improved version”.

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 (SD) approach can be used. It further states the following with respect to determination of the acceptable level of variation.

“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 section 9.447

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, SD_j , over replicates:

$$SD_j = \sqrt{\frac{\sum_{i=1}^n (y_{ij} - \bar{y}_j)^2}{(n-1)}}$$

$$SD = \frac{\sum_{j=1}^r SD_j}{r}$$

where y_{ij} is the observation on the i^{th} plant in the j^{th} plot, \bar{y}_j is the mean of the observations from the j^{th} plot, n is the number of plants measured in each plot and r is the number of replicates.

Step 2: Transformation of the SDs

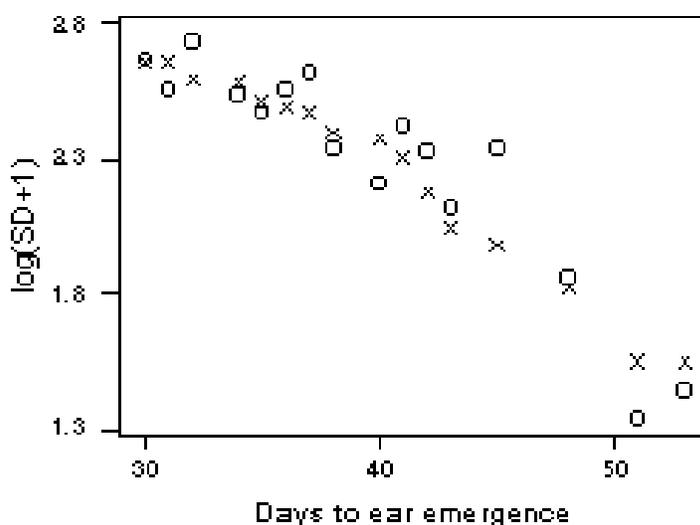
9.6.2 Transformation of SDs by adding 1 and converting to natural logarithms. The purpose of this transformation is to make the SDs more amenable to statistical analysis.

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

9.6.3 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 $Y_{i-4}, Y_{i-3}, \dots, Y_{i+4}$ where i represents the rank of the X value and Y_i is the corresponding Y value. For X values ranked 1st and 2nd the trend value is taken to be the mean of the first three values. In the case of the X value ranked 3rd the mean of the first five values are taken and for the X value ranked 4th the mean of the first seven values are used. A similar procedure operates for the four highest-ranked X values.

9.6.4 A simple example in Figure 1 illustrates this procedure for 16 varieties. The points marked "O" in Figure 1a 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.

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)



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

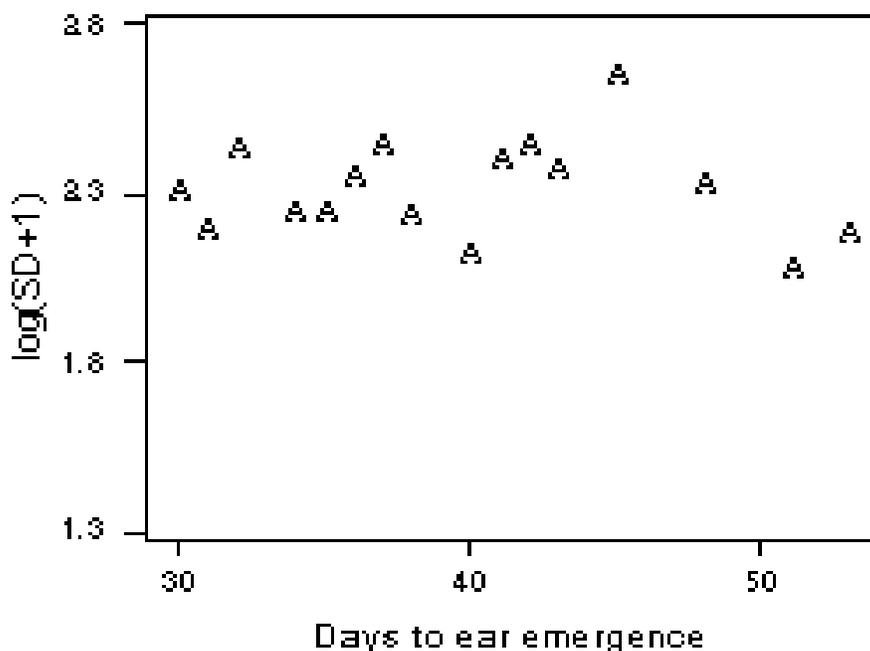
9.6.5 Once the trend values for the comparable varieties have been determined, the trend values for candidates are estimated using linear interpolation between the trend values of the nearest two comparable varieties as defined by their means for the characteristic. Thus if the trend values for the two 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 $X_i \leq X_c \leq X_{i+1}$, then the trend value T_c for the candidate is given by

$$T_c = \frac{(X_c - X_i)T_{i+1} + (X_{i+1} - X_c)T_i}{X_{i+1} - X_i}$$

9.6.6 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.

9.6.7 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 is for adjusted SD)



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

$$UC_p = SD_r + t_p \sqrt{V \left(\frac{1}{k} + \frac{1}{Rk} \right)}$$

where SD_r is the mean of adjusted log SDs for the comparable varieties, V is the variance of the adjusted log SDs after removing year effects, t_p is the one-tailed t-value for probability p with degrees of freedom as for V, k is the number of years and R is the number of comparable varieties.

9.7 Early decisions for a three-year test

9.7.1 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.

9.7.2 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%, a candidate variety can be rejected after two years if its uniformity exceeds the COYU criterion with probability level 0.2%.

9.7.3 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%, a candidate variety can be accepted after two years if its uniformity does not exceed the COYU criterion with probability level 2%.

9.7.4 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%. In this case, such varieties should be re-assessed after three years.

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

Variety	Character Means			Within Plot SD			Log (SD+1)		
	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, T_c , 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

$$T_c = \frac{(X_c - X_i)T_{i+1} + (X_{i+1} - X_c)T_i}{X_{i+1} - X_i} = \frac{(52 - 38) \times 2.28 + (63 - 52) \times 2.21}{63 - 38} = 2.28$$

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	$(2.25 + \dots + 2.42)/5 = 2.35$	$2.39 - 2.35 + 2.39 = 2.42$
R5	69	2.50	$(2.25 + \dots + 2.48)/7 = 2.38$	$2.50 - 2.38 + 2.39 = 2.52$
R4	71	2.42	$(2.25 + \dots + 2.32)/9 = 2.38$	$2.42 - 2.38 + 2.39 = 2.43$
R6	74	2.38	$(2.21 + \dots + 2.53)/9 = 2.41$	$2.38 - 2.41 + 2.39 = 2.36$
R8	75	2.48	$(2.39 + \dots + 2.34)/9 = 2.42$	$2.48 - 2.42 + 2.39 = 2.44$
R7	76	2.46	$(2.42 + \dots + 2.34)/7 = 2.42$	$2.46 - 2.42 + 2.39 = 2.43$
R11	76	2.32	$(2.48 + \dots + 2.34)/5 = 2.43$	$2.32 - 2.43 + 2.39 = 2.28$
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

Variety	Over-Year Means		Adj. Log (SD+1)		
	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:

$$UC_p = SD_r + t_p \sqrt{V \left(\frac{1}{k} + \frac{1}{Rk} \right)} = 2.15 + 3.118 \times \sqrt{0.0202 \times \left(\frac{1}{3} + \frac{1}{3 \times 11} \right)} = 2.42$$

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 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.

