UPOV

International Union for the Protection of New Varieties of Plants

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THE COMBINED-OVER-YEARS UNIFORMITY CRITERION (COYU)

Document prepared by the Office of the Union

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EXECUTIVE SUMMARY

1. The purpose of this document is to consider a proposal for a draft revision of document TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability", Section 9 "The Combined-Over-Years Uniformity Criterion (COYU)".

2. The TC is invited to:

(a) note the draft presented in the Annexes to this document for the revision of document TGP/8, Section 9 "The Combined-Over-Years Uniformity Criterion (COYU);"

(b) request the Technical Working Parties, at their sessions in 2021, to consider the proposed revision of document TGP/8, Section 9 "The Combined-Over-Years Uniformity Criterion (COYU);" on the basis of the draft presented in the Annexes to this document;

(c) note that evaluation versions of software for COYU Splines in both "R" and DUSTNT software are planned to be released in November 2020;

(d) note the expression of interest by experts from China, Finland, France and the United Kingdom to review the COYU Splines software;

(e) agree with the TWC to invite members to participate in a test campaign of the COYU Splines software until April 2021; and

(f) request that the TWC prepare a report of the results of the test campaign of the COYU Splines software for the TC for consideration in conjunction with the consideration of a revision of document TGP/8 by the TC at its fifty-seventh session.

3. The structure of this document is as follows:

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ANNEX I Draft revision of document TGP/8 on the current method of calculation of COYU
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. Previous background to this matter is provided in documents TC/55/4 and TC/55/4 Add. "TGP Documents".

6. The TC, at its fifty-fourth session[1], noted that the statistical development of the new method of calculation of COYU had been completed, including the establishment of the probability levels required to most closely match decisions using the current method for calculation of COYU. The TC noted the invitation by the TWC for the expert from the United Kingdom to draft a replacement section for document TGP/8 on the method of calculation of COYU (see document TC/54/25 "Report", paragraphs 221 to 224).

7. The TC, at its fifty-fifth session[2], considered documents TC/55/4 and TC/55/4 Add. (see document TC/55/25 "Report", paragraphs 154 to 156). The TC noted that the TWC, at its thirty-seventh session, had considered a draft replacement section for document TGP/8 on the method of calculation of COYU. The TC noted the invitation by the TWC for the expert from the United Kingdom to prepare a revised version of the draft guidance, to be presented to the TWC, at its thirty-eighth session.

DEVELOPMENTS AT THE TECHNICAL WORKING PARTIES

8. The TWV[3], TWO[4], TWA[5], and TWF[6], at their sessions in 2020, considered document TWP/4/11 "The Combined Over Years Uniformity Criterion (COYU)" (see documents TWV/54/12 "Report", paragraphs 33 to 37; TWO/52/11 "Report", paragraphs 16 to 20; TWA/49/7 "Report", paragraphs 21 to 26; and TWF/51/10 "Report", paragraphs 34 to 38).

9. The TWC[7] considered documents TWP/4/11 and TWC/38/6 "The Combined Over Years Uniformity Criterion (COYU)" (see document TWC/38/11 "Report", paragraphs 18 to 24).

10. The TWPs considered document TWP/4/11.

11. The TWPs noted the invitation by the TWC for members who use "R" or "DUST" Software to review the new COYU package to identify possible improvement points.

12. The TWPs noted the expression of interest by experts from China, Finland, France and the United Kingdom to review the new COYU package.

13. The TWPs noted the invitation for editorial suggestions to be communicated to the drafter from the United Kingdom on the proposed draft revision for document TGP/8, Section 9 "The Combined Over Years Uniformity Criterion (COYU)".

14. The TWPs noted the invitation for the expert from the United Kingdom to prepare a revised version of the draft guidance, to be presented to the TWC, at its thirty-eighth session.

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[1] held in Geneva on October 29 and 30, 2018

[2] held in Geneva on October 28 and 29, 2019,

[3] at its fifty-fourth session, hosted by Brazil and held via electronic means, from May 11 to 15, 2020

[4] at its fifty-second session, hosted by the Netherlands and held via electronic means, from June 8 to 12, 2020

[5] at its forty-ninth session, hosted by Canada and held via electronic means, from June 22 to 26, 2020

[6] at its fifty-first session, hosted by France and held via electronic means, from July 6 to 10, 2020

[7] at its thirty-eighth session, hosted by the United States of America and held via electronic means, from September 21 to 23, 2020

Technical Working Party on Agricultural Crops

15. The TWA, at its forty-ninth session, agreed that the COYU method was frequently used in the examination of agricultural crops and thanked the experts from the United Kingdom for the improvements to the method of calculation and its implementation in a new COYU package.

Technical Working Party on Automation and Computer Programs

Development of software incorporating the new COYU method

16. The TWC noted the progress on software development for COYU and the timetable for evaluation of the software. The TWC noted that evaluation versions in both "R" and DUSTNT software would be released in November 2020 and agreed to invite members to participate in a test campaign until April 2021. The TWC noted the expression of interest by experts from China, Finland, France and the United Kingdom to review the new COYU software.

Draft replacement section on the method of calculation of COYU for document TGP/8

17. The TWC considered the proposed draft text for document TGP/8, Sections 9 and 10, as reproduced in the Annexes to this document. The TWC noted that editorial suggestions provided to the drafter from the United Kingdom had been incorporated in the proposed draft revision for document TGP/8, Section 9 "The Combined Over Years Uniformity Criterion (COYU)" as presented in the Annexes to this document.

18. The TWC agreed that document TGP/8 should include two sections on the COYU criterion: one for the superseded version (moving average); and another for the improved method (splines). The TWC agreed that both sections were required for providing guidance to users of the different versions of the method.

