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# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS GENEVA

## DRAFT

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

#### **DOCUMENT TGP/10**

## **"EXAMINING UNIFORMITY"**

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to be considered by the

Technical Working Party for Vegetables (TWV), at its thirty-ninth session to be held in Nitra, Slovakia, from June 6 to 10, 2005

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# SECTION 1: ASSESSING UNIFORMITY ACCORDING TO THE FEATURES OF PROPAGATION

#### 1.1 Introduction

1.1.1 The variation in the expression of characteristics within varieties is the critical consideration in the assessment of uniformity. This variation has both genotypic and environmental components. The genotypic component is mainly influenced by the features of propagation. According to Article 8 of the 1991 Act of the UPOV Convention, uniformity of a variety is therefore considered on the basis of "... the variation that may be expected from the particular features of its propagation, …" The level of environmental variation depends on the interraction between individual plants and the environment. There is usually little environmental variation for qualitative characteristics. For quantitative characteristics, the level of environmental variation can differ from species to species and from characteristic to characteristic. [Pseudo-qualitative characteristics?]

(a) A low level of genotypic variation is expected for vegetatively propagated and truly self-pollinated varieties. Variation in the expression of characteristics within such varieties should result, predominantly, from environmental influences.

(b) Variation in the expression of characteristics within mainly self-pollinated varieties should also result, predominantly, from environmental influences but a low level of genotypical variation caused by some cross pollination is accepted. Therefore, more variation may be tolerated than for vegetatively propagated and truly self-pollinated varieties.

(c) In cross-pollinated varieties (including synthetic varieties) variation in the expression of characteristics within varieties results from both genotypical and environmental components. In relation to self-pollinated, vegetatively propagated and mainly self-pollinated varieties a higher genotypical variation is accepted. The overall level of variation is, therefore, generally higher in cross-pollinated and synthetic varieties.

(d) Genotypic variation in hybrid varieties depends on the type of hybrid (single- or multiple-cross), the level of genotypical variation in the parental lines (inbred lines or others) and the system for hybrid seed production (mechanical emasculation, system of male sterility etc.). The tolerance limits for uniformity are set according to the specific situation resulting from genotypic and environmental influences on the variation in the expression of characteristics.

1.1.2 As a result of the above, appropriate uniformity standards for the different types of varieties are developed according to the features of propagation (specific population standards).

1.1.3 The type of variation in the expression of characteristics within varieties determines how that characteristic is used to determine uniformity in the crop (off-types in case of discontinous variation or standard deviations in case of continous variation of characteristics). Thus, the uniformity of the crop may be determined by off-types alone, by standard deviations of the characteristics alone, or by off-types for some characteristics and by standard deviations for other characteristics.

## 1.2 Uniformity Assessment on the Basis of Off-Types

1.2.1 For characteristics with a low level of genotype and environmental variation it is possible to detect plants which are visually different to the variety and are considered as off-types. The General Introduction defines off-type as follows:

#### "6.4.1.1 Determination of Off-Types by Visual Assessment

A plant is to be considered an off-type if it can be clearly distinguished from the variety in the expression of any characteristic of the whole or part of the plant that is used in the testing of distinctness, taking into consideration the particular features of its propagation. This definition makes it clear that, in the assessment of uniformity, the standard for distinctness between off-types and a candidate variety is the same as for distinctness between a candidate variety and other varieties (see Chapter 5, section 5.5.2)."

In cases where off-types can be detected, the off-type procedure is recommended for the assessment of uniformity.

1.2.2 The proportion of off-types tolerated in a variety depends on the features of its propagation.

(a) In vegetatively propagated, truly self-pollinated and mainly self-pollinated varieties, the recommended limit for the number of off-types is based on an absolute population standard and a fixed acceptance probability (absolute population standard, see section 10.1.3, "absolute" because it is fixed in a general way). The population standard and the acceptance probability as well as the acceptable number of off types for a given sample size are specified in the individual Test Guidelines. The absolute population standard is fixed on the basis of experience.