9.910 Implementing COYU

The COYU criterion can be applied using COYU 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> <https://www.afbini.gov.uk/articles/distinctness-uniformity-and-stability-trials-dust-software>.

9.4011 COYU software

9.4011.1 DUST computer program

9.4011.1.1 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.

9.4011.1.2 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.

9.4011.1.3 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.

9.4011.1.4 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.

9.4011.1.5 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.

Table A1: Example of summary output from COYU program

**** OVER-YEARS UNIFORMITY ANALYSIS SUMMARY ****

WITHIN-PLOT STANDARD DEVIATIONS AS % MEAN OF
REFERENCE VARIETY SDS

	CHARACTERISTIC NUMBER				
	5	60	8	10	11
R1	100	100	95 1	100	97 97
R2	105	106	98	99	104 101
R3	97	103	92 1	103	96 98
R4	102	99	118 2	105	101 101
R5	102	99	116 3	95	104 110
R6	103	102	101	99	97 104
R7	100	95	118 2	102 1	98 99
R8	97	98	84	95	97 93
R9	97	105	87	99	101 99
R10	104	100	96	105 1	96 102
R11	99	96	112	99	101 98
R12	100	97	99 1	103	105 106
R13	95	96	101	100	96 101
R14	105	103	90	97	101 97
R15	102	100 1	89	105	105 1 101
R16	99	98	92 1	98	102 98
R17	97	101	98	101	101 95
R18	99	97	96	96	102 99
R19	103	101	105	102	100 98
R20	104	99	93	91	100 102
R21	97	94	103	97	100 102
R22	101	110*1	112	107 1	103 1 101
R23	94	101	107	99	104 97
R24	99	97	95	99	100 103
R25	104 1	103	93 1	99	101 96
R26	98	97	111 2	96	102 1 106
R27	102	99	106 1	99	103 107
R28	101	106	90	95	101 101
R29	101	105	83	102	94 93
R30	99	96	97	99	95 100
R31	99	102	107	107 1	102 99
R32	98	93	111 2	102	98 103
R33	104	102 1	107 1	103	100 97
R34	95	94	82	95	97 96
R35	100	102	95	100	99 94
R36	99	98	111 1	99	100 103
R37	100	107 1	107	101	100 107
R38	95	97	102	107 1	97 101
R39	99	99	90	98	101 100
R40	104	102	112 1	100	101 97
C1	100 1	106	113 2	104 1	106 1 106
C2	103	101	98	97	101 109
C3	97	93	118 2	98	99 109
C4	102	101	106	103	99 101
C5	100	104	99	103	100 107
C6	101	102	103	100	103 107
C7	96	98	106	97	102 103
C8	101	105 1	116 2	103	103 93
C9	99	99	90 2	91	97 98

CHARACTERISTIC

5	SPRING	60	NATURAL SPRIN
8	DATE OF EAR	10	HEIGHT AT EAR
11	WIDTH AT EAR	14	LENGTH OF FLA
15	WIDTH OF FLAG	24	EAR LENGTH

SYMBOLS

- * - SD EXCEEDS OVER-YEARS CRITERION AFTEF
- + - SD EXCEEDS OVER-YEARS CRITERION AFTEF
- : - SD NOT YET ACCEPTABLE AFTER 2 YEARS
- 1, 2, 3 - THE NUMBER OF OCCASIONS THE WITHIN-YE

Table A2: Example of supplementary DUST output for date of ear emergency (char.8)