19. The TWC agreed the following amendments to the draft guidance in Annex I to document TWC/38/6:

- Title: to be amended to read: "9. THE COMBINED-OVER-YEARS UNIFORMITY CRITERION (COYU) – MOVING AVERAGE (SUPERSEDED VERSION)

- Section 9.1: to amend last paragraph to read: "This section describes the previous version of COYU, which since 2020 has been superseded by an improved method using splines. It is recommended that the improved version be used. Please see TGP/8, section 10 "The Combined-over-years uniformity criterion (COYU) – improved version (splines)"."

- To delete superfluous references to COYD throughout the section

20. The TWC agreed the following amendments to the draft guidance in Annex II to document TWC/38/6:

- Title: to be amended to read: "10. THE COMBINED-OVER-YEARS UNIFORMITY CRITERION (COYU) – SPLINES (IMPROVED VERSION)

- Section 10.1: to amend last paragraph to read: "This section describes the improved method of COYU using splines, which supersedes the previous version (see TGP/8, section 9 "The Combined-over-years uniformity criterion (COYU) – superseded version (moving average)". It is recommended that this improved version be used."

- Section 10.4.1, third element to read as follows: "Estimation of the relationship between the SD and mean in each year. The method used is based on splines fitted to log SDs of comparable varieties."

- To delete superfluous references to COYD throughout the section

- Paragraph 10.7.2 to read "early acceptance" instead of "early rejection" (see also TWC/35/6 paragraph 23)

- Paragraph 10.8.4 to clarify paragraph: "…example set out in section 10.11…" instead of "…example set out in section 10.8…"

- Annex II, page 13, Fig.3, to read: “Decision after 3rd cycle non uniform pu3=0.003” instead of “Decision after 3rd cycle non uniform pu3=0.03”

21. The TWC agreed that, once the amendments above had been incorporated, the draft guidance provided in Annexes I and II to document TWC/38/6 should be proposed to the Technical Committee for inclusion in a future revision of document TGP/8.

NEXT STEPS

22. The TC may wish to invite the TWPs, at their sessions in 2021, to consider the draft guidance in the Annexes to this document as the basis for revising document TGP/8 “Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability”, Section 9 “The Combined-Over-Years Uniformity Criterion (COYU)”, subject to the amendments indicated in the text.

23. The TC is invited to:

(a) note the draft presented in the Annexes to this document for the revision of document TGP/8, Section 9 “The Combined-Over-Years Uniformity Criterion (COYU);”

(b) request the Technical Working Parties, at their sessions in 2021, to consider the proposed revision of document TGP/8, Section 9 “The Combined-Over-Years Uniformity Criterion (COYU);” on the basis of the draft presented in the Annexes to this document;

(c) note that evaluation versions of software for COYU Splines in both “R” and DUSTNT software are planned to be released in November 2020;

(d) note the expression of interest by experts from China, Finland, France and the United Kingdom to review the COYU Splines software;

(e) agree with the TWC to invite members to participate in a test campaign of the COYU Splines software until April 2021; and

(f) request that the TWC prepare a report of the results of the test campaign of the COYU Splines software for the TC for consideration in conjunction with the consideration of a revision of document TGP/8 by the TC at its fifty-seventh session.

[Annex follows]

ANNEX I

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) – SUPERSEDED VERSION (MOVING AVERAGE)

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").

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

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.~~11~~7

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:

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

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

where y_{ij} is the observation on the i^{th} plant in the j^{th} plot, $\mathbf{y}_j$ 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

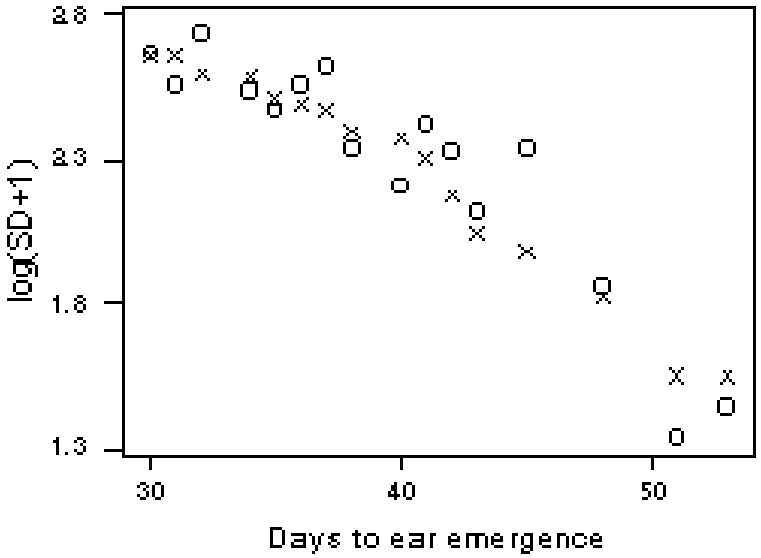
9.6.2 Transformation of SDs by adding 1 and converting to natural logarithms. The purpose of this transformation is to make the SDs more amenable to statistical analysis.

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}, ... , 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 "0" 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