(b) In cross-pollinated varieties including synthetic varieties, most quantitative characteristics show continuous variation within varieties. In these cases uniformity should be assessed on the basis of standard deviations (see section 1.3). If, especially in qualitative characteristics, the great majority of individuals of a variety have the same expression, plants with a clearly different expression can be detected as off-types (e.g. root color in fodder beet). In such cases the off-type procedure is appropriate. The number of off-types of a candidate variety should not significantly exceed the number found in comparable varieties already known. Comparable varieties are varieties of the same type within the same or closely related species that have been previously examined and considered to be sufficiently uniform.

1.2.3 If the number of comparable varieties is sufficiently high to give a representative mean number of off-types, the comparable varieties can be used as the basis for the calculation of an appropriate population standard which is applied with a fixed acceptance probability (relative population standard, see section 3.2, "relative" because it is fixed in comparison to other varieties). If the calculated relative population standard would be too stringent, e.g. more stringent than the standard for the same sample size in self-pollinated varieties, an appropriate absolute population standard should be fixed on the basis of experience.

1.2.4 An appropriate absolute population standard which is fixed on the basis of experience may also be applied in the case of new species (see TGP/13) or in cases where the number of comparable varieties is very low and may not be representative for that type of variety.

1.2.5 If off-types cannot be detected visually, uniformity must be assessed on the basis of standard deviations. In some cases it may be appropriate to detect off-types in measurements taken from individual plants. Guidance for such procedures is given in section 10.3.x.

<u>*Remark BR</u></u>: Guidance for detection of off-types in measurements of individual plants is not yet available! Should be developed by TWC?*</u>

# 1.3 Uniformity Assessment on the Basis of Standard Deviations

1.3.1 If the detection of off-types is not possible because of considerable genotypic and/or environmental variation within varieties, uniformity should be assessed after taking this variation into account. The variability of a candidate variety should not significantly exceed the variability of comparable varieties already known. The comparison between a candidate variety and comparable varieties is carried out on the basis of standard deviations calculated from individual plant observations.

1.3.2 If the conditions for the application of the COYU procedure are fulfilled, COYU is the recommended statistical method for this comparison (see section 2.1). This procedure calculates the 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.

1.3.3 If the conditions for the application of the COYU procedure are not fulfilled e.g. the test is performed for only one year or the number of tested varieties is too small, other appropriate statistical methods should be used for the comparison of standard deviations (e.g. 1,6 x variance, long term LSD).

<u>*Remark BR</u>: Guidance for other methods than COYU is still to be developed by TWC. To be included in TGP/10.3 or in TGP/8.*</u>

## 1.4 Uniformity Assessment for Varieties with Segregating Characteristics

1.4.1 For multiple cross hybrids and synthetic varieties, a segregation of certain characteristics, in particular qualitative characteristics, is accepted if it is compatible with the expression of the parental lines and the method of propagating the variety. If the inheritance of a segregating characteristic is known, the variety is considered to be uniform if the characteristic behaves in the predicted manner. Guidance for assessing consistency with the predicted segregation ratio is provided in section 3.

1.4.2 If the inheritance of a clear-cut segregating characteristic is not known, the observed segregation ratio should be described. An assessment of uniformity is not possible for these characteristics. (The rules outlined for predictable segregation ratios in section 10.3.3 should be used for testing stability.)

1.4.3 In quantitative characteristics segregation in multiple hybrids may result in a continuous variation. In such cases uniformity is assessed as in cross-pollinated varieties on the basis of relative uniformity standards calculated from the range of variation of comparable varieties (see section 10.2.2).

# SECTION 2: METHODS FOR ASSESSING UNIFORMITY ON THE BASIS OF STANDARD DEVIATIONS

## 2.1 The Combined-Over-Years Uniformity Criterion (COYU)

#### 2.1.1 Summary

2.1.1.1 When the uniformity of plants of a variety is to be judged on the basis of quantitative characteristics then the standard deviation (SD) can be used to summarise the spread of the observations. A new variety can then be tested for uniformity by comparing its SD with that of reference varieties. There are several possible ways of assessing uniformity based on the SD. Here the Combined-Over-Years Uniformity (COYU) criterion is described.