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

VARIETY	OVER-YEARS			INDIVIDUAL YEARS									
	CHAR.	ADJ.	UNADJ	CHAR. MEAN			LOG (SD+1)			ADJ LOG(SD+1)			
	MEAN	LOG SD	LOG SD	88	89	90	88	89	90	88	89	90	
REFERENCE													
R3	38.47	1.823	2.179	39.07	41.21	35.12	2.02	2.18	2.34X	1.73	1.78	1.96	
R5	50.14	2.315	2.671	48.19	53.69	48.54	2.52X	2.74X	2.76X	2.23	2.33	2.39	
R16	59.03	1.833	2.179	57.25	63.33	56.50	2.28X	2.24	2.01	1.96	1.73	1.81	
R26	63.44	2.206	2.460	61.00	66.53	62.81	2.50X	2.75X	2.13	2.18	2.33	2.11	
R9	63.99	1.739	1.994	62.92	68.32	60.72	2.21	2.03	1.74	1.96	1.64	1.62	
R12	66.12	1.964	2.086	67.89	65.35	65.12	2.07	2.58X	1.60	1.97	2.14	1.78	
R33	67.58	2.124	2.254	66.66	71.54	64.53	2.55X	2.26	1.95	2.32	1.92	2.12	
R1	67.87	1.880	1.989	69.07	70.64	63.90	1.60	2.45X	1.93	1.60	2.08	1.96	
R20	68.74	1.853	1.893	67.17	74.31	64.74	2.05	1.95	1.68	1.92	1.75	1.89	
R25	68.82	1.853	1.905	68.28	72.38	65.81	1.83	2.39X	1.49	1.75	2.09	1.72	
R18	69.80	1.899	1.853	68.61	75.22	65.58	1.88	1.84	1.84	1.82	1.80	2.08	
R30	70.53	1.919	1.864	70.36	75.08	66.15	2.04	1.84	1.71	2.00	1.78	1.98	
R13	70.63	2.005	2.000	70.23	75.00	66.66	1.97	2.03	2.01	1.91	1.86	2.24	
R32	71.49	2.197	2.238	70.03	74.98	69.44	2.32X	2.45X	1.94	2.31	2.27	2.01	
R34	72.09	1.630	1.545	71.32	77.35	67.59	1.57	1.49	1.58	1.54	1.58	1.78	
R40	72.24	2.222	2.178	72.71	75.07	68.95	2.25X	2.26	2.03	2.29	2.16	2.22	
R23	72.40	2.122	2.058	69.72	78.39	69.10	2.11	2.14	1.93	2.16	2.14	2.06	
R29	72.66	1.657	1.580	73.13	75.80	69.04	1.46	1.63	1.65	1.47	1.69	1.81	
R7	73.19	2.341	2.342	72.23	75.80	71.52	2.62X	2.30X	2.10	2.61	2.30	2.11	
R24	73.19	1.888	1.796	74.00	76.37	69.20	1.62	1.84	1.93	1.71	1.91	2.04	
R19	73.65	2.083	2.049	73.32	76.06	71.57	1.96	2.05	2.14	1.96	2.13	2.16	
R2	73.85	1.946	1.897	72.98	78.16	70.42	1.76	1.96	1.97	1.79	2.02	2.03	
R31	74.23	2.119	2.012	73.73	78.23	70.71	2.05	1.86	2.13	2.25	1.94	2.17	
R37	74.38	2.132	2.020	74.87	76.95	71.32	1.97	2.04	2.04	2.23	2.11	2.06	
R11	74.60	2.224	2.150	73.87	78.07	71.87	2.21	2.08	2.16	2.36	2.10	2.21	
R38	74.76	2.029	1.916	76.11	78.24	69.93	1.84	2.15	1.75	1.98	2.24	1.87	
R8	74.83	1.677	1.593	74.27	78.77	71.45	1.62	1.55	1.61	1.75	1.64	1.64	
R15	75.54	1.760	1.682	75.72	78.68	72.22	1.53	1.79	1.73	1.64	1.84	1.80	
R10	75.64	1.915	1.847	73.47	79.24	74.23	1.87	1.66	2.00	1.99	1.78	1.98	
R22	75.68	2.228	2.133	74.57	79.17	73.32	2.18	2.21	2.01	2.40	2.26	2.03	
R14	75.84	1.797	1.688	74.53	79.56	73.43	1.54	1.63	1.90	1.70	1.76	1.93	
R17	76.13	1.942	1.832	75.34	79.09	73.96	1.65	2.04	1.81	1.90	2.10	1.83	
R39	76.83	1.781	1.676	75.49	80.50	74.50	1.56	1.51	1.96	1.72	1.70	1.92	
R35	77.22	1.886	1.773	76.67	80.85	74.15	1.73	1.67	1.92	1.88	1.85	1.93	
R4	77.78	2.349	2.268	76.80	81.22	75.33	2.36X	2.13	2.31X	2.52	2.33	2.20	
R36	77.98	2.209	2.173	78.97	79.85	75.11	2.13	2.15	2.25X	2.24	2.21	2.18	
R6	78.73	2.009	1.935	77.53	82.88	75.78	2.00	1.75	2.06	2.03	2.09	1.91	
R27	78.78	2.116	2.098	77.61	80.03	78.69	1.80	2.25	2.24X	1.87	2.39	2.09	
R28	79.41	1.785	1.722	78.28	81.99	77.97	1.68	1.43	2.05	1.79	1.67	1.89	
R21	80.52	2.045	1.950	77.43	85.02	79.11	1.98	1.75	2.13	2.07	2.09	1.98	

CANDIDATE	CHAR.	ADJ.	UNADJ	88	89	90	LOG (SD+1)	LOG (SD+1)	LOG (SD+1)	ADJ LOG(SD+1)	ADJ LOG(SD+1)	ADJ LOG(SD+1)
C1	64.03	2.252	2.438	63.85	63.33	64.92	2.49X	2.81X	2.02	2.25	2.29	2.21
C2	86.11	1.940	1.837	84.83	88.63	84.85	1.79	1.71	2.01	1.90	2.05	1.87
C3	82.04	2.349	2.248	82.26	87.45	76.40	2.37X	2.03	2.35X	2.48	2.37	2.20
C4	78.63	2.104	2.033	78.01	82.17	75.72	2.05	2.01	2.04	2.15	2.27	1.90
C5	72.99	1.973	1.869	71.98	79.40	67.59	1.95	1.78	1.88	1.93	1.90	2.08
C6	83.29	2.050	1.947	84.10	85.57	80.21	2.05	1.69	2.10	2.16	2.03	1.96
C7	83.90	2.100	1.997	84.12	87.99	79.60	1.93	1.95	2.11	2.04	2.29	1.97
C8	83.50	2.304	2.201	82.43	85.98	82.08	2.27X	2.00	2.34X	2.38	2.33	2.20
C9	51.89	1.788	2.157	52.35	55.77	47.56	1.83	2.34X	2.31X	1.52	1.91	1.93

MEAN OF REFERENCE	CHAR.	ADJ.	UNADJ	88	89	90	LOG (SD+1)	LOG (SD+1)	LOG (SD+1)	ADJ LOG(SD+1)	ADJ LOG(SD+1)	ADJ LOG(SD+1)
	71.47	1.988		70.78	74.97	68.65	1.97	2.03	1.96	1.99	1.99	1.99

UNIFORMITY CRITERION	PROB. LEVEL
3-YEAR REJECTION	2.383 0.002
2-YEAR REJECTION	2.471 0.002
2-YEAR ACCEPTANCE	2.329 0.020

**** ANALYSIS OF VARIANCE OF ADJUSTED LOG(SD+1) ****

	DF	MS	F RATIO
YEARS	2	0.06239	
VARIETIES	39	0.11440	5.1
RESIDUAL	78	0.02226	
TOTAL	119	0.05313	

SYMBOLS

- * - SD EXCEEDS OVER-YEARS UNIFORMITY CRITERION AFTER 3 YEARS.
- + - SD EXCEEDS OVER-YEARS UNIFORMITY CRITERION AFTER 2 YEARS.
- : - SD NOT YET ACCEPTABLE ON OVER-YEARS CRITERION AFTER 2 YEARS.
- X - SD EXCEEDS 1.265 TIMES MEAN OF REFERENCE VARIETIES

9.4412 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 (p_{u2} , p_{nu2} and p_{u3}) 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 :-
p_{u2}	uniform in a characteristic after 2 cycles
p_{nu2}	non-uniform after 2 cycles
p_{u3}	uniform in a characteristic after 3 cycles

In Figures 1 to 4 the COYU criterion calculated using say the probability level p_{u2} is denoted by $UC_{p_{u2}}$ 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 (p_{u3}), whereas Case C requires two (p_{u2} and p_{u3}).