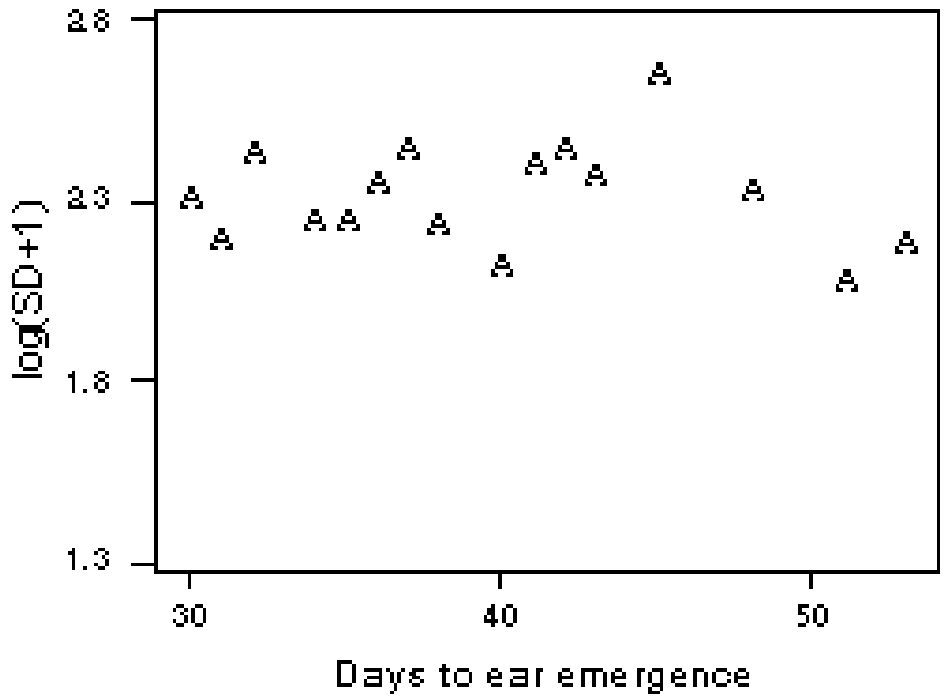
9.6.5 Once the trend values for the comparable varieties have been determined, the trend values for candidates are estimated using linear interpolation between the trend values of the nearest two comparable varieties as defined by their means for the characteristic. Thus if the trend values for the two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

	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, 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

$$T_c = \frac{(X_C - X_i)T_{i+1} + (X_{i+1} - X_C)T_i}{X_{i+1} - X_i} = \frac{(52-38)\text{x}2.28 + (63-52)\text{x}2.28}{63-38} = 2.28$$

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 + . . . + 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:

$$UC_p = SD_r + t_p\sqrt{V\left(\frac{1}{k}+\frac{1}{Rk}\right)} = 2.15 + 3.118x\sqrt{0.0202x\left(\frac{1}{3}+\frac{1}{3x11}\right)} = 2.42$$

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. ~~9~~10 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. ~~10~~11 COYU software

9. ~~10~~11.1 DUST computer program

9. ~~10~~11.1.1 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~~11.1.2 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~~11.1.3 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~~11.1.4 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~~11.1.5 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 AFTER
+ SD EXCEEDS OVER-YEARS CRITERION AFTER
: SD NOT YET ACCEPTABLE AFTER 2 YEARS W
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) ****

	OVER-YEARS			INDIVIDUAL YEARS								
VARIETY	CHAR. MEAN	ADJ. LOG SD	UNADJ LOG SD	CHAR. MEAN 88	CHAR. MEAN 89	CHAR. MEAN 90	LOG (SD+1) 88	LOG (SD+1) 89	LOG (SD+1) 90	ADJ LOG(SD+1) 88	ADJ LOG(SD+1) 89	ADJ LOG(SD+1) 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												
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	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.~~11~~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}).

Table 1	COYU		
CASE	p_{u2}	p_{nu2}	p_{u3}
A			
B			
C			
D			

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

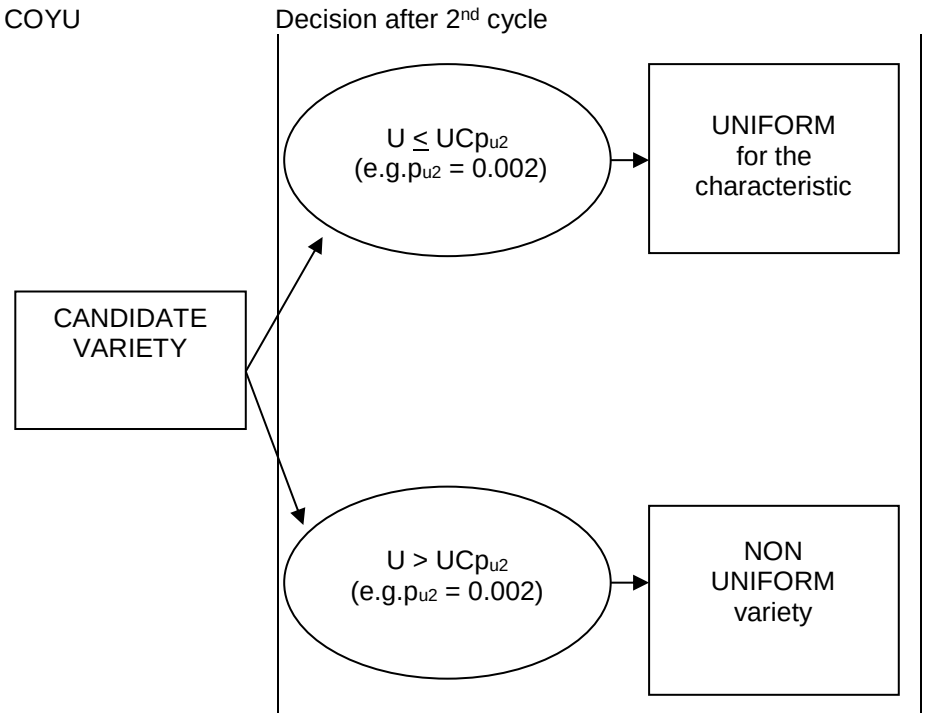
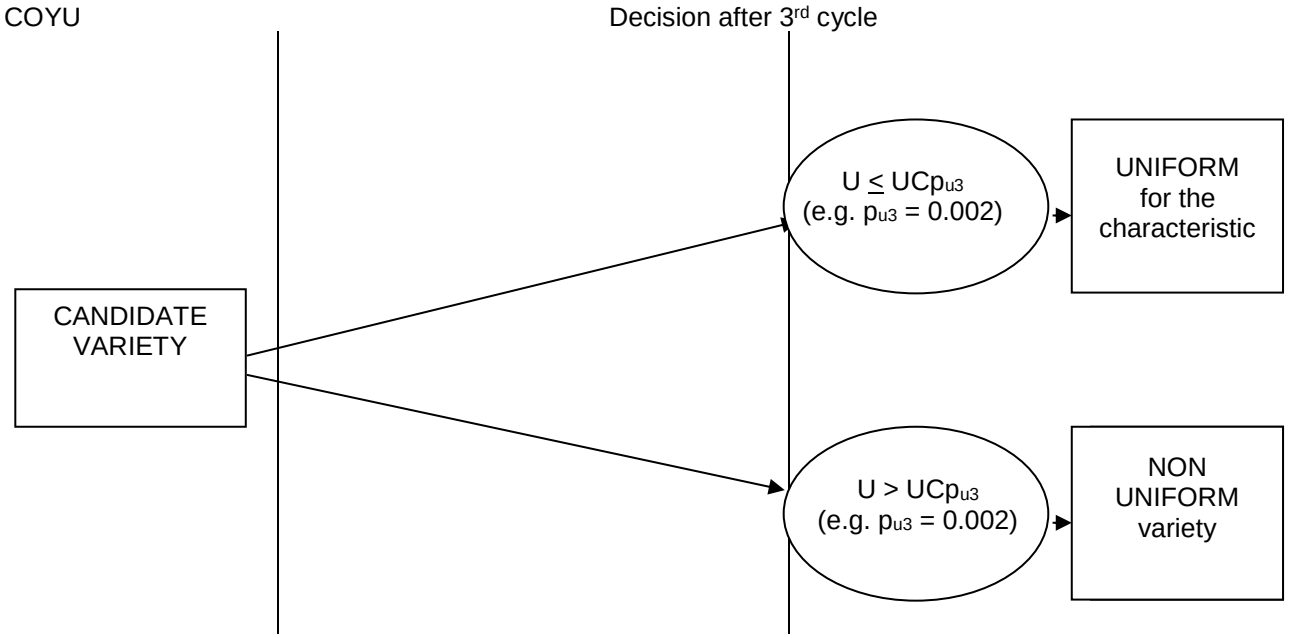