2.1.1.2 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.

2.1.1.3 The technique involves ranking reference 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 comparable varieties.

2.1.1.4 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 reference varieties using a standard t-test.

2.1.1.5 COYU, in effect, compares the uniformity of a candidate with that of the reference 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.

## 2.1.2 Introduction

2.1.2.1 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.

2.1.2.2 This section outlines a procedure known as the combined-over-years uniformity (COYU) criterion. COYU assesses the uniformity of a variety relative to reference 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.

2.1.2.3 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.

# 2.1.3 The COYU Criterion

2.1.3.1 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 section 2.1.4 below.

- 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 reference varieties ordered by their means.
- Adjustments of log SDs of candidate and reference 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 reference 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.
- 2.1.3.2 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 references varieties.

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#### 2.1.4 Recommendations on COYU

- 2.1.4.1 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.

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

2.1.4.3 The probability level "p" used to determine the uniformity criterion depends on the crop. Recommended probability levels are given in TGP/10.1.

2.1.4.4 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.

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

## 2.1.5 Mathematical details

## *Step 1: Derivation of the within-plot standard deviation*

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

$$SD_{j} = \sqrt{\frac{\sum_{i=1}^{n} (y_{ij} - y_{j})^{2}}{(n-1)}}$$
$$SD = \frac{\sum_{j=1}^{r} SD_{j}}{r}$$

where  $y_{ij}$  is the observation on the i<sup>th</sup> plant in the j<sup>th</sup> plot,  $y_j$  is the mean of the observations from the j<sup>th</sup> plot, n is the number of plants measured in each plot and r is the number of replicates.

#### Step 2: Transformation of the SDs

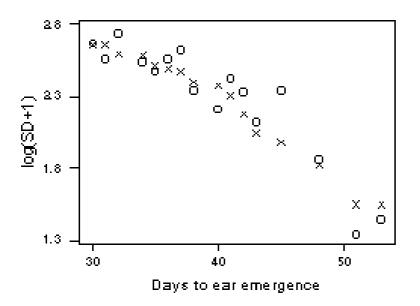
2.1.5.2 Transformation of SDs by adding 1 and converting to natural logarithms. The purpose of this transformation is to make the SDs more amenable to statistical analysis.

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

2.1.5.3 For each year separately, the form of the average relationship between SD and characteristic mean is estimated for the reference varieties. The method of estimation is a 9-point moving average. The log SDs (the Y variate) and the means (the X variate) for each variety are first ranked according to the values of the mean. For each point  $(X_i, Y_i)$  take the trend value  $T_i$  to be the mean of the values  $Y_{i-4}, Y_{i-3}, ..., Y_{i+4}$  where i represents the rank of the X value and  $Y_i$  is the corresponding Y value. For X values ranked  $1^{st}$  and  $2^{nd}$  the trend value is taken to be the mean of the first three values. In the case of the X value ranked  $3^{rd}$  the mean of the first five values are taken and for the X value ranked  $4^{th}$  the mean of the first seven values are used. A similar procedure operates for the four highest-ranked X values.

2.1.5.4 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

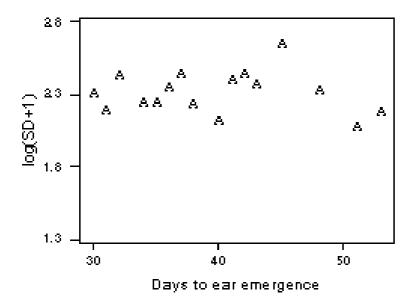
2.1.5.5 Once the trend values for the reference varieties have been determined, the trend values for candidates are estimated using linear interpolation between the trend values of the nearest two reference varieties as defined by their means for the characteristic. Thus if the trend values for the two reference 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

$$T_{c} = \frac{(X_{C} - X_{i})T_{i+1} + (X_{i+1} - X_{C})T_{i}}{X_{i+1} - X_{i}}$$

2.1.5.6 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.