CASE	COYU		
	p_{u2}	p_{nu2}	p_{u3}
A			
B			
C			
D			

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

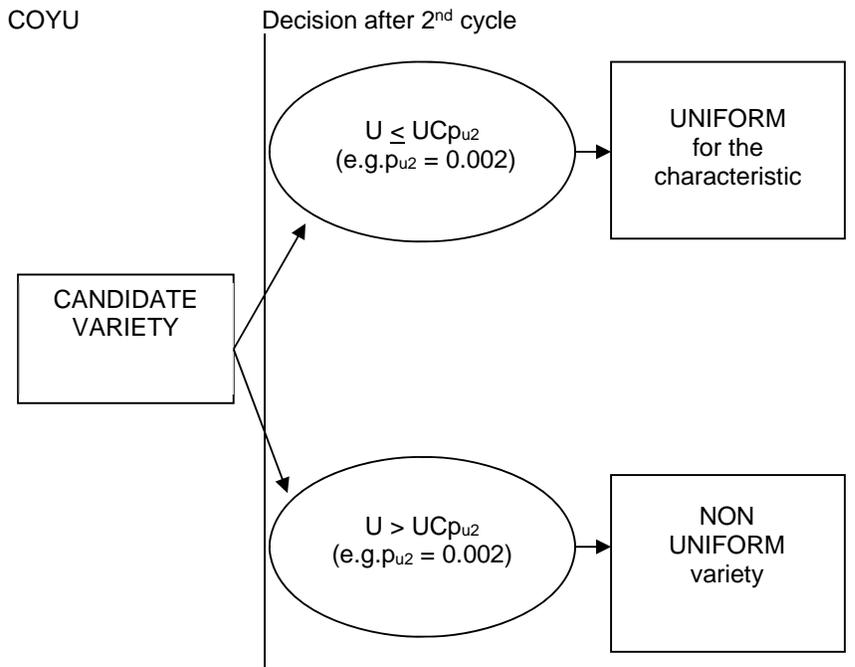
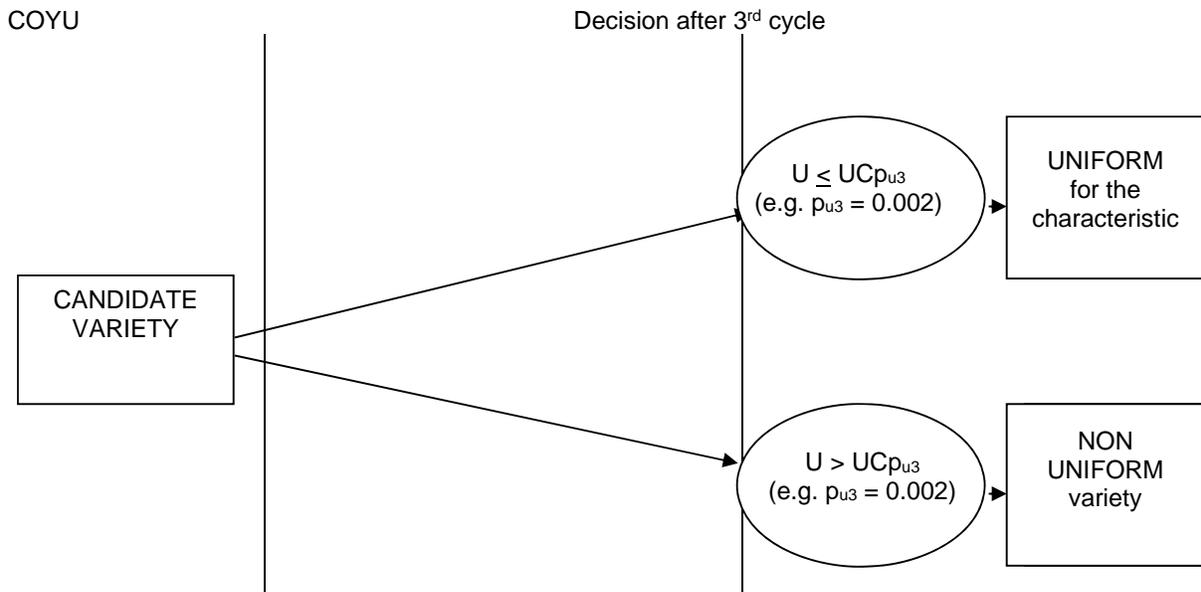


Figure 2. COYD and COYU decisions and standard probability levels (p_i) in Case B