Figure 2. COYD and COYU decisions and standard probability levels (p_i) in Case B



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

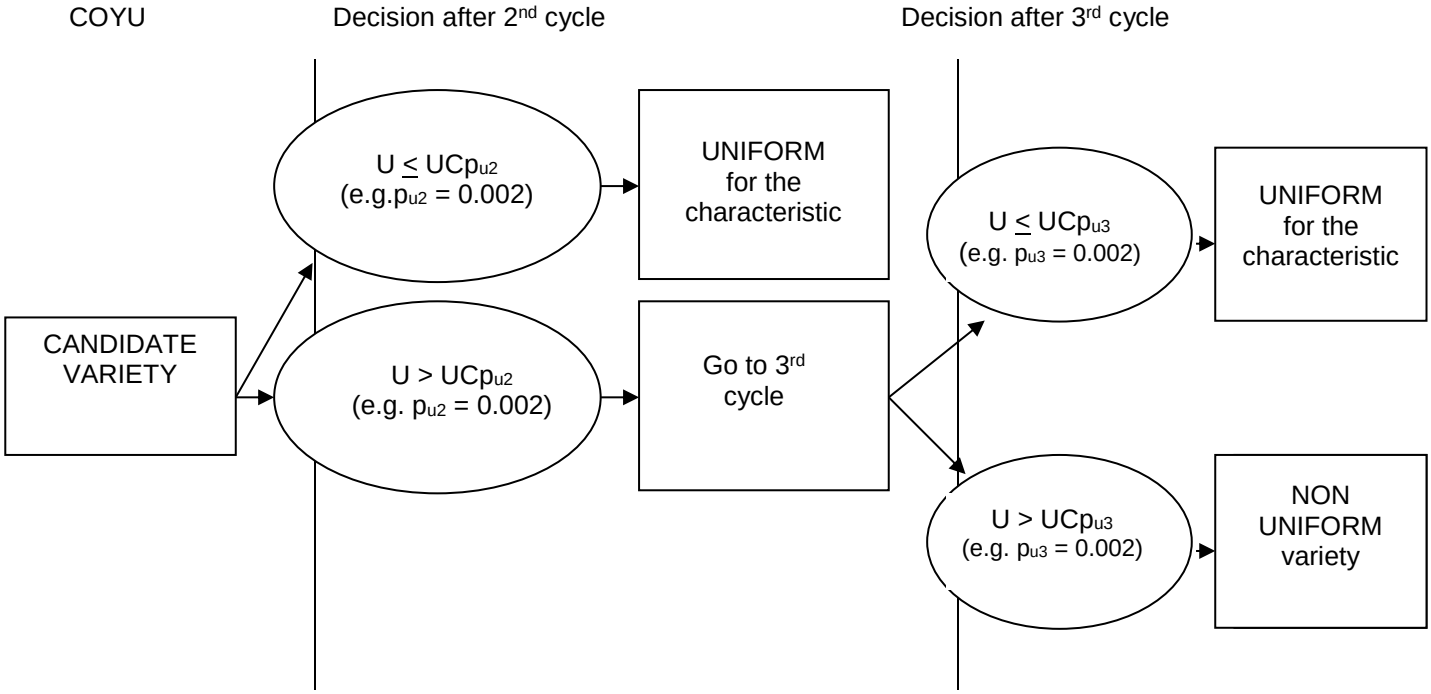
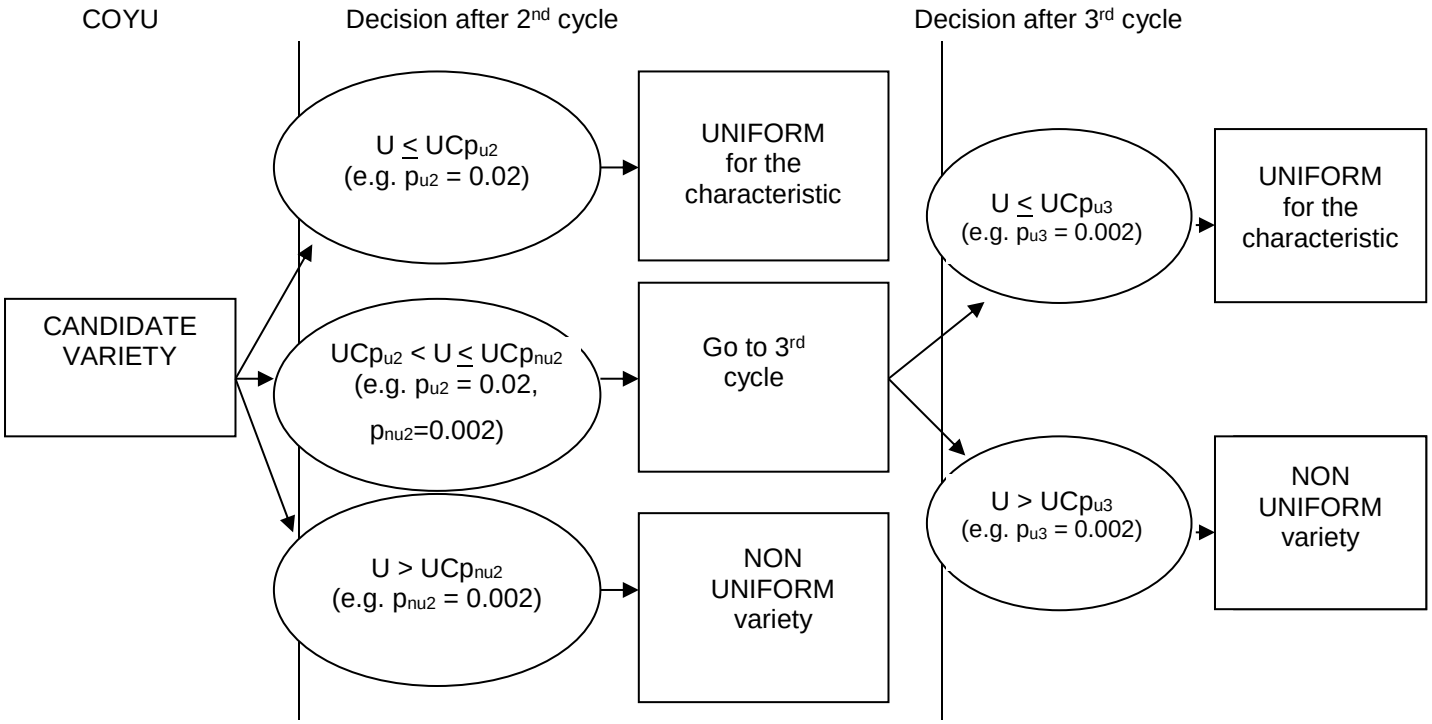