2.1.5.7 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 6: Calculation of the uniformity criterion

2.1.5.8 An estimate of the variability in the uniformity of the reference 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.

2.1.5.9 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 reference 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 reference varieties.

### 2.1.6 Early decisions for a three-year test

2.1.6.1 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.

2.1.6.2 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%.

2.1.6.3 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%.

2.1.6.4 Some varieties may fail to be rejected or accepted after two years. In the example set out in paragraphs 26 and 27, 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.

## 2.1.7 Example of COYU calculations

2.1.7.1 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 reference varieties (R1 to R11) and one candidate (C1). The data is tabulated in Table 1.

	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

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

$$T_{c} = \frac{(X_{C} - X_{i})T_{i+1} + (X_{i+1} - X_{C})T_{i}}{X_{i+1} - X_{i}} = \frac{(52 - 38)x2.28 + (63 - 52)x2.28}{63 - 38} = 2.28$$

Variety	Ranked mean	Log (SD+1)	Trend Value	Adj. Log (SD+1)
	(X)	(Y)	Т	
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	$(2.25 + \ldots + 2.42)/5 = 2.35$	2.39 - 2.35 + 2.39 = 2.42
R5	69	2.50	$(2.25 + \ldots + 2.48)/7 = 2.38$	2.50 - 2.38 + 2.39 = 2.52
R4	71	2.42	$(2.25 + \ldots + 2.32)/9 = 2.38$	2.42 - 2.38 + 2.39 = 2.43
R6	74	2.38	$(2.21 + \ldots + 2.53)/9 = 2.41$	2.38 - 2.41 + 2.39 = 2.36
R8	75	2.48	$(2.39 + \ldots + 2.34)/9 = 2.42$	2.48 - 2.42 + 2.39 = 2.44
R7	76	2.46	$(2.42 + \ldots + 2.34)/7 = 2.42$	2.46 - 2.42 + 2.39 = 2.43
R11	76	2.32	$(2.48 + \ldots + 2.34)/5 = 2.43$	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

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

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

	Over-	Year Means	Ad	j. 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

Table 3: Example data-set – adjusted log(SD+1) for all three years with over-year means
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2.1.7.4 The analysis of variance table for the adjusted log SDs is given in Table 4 (based on reference varieties only). The variability in the uniformity of reference 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	Sums of	Mean
	freedom	squares	squares
Year	2	1.0196	0.5098
Varieties within years (=residual)	30	0.6060	0.0202
Total	32	1.6256	

#### 2.1.7.5 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.

2.1.7.6 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.

#### 2.1.8 Implementing COYU

The COYU criterion can be applied using the DUST software package for the statistical analysis of DUS data. This is available from the Dr. Sally Watson, Biometrics Division, Department of Agriculture for Northern Ireland, Newforge Lane, Belfast BT9 5PX, UK . Sample outputs are given in Appendix A.

# APPENDIX A: COYU Software

## 1 DUST Computer program

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.

1.2 In Table A1, the adjusted SD for each variety is expressed as a percent of the mean SD for all reference 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.

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.

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.