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 (p_i) in Case C

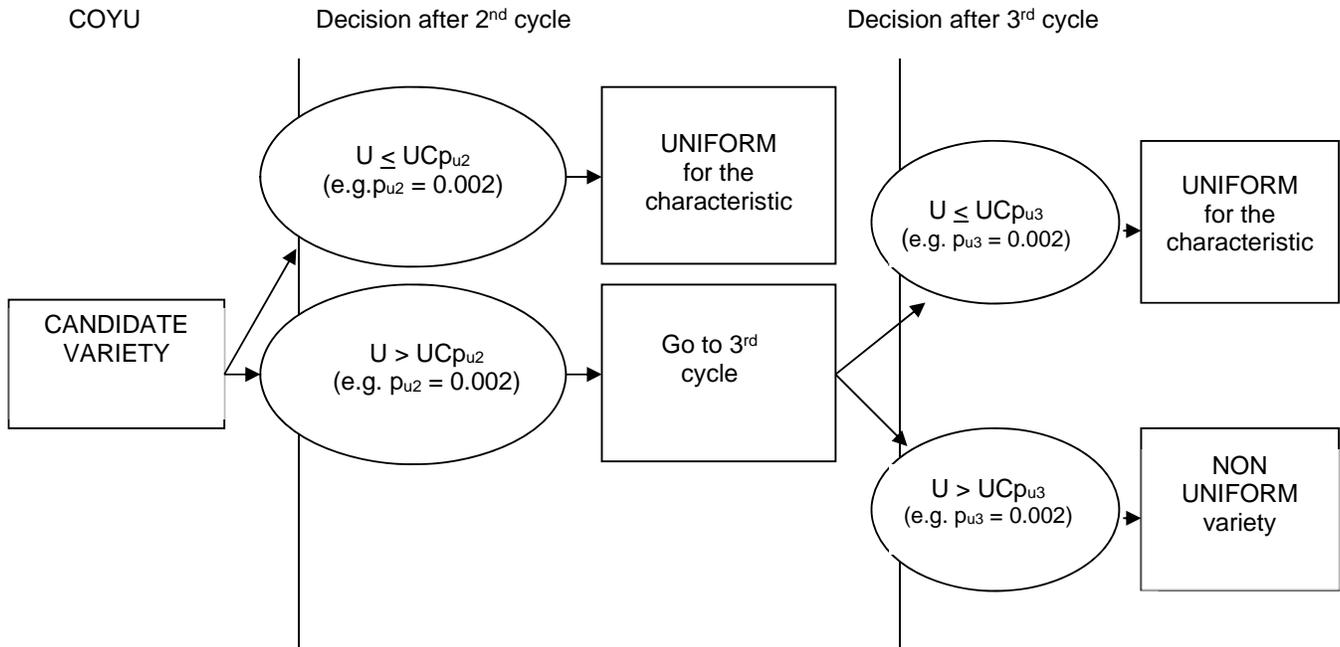
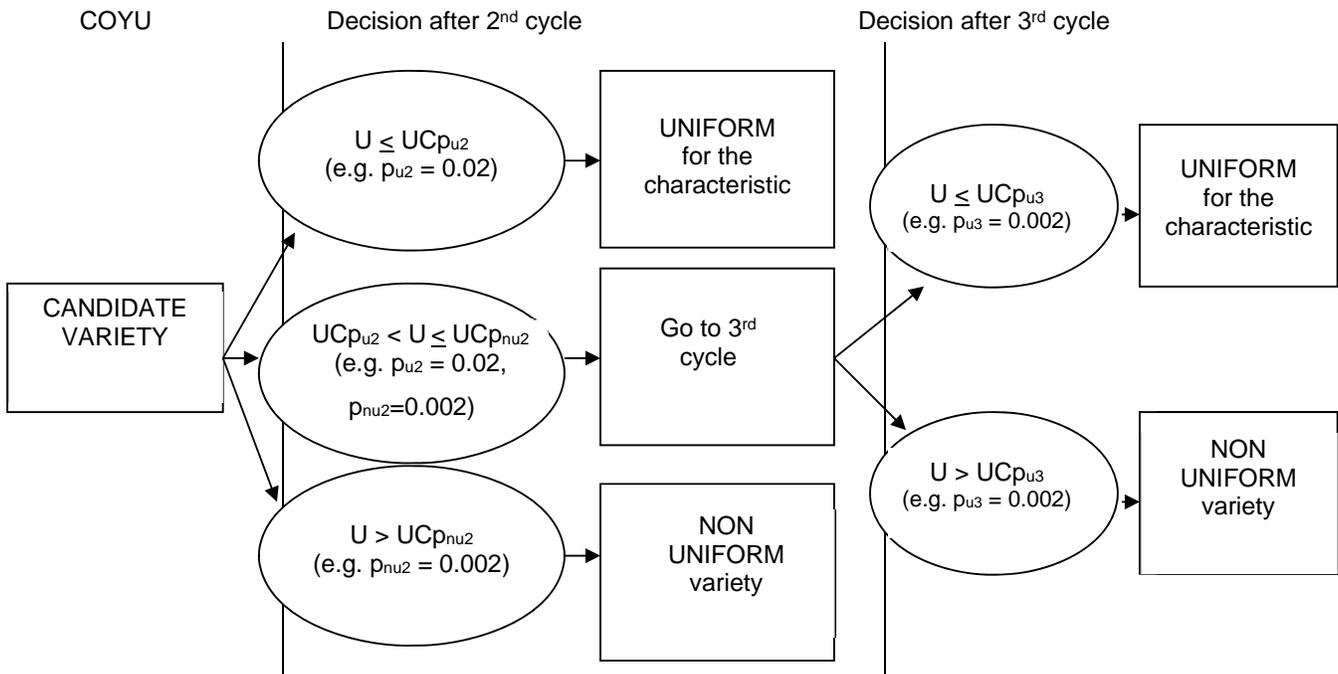


Figure 4. COYD and COYU decisions and standard probability levels (p_i) in Case D



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

PROPOSED DRAFT TEXT FOR DOCUMENT TGP/8, SECTION 10

Note for Draft version

~~Strikethrough~~ (highlighted in grey) indicates deletion from the text proposed in document TWC/37/7.

Underlining (highlighted in grey) indicates insertion to the text proposed in document TWC/37/7.

9-10. THE COMBINED-OVER-YEARS UNIFORMITY CRITERION (COYU) – IMPROVED VERSION**9-10.1 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”).

This document describes the improved version of COYU, which supersedes the previous version (see TGP/8, section 9 “The Combined-over-years uniformity criterion – pre-2020 version (COYU)”. It is recommended that this improved version be used.

9-10.2 Summary

9-10.2.1 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.

“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.”

9-10.2.2 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.

9-10.2.3 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.

9-10.2.4 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.

9-10.2.5 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.

9-10.3 Introduction

9-10.3.1 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.

9-10.3.2 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.

9-10.3.3 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.

9-10.4 The COYU Criterion

9-10.4.1 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 9-10.6.

- 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.

9-10.4.2 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.

9-10.5 Use of COYU

9-10.5.1 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.

9-10.5.2 A variety is considered to be uniform for a characteristic if its mean adjusted log SD does not exceed the uniformity criterion.

9-10.5.3 The probability level “p” used to determine the uniformity criterion depends on the crop. Recommended probability levels are given in section 9-10.7 and 9-10.8.

9-10.5.4 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.

9-10.5.5 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 12 comparable varieties for a COYU test based on two years of trials and 11 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.

9-10.6 Mathematical details

Step 1: Derivation of the within-plot standard deviation

9-10.6.1 Within-plot standard deviations for each variety in each year are calculated by averaging the plot between-plant standard deviations, SD_j , over replicates:

$$SD_j = \sqrt{\frac{\sum_{i=1}^n (y_{ij} - \bar{y}_j)^2}{(n-1)}}$$

$$SD = \frac{\sum_{j=1}^r SD_j}{r}$$

where y_{ij} is the observation on the i^{th} plant in the j^{th} plot, \bar{y}_j is the mean of the observations from the j^{th} plot, n is the number of plants measured in each plot and r is the number of replicates.

Step 2: Transformation of the SDs

9-10.6.2 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.