Figure 4. COYD and COYU decisions and standard probability levels (p_i) in Case D



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

[Annex II follows]

ANNEX II

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 (SPLINES)

~~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 section describes the improved method of COYU using splines, which supersedes the previous version (see TGP/8, section 9 "The Combined-over-years uniformity criterion (COYU) – superseded version (moving average)". 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 splines fitted to log SDs of comparable varieties.
- 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.~~10.5Use 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.~~10.6Mathematical 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:

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

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

where y_{ij} is the observation on the i^{th} plant in the j^{th} plot, $\mathbf{y}_j$ 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.

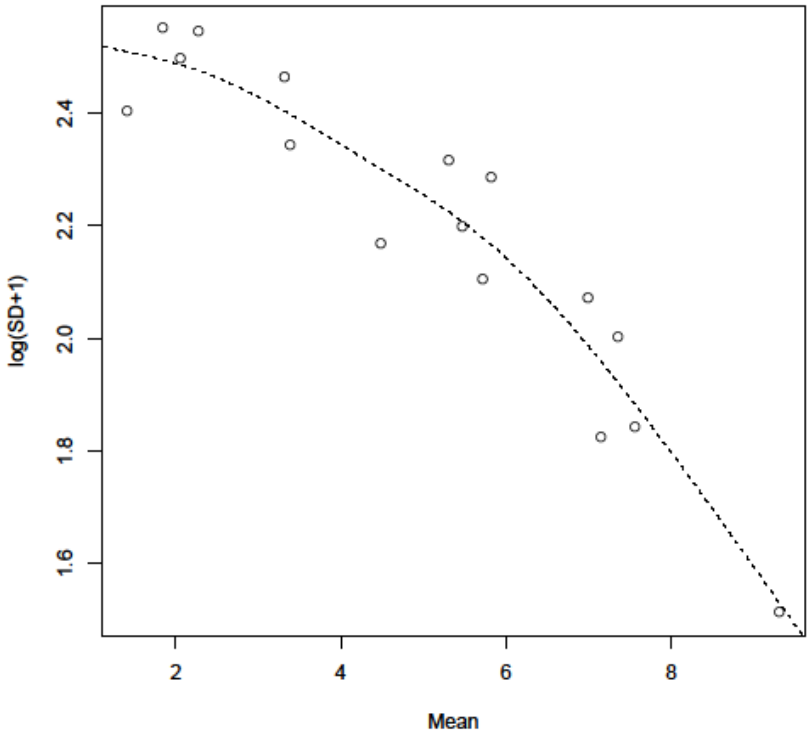
Step 3: Estimation of the relationship between the SD and mean in each year

~~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*)