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

#### WITHIN-PLOT STANDARD DEVIATIONS AS % MEAN OF

#### CHARACTERISTIC

	5	60	8	10	11
R1         R2         R3         R4         R5         R6         R7         R8         R10         R11         R12         R13         R14         R15         R14         R15         R18         R19         R19         R10         R11         R12         R13         R14         R15         R14         R15         R14         R15         R14         R15         R18         R20         R21         R23         R23         R24         R25         R26	5 100 105 97 102 102 103 100 97 97 104 99 105 105 102 99 97 99 103 104 97 101 94 99 101 94 98	60 100 106 103 99 95 98 105 100 96 97 96 103 100 97 96 103 101 97 101 99 94 110*1 101 97 103 97	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	10 99 103 105 95 99 102 1 95 99 103 100 97 105 98 101 96 102 91 97 107 1 99 99 99 99 99 99 99 99 99	11 97 97 104 101 96 98 101 101 104 110 97 104 98 99 97 93 101 99 96 102 101 98 105 106 96 101 101 97 105 1 101 102 98 101 95 102 99 100 98 100 102 100 102 103 1 101 104 97 100 103 101 96 102 1 106
R29         R30         R31         R32         R33         R34         R35         R36         R37         R38         R39         R39         C1         C2         C3         C4         C5         C6         C7         C8         C9         C1	101 101 99 98 104 95 100 95 99 104 100 103 97 102 100 101 96 101 99	106 105 96 102 93 102 1 94 102 98 107 1 97 99 102 106 101 93 101 104 102 98 105 1 99	90 83 97 107 111 2 107 1 82 95 111 1 107 102 90 112 1 113 2 98 118 2 106 99 103 106 116 2 90 2	102 99 107 1 102 103 95 100 99 101 107 1 98 100 104 1 97 98 103 103 103 103 97	$\begin{array}{cccccc} 101 & 101 \\ 94 & 93 \\ 95 & 100 \\ 102 & 99 \\ 98 & 103 \\ 100 & 97 \\ 97 & 96 \\ 99 & 94 \\ 100 & 103 \\ 100 & 107 \\ 97 & 101 \\ 101 & 100 \\ 101 & 97 \\ 106 & 1 & 106 \\ 101 & 109 \\ 99 & 101 \\ 100 & 107 \\ 103 & 107 \\ 103 & 107 \\ 103 & 93 \\ 97 & 98 \end{array}$

#### CHARACTERISTIC

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

#### SYMBOLS

-----

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

OVER-YEARS				INDIVIDUAL YEARS								
VARIETY			UNADJ	C	HAR. ME	AN	LO	G (SD+1	.)	ADJ	LOG (SI	D+1)
		LOG SD	LOG SD	88	89	90	88	89	90	88	89	90
REFERENCE												
R3						35.12				1.73		
			2.671			48.54				2.23		
R16	59.03	1.833	2.179	5/.25	63.33	56.50	2.28X	2.24	2.01	1.96	1./3	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 R12	65.99	1.739	2.460 1.994 2.086	62.92	66.32	60.72	2.21	2.03	1.74	1.90	1.04	1.02
R12 R33	60.12 67 E0	2 1 2 4	2.254	67.89	05.35 71 E4	64 52	2.07	2.584	1.00	1.9/	2.14	1.70
R33 R1	67 87	1 880	1.989	60.00	70.64	63 00	2.55A 1.60	2.20 2.45V	1.95	2.32	2 08	2.12
R20	68 74	1 953	1 903	67 17	70.04	64 74	2.05	1 95	1 68	1 92	2.00	1 80
R25	68 82	1 853	1.893 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	1.905 1.853 1.864 2.000 2.238 1.545 2.178 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 1.897 2.012 2.020 2.150	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 1.593	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 1.688 1.832 1.676 1.773	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 R35	70.03	1.701	1.070	75.49	00.50	74.50	1.50	1.51	1.90	1.72	1.70	1.92
R35 R4	77 70	1.000	2.268	76.07	01.05	74.15	1./3 0.26V	1.0/	1.92 0.01v	1.00	1.05	1.93
R36	77 00	2.349	2.208	70.00	01.22 70.0E	75.33	2.30A	2.15	2.31A 2.2EV	2.52	2.33	2.20
R6	78 73	2.209	1.935	77 53	82 88	75 78	2.15	1 75	2.254	2.27	2.21	1.91
R0 R27	78 78	2.009	2.098	77 61	80 03	79 69	1 80	2 25	2.00 2.24v	2.03	2.09	2.09
R28	70.70	1 785	1 722	78 28	Q1 QQ	77 97	1 68	1 43	2.241	1 70	1 67	1.89
R21	80 52	2 045	1.722	77 43	85 02	79 11	1 98	1 75	2.05	2 07	2 09	1.98
1101	00.52	2.015	2.000		00.02	///	1.50	1.75	2.13	2.07	2.05	2.50
CANDIDATE												
	64.03	2.252	2.438	63.85	63.33	64.92	2.49X	2.81X	2.02	2.25	2.29	2.21
			1.837									
			2.248									
C4			2.033				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	1.947	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
			2.157									
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	CRITERI											
			PROB. LEV	ЪĽ								
3-YEAR REG												
2-YEAR REG												
2-YEAR ACC	CEPTANCE	5 2.329	0.020									
**** **	JAT.VOTO	0	ANCE OF AD	יישייטודו	LOCIED	.1) *** *						
AI	CTG1UM	OF AWKT	LANCE OF AL	OUDIED	70G(2D+							
	DF	MS	F RATIO									
YEARS	2	0.0623										