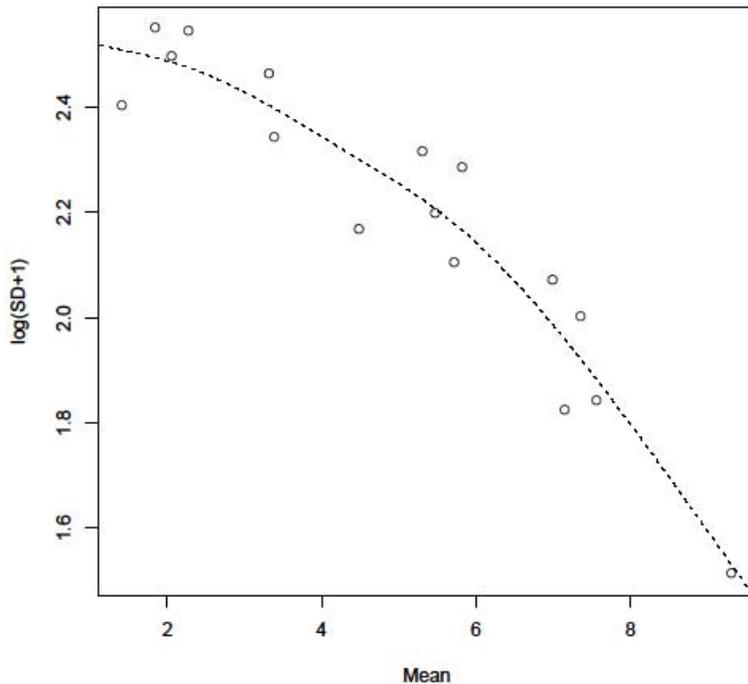
Step 3: Estimation of the relationship between the SD and mean in each year

9-10.6.3 The revised version of COYU uses the method of splines rather the moving average approach used in the previous procedure.

9-10.6.4 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 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.

9-10.6.5 A simple example using simulated data illustrates this procedure for 16 varieties in Figure 1. The points marked "O" in Figure 1 represent the log SDs and the corresponding means of 16 varieties. The dashed line represents the fitted smoothing spline.

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



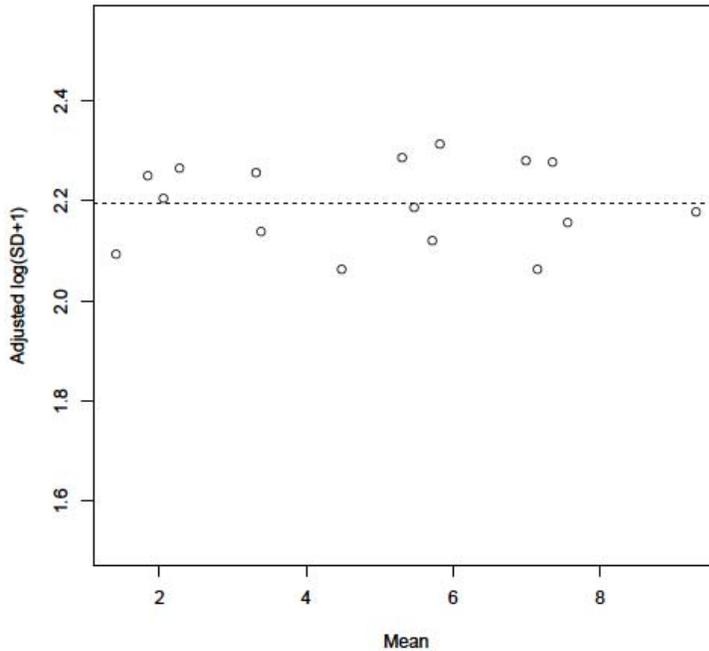
Step 4: Adjustment of transformed SD values based on estimated SD-mean relationship

9-10.6.6 Once the trend values for the comparable varieties have been determined, the trend values for candidates are estimated using predictions from the spline.

9-10.6.7 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.

9-10.6.8 The results for the simple example with 16 varieties are illustrated in Figure 2.

Figure 2: Adjusting for association between SD and mean (symbol O is for adjusted SD, dashed line is the grand mean)



Step 5: Calculation of the uniformity criterion

9-10.6.9 The maximum allowable standard deviation (the uniformity criterion), based on k years of trials, is in the form

$$UC = SD_r + t_p \sqrt{V_c}$$

where SD_r is the mean of adjusted log SDs for the comparable varieties, V_c is a variance specific to the candidate variety (related to the uncertainty of the spline prediction), t_p 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).

9-10.6.10 The uniformity criterion is specific to the candidate and depends on its level of expression relative to the comparable varieties.

9-10.7 Probability levels

9-10.7.1 For the current procedure, it is recommended that a probability level of 0.3% is used. This level has been chosen to align decisions with the previous procedure, where a probability level 0.1% was commonly used.

10.7.2 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.

9-10.8 Early decisions for a three-year test

~~9-10.8.1~~ 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.

~~9-10.8.2~~ 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 0.3%, a candidate variety can be rejected after two years if its uniformity exceeds the COYU criterion with probability level 0.3%.

~~9-10.8.3~~ 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 0.3%, a candidate variety can be accepted after two years if its uniformity does not exceed the COYU criterion with probability level 2%.

~~9-10.8.4~~ Some varieties may fail to be rejected or accepted after two years. In the example set out in section ~~9-10.8~~, a variety might have a uniformity that exceeds the COYU criterion with probability level 2% but not the criterion with probability level 0.3%. 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.

9-10.9 Extrapolation

~~9-10.9.1~~ If a candidate has a level of expression in a characteristic outside that seen in other seen in other varieties, we call this “extrapolation”.

~~9-10.9.2~~ 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.”*

~~9-10.9.3~~ If the level of expression is very different from other varieties in test, it should be considered whether these varieties are actually comparable.

~~9-10.9.3~~ The COYU ~~procedure software~~ 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.

~~9-10.9.4~~ 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.

~~9-10.9.4~~ 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.

~~9-10.9.5~~ 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.

9-10.10 Implementing COYU

~~9-10.9.1~~ 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> <https://www.afbini.gov.uk/articles/distinctness-uniformity-and->

[stability-trials-dust-software](https://github.com/BiomathematicsAndStatisticsScotland/coyus/). There is also an R package. This can be found at <https://github.com/BiomathematicsAndStatisticsScotland/coyus/>.

9.10.11 Example of the use of COYU software

9.10.11.1 DUST computer program

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

9.10.11.1.2 Table A1 shows an example of the detailed results for a characteristic (date of ear emergence). This was for a two-year test. In this case, neither candidate exceeds the COYU criterion (with probability 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. Note a '!' indicates a problem with extrapolation for candidate C1, with a rather large extrapolation factor of 6.0. This is clear in the Figure.