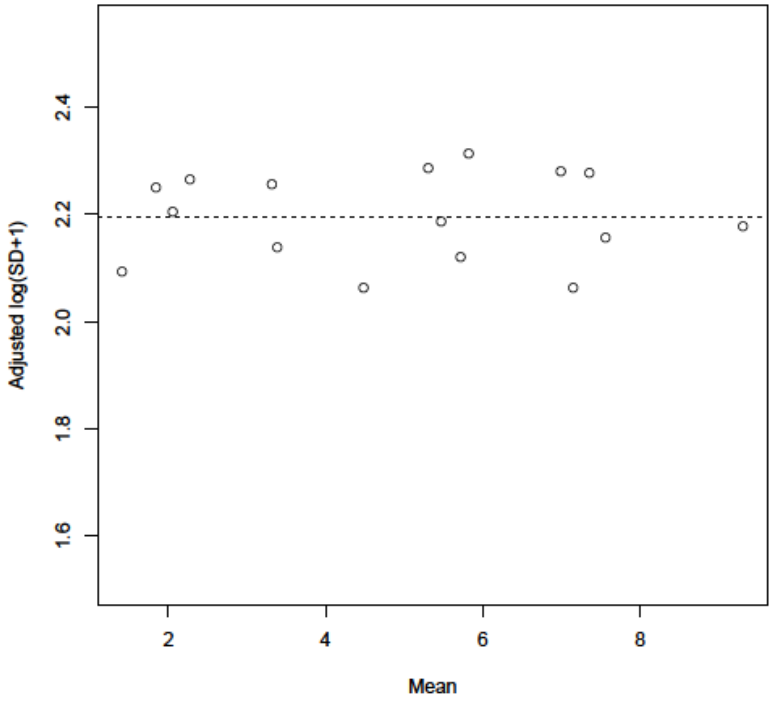
Step 4: Adjustment of transformed SD values based on estimated SD-mean relationship

~~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

$$\mathrm{UC} = \mathrm{SD}_r + t_p\sqrt{V_c}$$

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 Probablity 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 acceptance 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.~~10.11, 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. 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 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. 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) ****

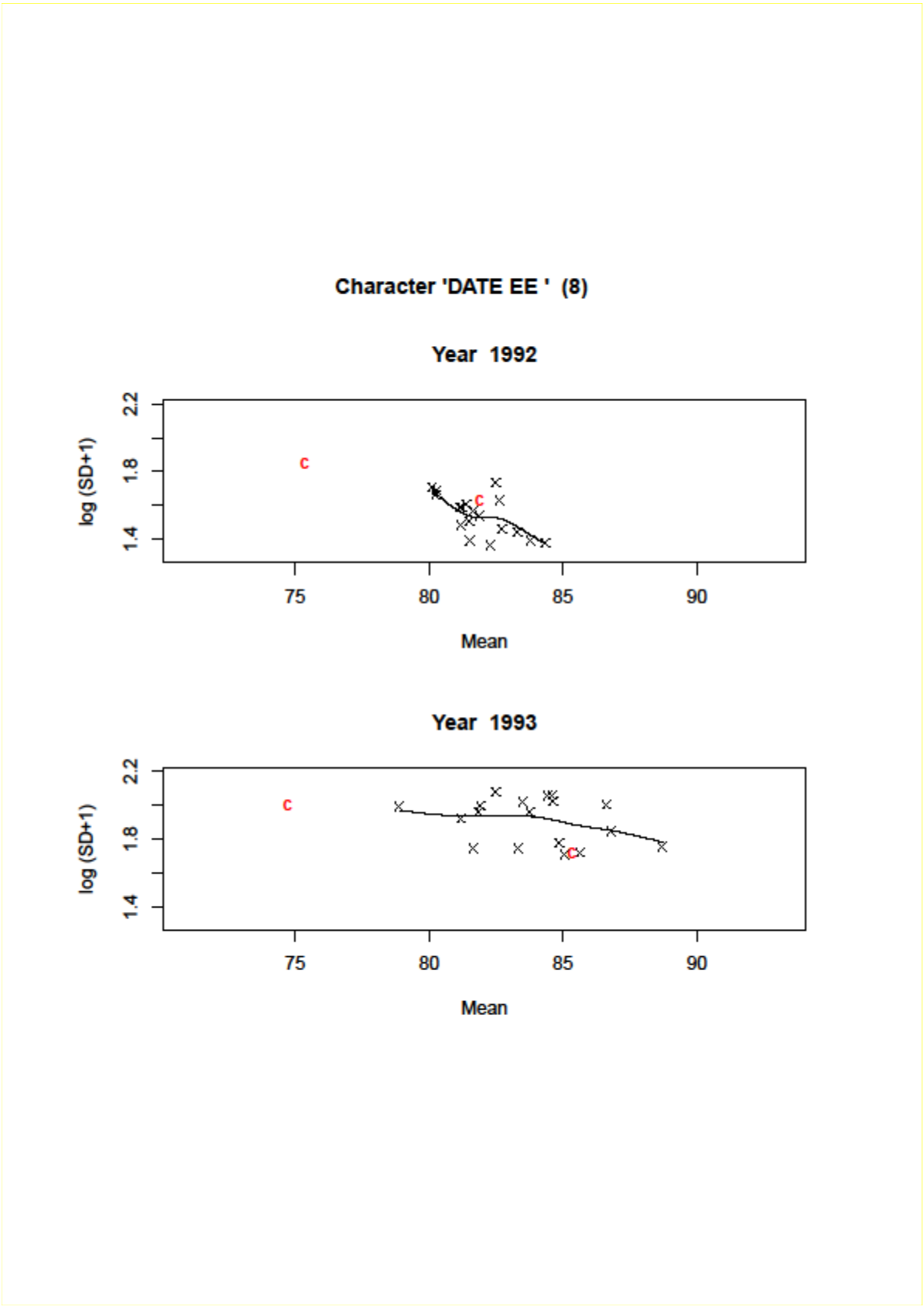
AFP	VARIETY	Extrapolation	Char_Mean	Adj_LogSD	Unadj_Log_SD	Mean_y1	Mean_y2	Log(SD+1)_y1	Log(SD+1)_y2
CANDIDATE									
101	C1	6.0	75.0!	1.45	1.92	75.3	74.7	1.85	2.00
102	C2	-	83.6	1.69	1.67	81.9	85.3	1.63	1.71
REFERENCE	MEANS			82.9	1.73				
REFERENCE									
1	R1		81.9	1.76	1.77	84.4	88.7	1.38	1.76
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}).