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

# SECTION 3: METHODS FOR ASSESSING UNIFORMITY ON THE BASIS OF OFF-TYPES

## **3.1** Fixed Population Standard

## 3.1.1 Summary

3.1.1.1 This section describes the method of assessing uniformity by comparing the number of off-types observed to a fixed population standard. This is of particular use for self-pollinated and vegetatively propagated crops.

3.1.1.2 Methods for assessing uniformity using off-types for other types of crop are in development.

3.1.1.3 The maximum number of off-types that is acceptable should be chosen so that the probability of rejecting a candidate variety that should meet the crop standard is small. On the other hand the probability of accepting a candidate variety that has many more off-types than the standard of that crop should also be low.

3.1.1.4 The methods described here address the problem of choosing the maximum permitted number of off-types for different standards and sample sizes so that the probability of making errors is known and acceptable. The methods involve establishing a standard for the crop in question and then choosing the sample size and the number of off-types that best satisfy the risks that can be tolerated.

3.1.1.5. This document also outlines procedures for when more than a single test (more than one year for instance) is used and explains the possibility of using sequential tests to minimize testing effort.

## 3.1.2 Introduction

3.1.2.1 When testing for uniformity on the basis of a sample, there will always be some risk of making a wrong decision. The risks can be reduced by increasing the sample size but at a greater cost. The aim of the statistical procedure described here is to achieve an acceptable balance between risks.

3.1.2.2 The procedures described below require the user to define an acceptance standard (called the *population standard*) for the crop in question. The methods described then show how to determine the sample size and the maximum number of off-types allowed for various levels of risks.

3.1.2.3 The population standard is the maximum percentage of off-types that would be accepted if all individuals of the variety could be examined.

#### 3.1.3 Recommendations on the fixed population standard method of assessing uniformity by the number of off-types

3.1.3.1 This method is recommended for use in assessing the uniformity by number of off-types with a fixed population standard.

3.1.3.2 The sample size and acceptable number of off-types employed depend on the crop. Recommended sample sizes and acceptable numbers of off-types for different crops are given in [to be developed].

# 3.1.4 Errors in testing for off-types

3.1.4.1 As mentioned, there will be some risk of making wrong decisions. Two types of error exist:

(a) Declaring that the variety lacks uniformity when it in fact meets the standard for the crop. This is known as "type I error."

(b) Declaring that the variety is uniform when it in fact does not meet the standard for the crop. This is known as "type II error."

3.1.4.2 The types of error can be summarized in the following table:

	Decision made on variety			
True state of the variety	Acceptance as uniform	Rejection as non-uniform		
uniform	correctly accepted	type I error		
non-uniform	type II error	correctly rejected		

3.1.4.3 The probability of correctly accepting a uniform variety is called the acceptance probability and is linked to the probability of type I error by the relation:

"Acceptance probability" + "probability of type I error" = 100%

3.1.4.4 The probability of type II error depends on "how non-uniform" the candidate variety is. If it is much more non-uniform than the population standard then the probability of type II error will be small and there will be a small probability of accepting such a variety. If, on the other hand, the candidate variety is only slightly more non-uniform than the standard, there is a large probability of type II error. The probability of acceptance will approach the acceptance probability for a variety with a level of uniformity near to the population standard.

3.1.4.5 Because the probability of type II error is not fixed but depends on "how nonuniform" the candidate variety is, this probability can be calculated for different degrees of non-uniformity. This document gives probabilities of type II error for three degrees of nonuniformity: 2.5 and 10 times the population standard.