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

8 - DATE EE

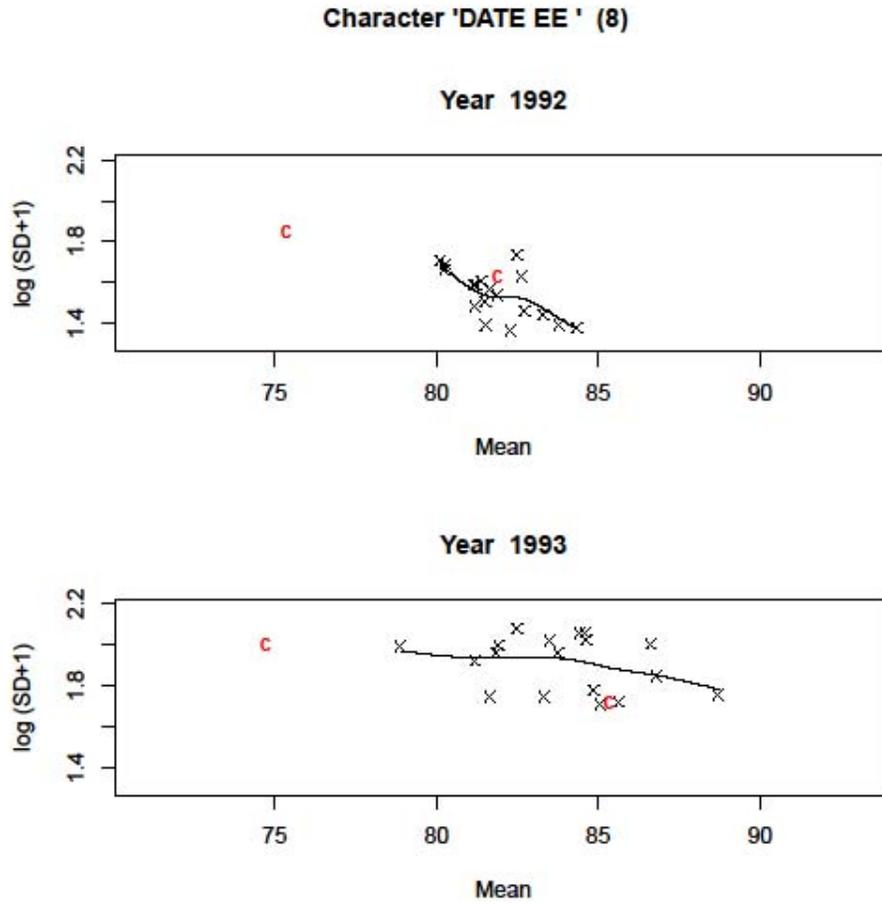
**** UNIFORMITY ANALYSIS OF BETWEEN-PLANT STANDARD DEVIATIONS (SD) ****

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



9-10.11.1.3 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 C1 exhibits signs of extrapolation in several characteristics. So the expert would be advised to look at this candidate with care.

TABLE A2: Example of summary output from the COYUS program

CANDIDATE SUMMARY

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

		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	

9-10.11.1.4 The COYUS program also outputs a comma-separated value formatted file of results to allow easy transfer to Excel.

9-10.12 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 (p_{u2} , p_{nu2} and p_{u3}) 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 :-
p_{u2}	uniform in a characteristic after 2 cycles
p_{nu2}	non-uniform after 2 cycles
p_{u3}	uniform in a characteristic after 3 cycles

In Figures 1 to 4 the COYU criterion calculated using say the probability level p_{u2} is denoted by UCp_{u2} 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 (p_{u3}), whereas Case C requires two (p_{u2} and p_{u3}).

CASE	COYU		
	p_{u2}	p_{nu2}	p_{u3}
A			
B			
C			
D			

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

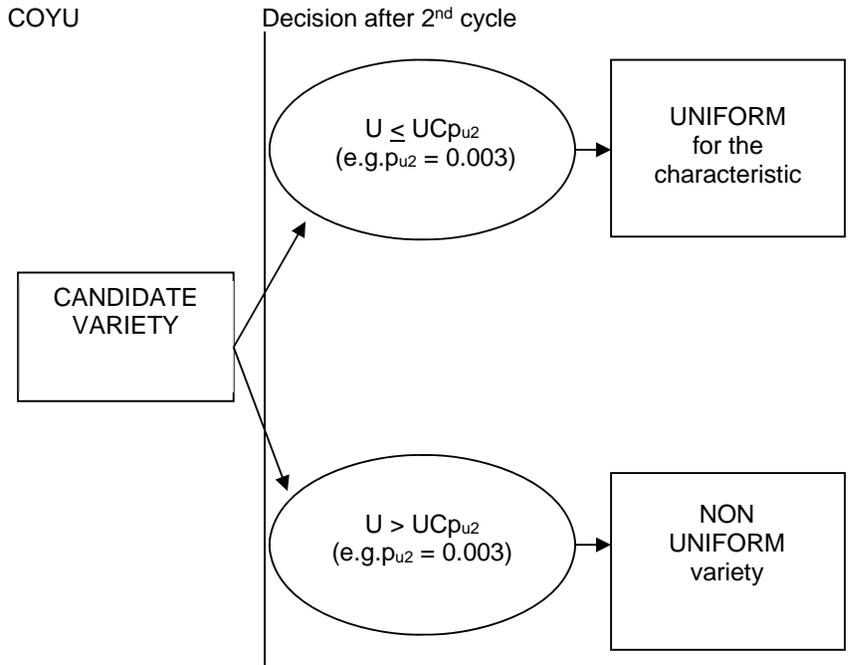
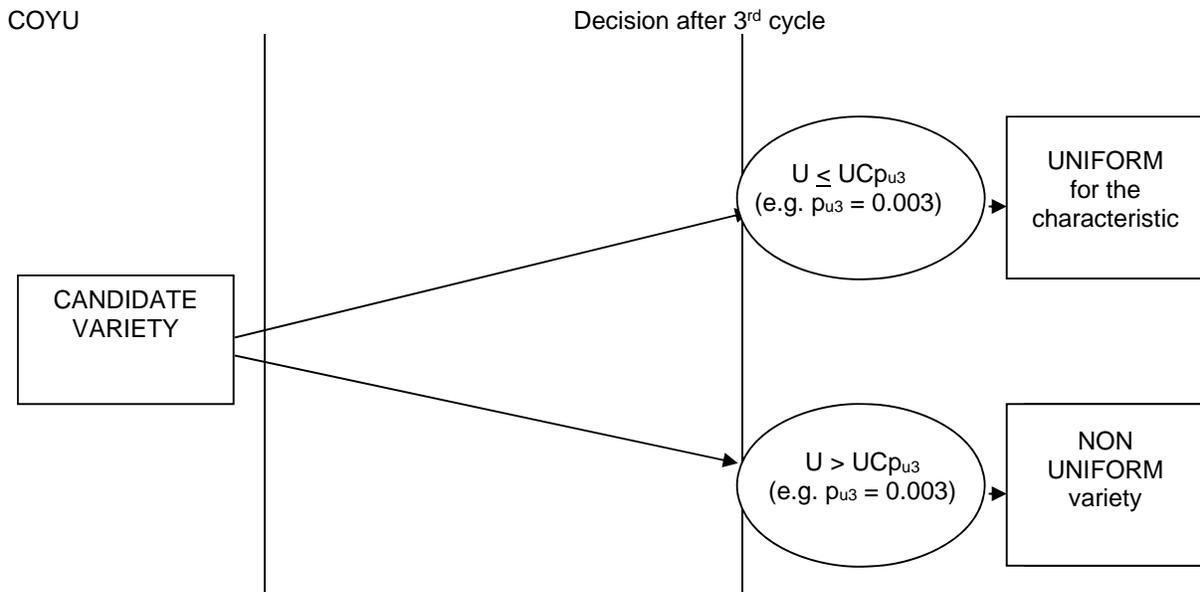