Table 1	COYU		
CASE	p_{u2}	p_{nu2}	p_{u3}
A			
B			
C			
D			

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

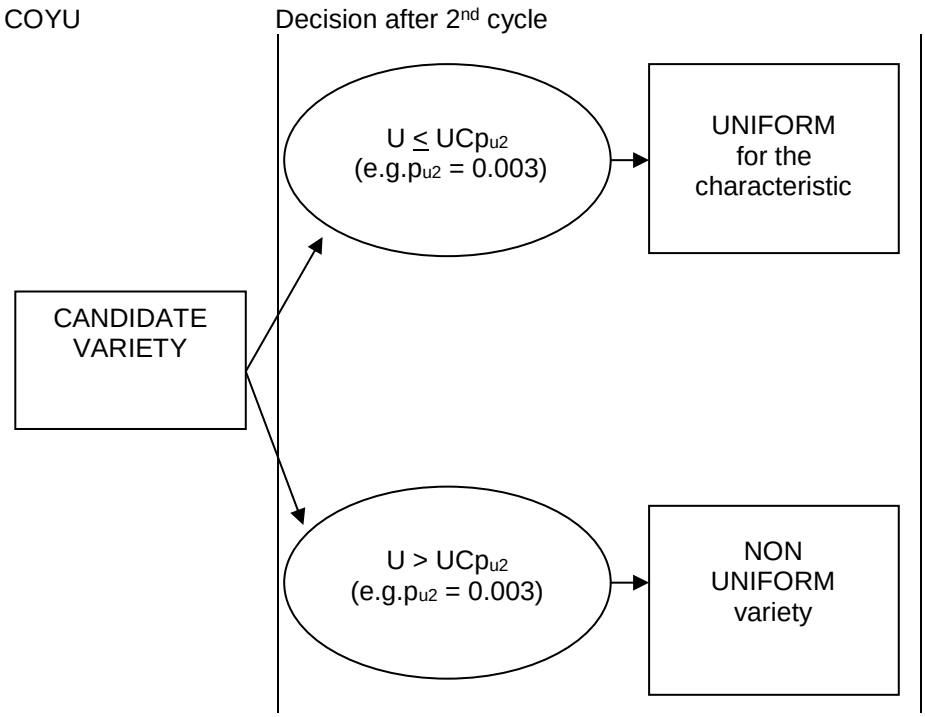
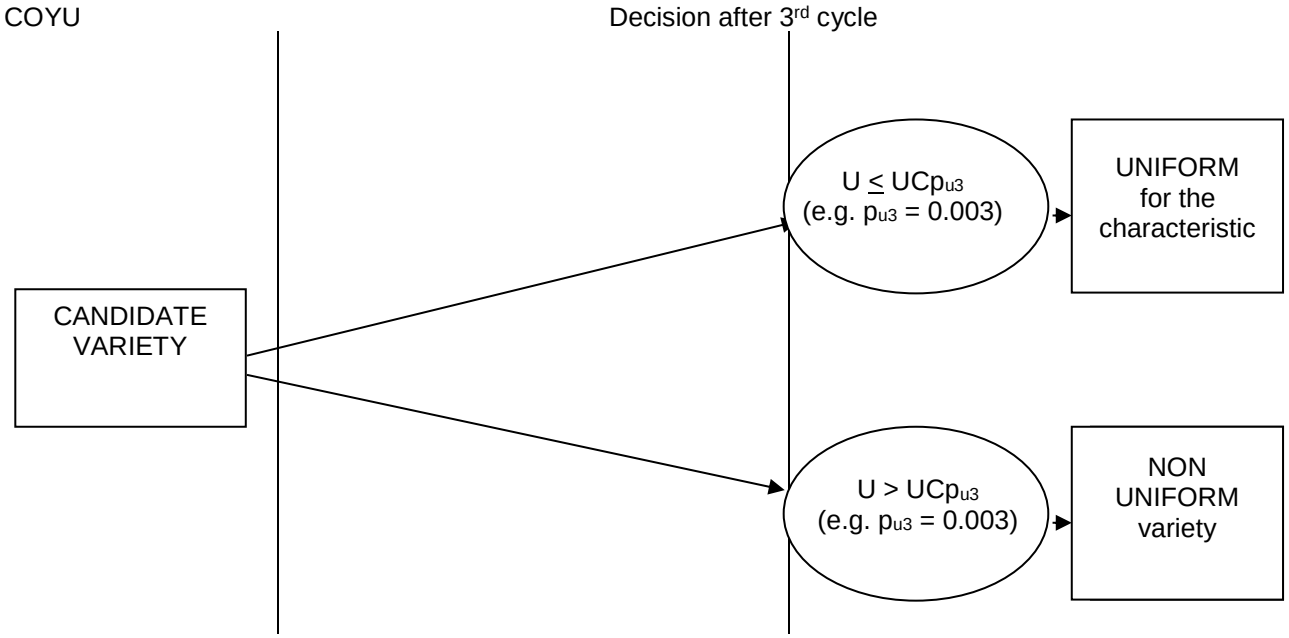


Figure 2. COYD and COYU decisions and standard probability levels (p_i) in Case B