3.1.4.6 In general, the probability of making errors will be decreased by increasing the sample size and increased by decreasing the sample size.

3.1.4.7 For a given sample size, the balance between the probabilities of making type I and type II errors may be altered by changing the number of off-types allowed.

3.1.4.8 If the number of off-types allowed is increased, the probability of type I error is decreased but the probability of type II error is increased. On the other hand, if the number of off-types allowed is decreased, the probability of type I errors is increased while the probability of type II errors is decreased.

3.1.4.9 By allowing a very high number of off-types it will be possible to make the probability of type I errors very low (or almost zero). However, the probability of making type II errors will now become (unacceptably) high. If only a very small number of off-types is allowed, the result will be a small probability of type II errors and an (unacceptably) high probability of type I errors. This will be illustrated by examples.

#### 3.1.5 Examples

#### Example 1

3.1.5.1 From experience, a reasonable standard for the crop in question is found to be 1%. So the population standard is 1%. Assume that a single test with a maximum of 60 plants is used. From tables 4, 10 and 16 (chosen to give a range of target acceptance probabilities), the following schemes are found:

Scheme	Sample size	Target acceptance probability <sup>*</sup>	Maximum number of off-types
a	60	90%	2
b	53	90%	1
с	60	95%	2
d	60	99%	3

See paragraph 54

3.1.5.2 From the figures 4, 10 and 16, the following probabilities are obtained for the type I error and type II error for different percentages of off-types (denoted by  $P_2$ ,  $P_5$  and  $P_{10}$  for 2, 5 and 10 times the population standard).

Scheme	Sample size	Maximum number of off-types	Probabilities of error (%)			
			Type I Type II			
				$P_2 = 2\%$	$P_5 = 5\%$	$P_{10} = 10\%$
а	60	2	2	88	42	5
b	53	1	10	71	25	3
с	60	2	2	88	42	5
d	60	3	0.3	97	65	14

3.1.5.3 The table lists four different schemes and they should be examined to see if one of them is appropriate to use. (Schemes a and c are identical since there is no scheme for a sample size of 60 with a probability of type I error between 5 and 10%). If it is decided to ensure that the probability of a type I error should be very small (scheme d) then the probability of the type II error becomes very large (97, 65 and 14%) for a variety with 2.5 and 10% of off-types, respectively. The best balance between the probabilities of making the two types of error seems to be obtained by allowing one off-type in a sample of 53 plants (scheme b).

# Example 2

3.1.5.4 In this example, a crop is considered where the population standard is set to 2% and the number of plants available for examination is only 6.

3.1.5.5 Using the tables and the figures 3, 9 and 15, the following schemes a-d are found:

Sche- me	Sample size	Acceptance probability	Maximum number of off-types	Probability of error (%)			
				Type I Type II			
					$P_2 = 4\%$	$P_5 = 10\%$	$P_{10} = 20\%$
а	6	90	1	0.6	98	89	66
b	5	90	0	10	82	59	33
c	6	95	1	0.6	98	89	66
d	6	99	1	0.6	98	89	66
e	6		0	11	78	53	26

3.1.5.6 Scheme e of the table is found by applying the formulas (1) and (2) shown later in this document.

3.1.5.7 This example illustrates the difficulties encountered when the sample size is very low. The probability of erroneously accepting a non-uniform variety (a type II error) is large for all the possible situations. Even when all five plants must be uniform for a variety to be accepted (scheme b), the probability of accepting a variety with 20% of off-types is still 33%.

3.1.5.8 It should be noted that a scheme where all six plants must be uniform (scheme e) gives slightly smaller probabilities of type II errors, but now the probability of the type I error has increased to 11%.

3.1.5.9 However, scheme e may be considered the best option when only six plants are available in a single test for a crop where the population standard has been set to 2%.

## Example 3

3.1.5.10 In this example we reconsider the situation in example 1 but assume that data are available for two years. So the population standard is 1% and the sample size is 120 plants (60 plants in each of two years).