Figure 2. COYD and COYU decisions and standard probability levels (p_i) in Case B



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 (p_i) in Case C

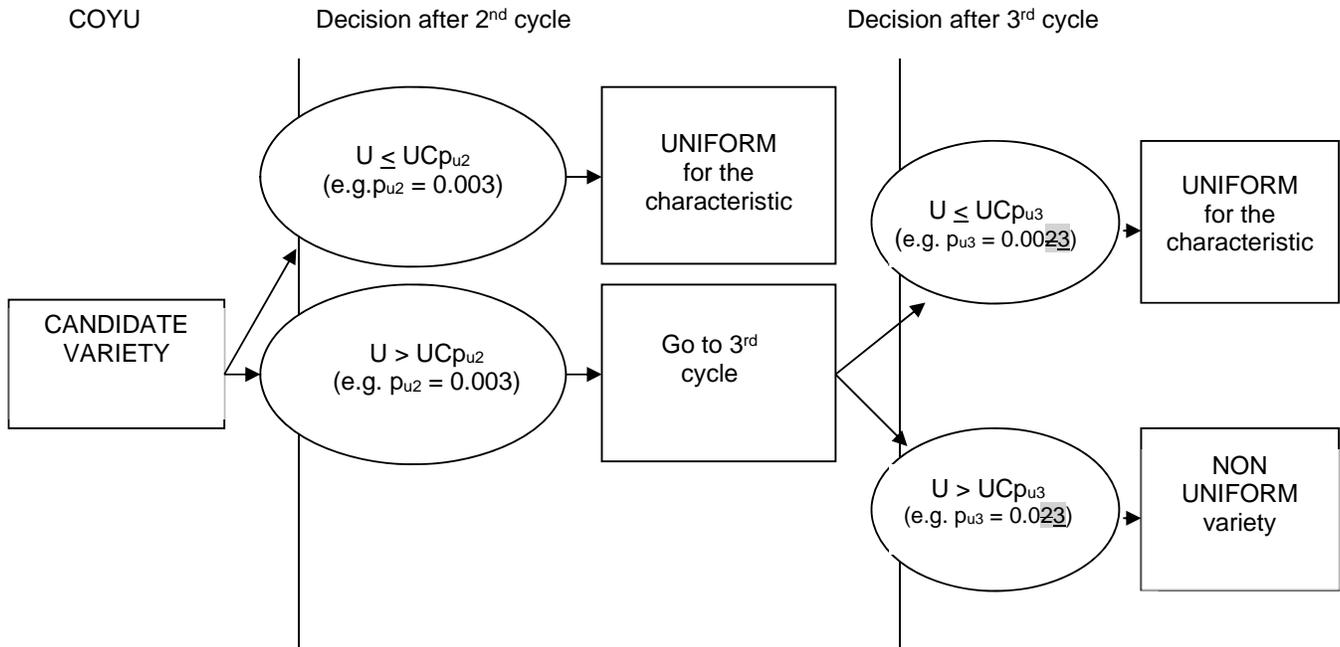
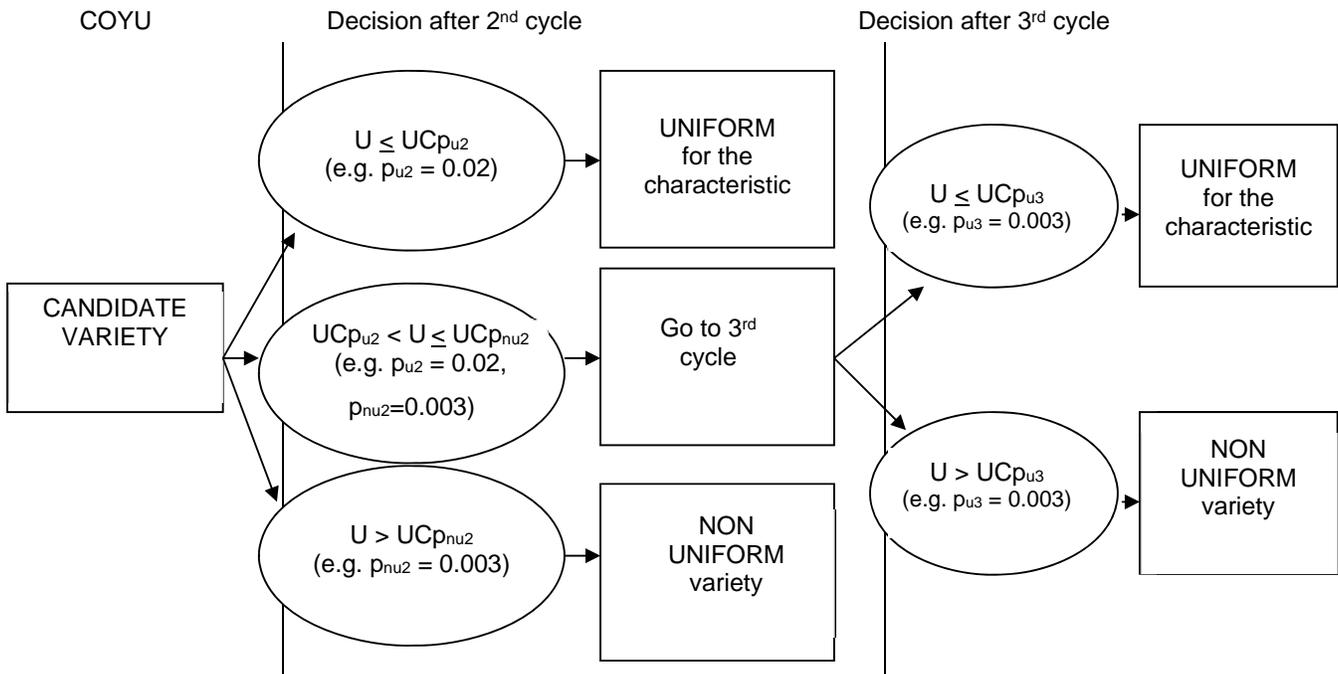


Figure 4. COYD and COYU decisions and standard probability levels (p_i) in Case D



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

9.10.13 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 II and of document]