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

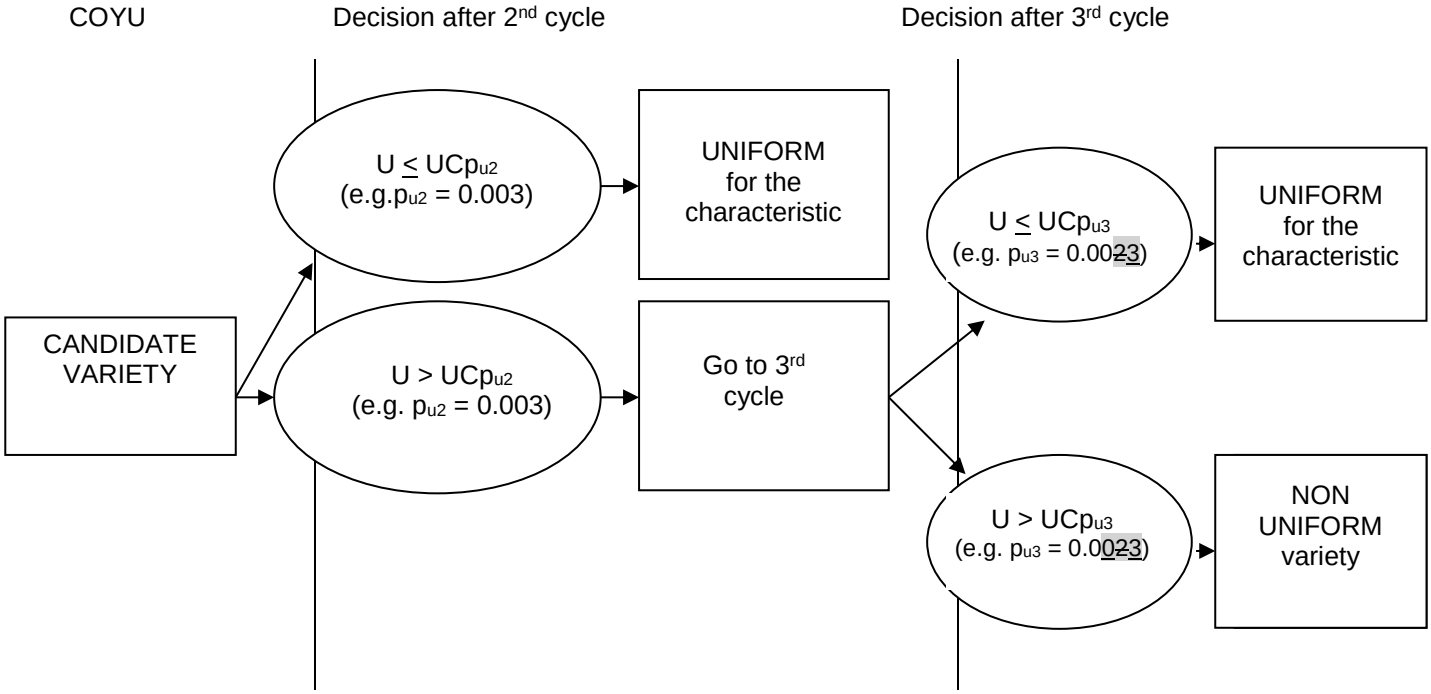
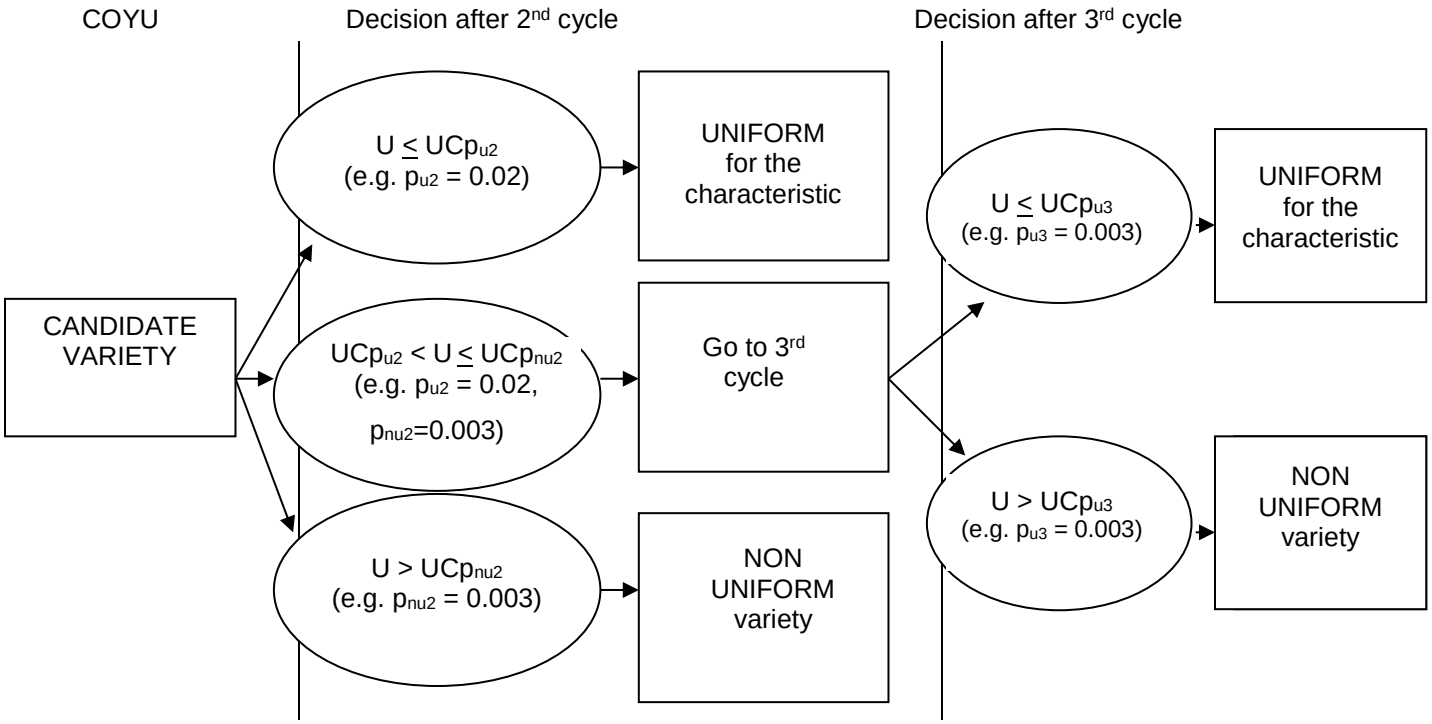


Figure 4. COYD and COYU decisions and standard probability levels (p_i) in Case D



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]