3.1.5.11 The following schemes and probabilities are obtained from the tables and figures 4, 10 and 16:

Sche- me	Sample size	Acceptance probability	Maximum number of off-types	Probability of error (%)			
				Type I Type II			
					$P_2 = 2\%$	$P_5 = 5\%$	$P_{10} = 10\%$
а	120	90	3	3	78	15	<0.1
b	110	90	2	10	62	8	<0.1
c	120	95	3	3	78	15	<0.1
d	120	99	4	0.7	91	28	1

3.1.5.12 Here the best balance between the probabilities of making the two types of error is obtained by scheme c, i.e. to accept after two years a total of three off-types among the 120 plants examined.

3.1.5.13 Alternatively a two-stage testing procedure may be set up. Such a procedure can be found for this case by using formulae (3) and (4) later in this document.

Scheme	Sample size	Acceptance probability	Largest number for acceptance after year 1	Largest number before reject in year 1	Largest number to accept after 2 years
e	60	90	can never accept	2	3
f	60	95	can never accept	2	3
g	60	99	can never accept	3	4
h	58	90	1	2	2

3.1.5.14 The following schemes can be obtained:

3.1.5.15 Using the formulas (3), (4) and (5) the following probabilities of errors are obtained:

Scheme	Probability of error (%)				Probability
	Type I		of testing in a second		
		$P_2 = 2\%$	year		
e	4	75	13	0.1	100
f	4	75	13	0.1	100
g	1	90	27	0.5	100
h	10	62	9	0.3	36

3.1.5.16 Schemes e and f (which are identical) result in a probability of 4% for rejecting a uniform variety (type I error) and a probability of 13% for accepting a variety with 5% off-types (type II error). The decision is:

- Never accept the variety after 1 year
- More than 2 off-types in year 1: reject the variety and stop testing
- Between and including 0 and 2 off types in year 1: do a second year test
- At most 3 off-types after 2 years: accept the variety
- More than 3 off-types after 2 years: reject the variety

3.1.5.17 Alternatively, scheme h may be chosen but scheme g seems to have a too large probability of type II errors compared with the probability of type I error.

3.1.5.18 Scheme h has the advantage of often allowing a final decision to be taken after the first test (year) but, as a consequence, there is a higher probability of a type I error.

## Example 4

3.1.5.19 In this example, we assume that the population standard is 3% and that we have 8 plants available in each of two years.

Scheme	Sample size	Acceptance	Maximum	Р	robability o	of error (%)	)
		probability	number of off-types	Type I		Type II	
					$P_2 = 6\%$	$P_5 = 15\%$	$P_{10} = 30\%$
a	16	90	1	8	78	28	3
b	16	95	2	1	93	56	10
c	16	99	3	0.1	99	79	25

3.1.5.20 From the tables and figures 2, 8 and 14, we have:

3.1.5.21 Here the best balance between the probabilities of making the two types of error is obtained by scheme a.

# 3.1.6 Introduction to the tables and figures

3.1.6.1 In the TABLES AND FIGURES section (section 3.2.13), there are 21 table and figure pairs corresponding to different combinations of population standard and acceptance probability. These are design to be applied to a single off-type test. An overview of the tables and the figures are given in table A.

3.1.6.2 Each table shows the maximum numbers of off-types (k) with the corresponding ranges in sample sizes (n) for the given population standard and acceptance probability. For example, in table 1 (population standard 5%, acceptance probability  $\ge$  90%), for a maximum set at 2 off-types, the corresponding sample size (n) is in the range from 11 to 22. Likewise, if the maximum number of off-types (k) is 10, the corresponding sample size (n) to be used should be in the range 126 to 141.

3.1.6.3 For small sample sizes, the same information is shown graphically in the corresponding figures (figures (1 to 21). These show the actual risk of rejecting a uniform variety and the probability of accepting a variety with a true proportion of off-types 2 times (2P), 5 times (5P) and 10 times (10P) greater than the population standard. (To ease the reading of the figure, lines connect the risks for the individual sample sizes, although the probability can only be calculated for each individual sample size).

Population standard %	Acceptance probability %	See table and figure no.
10	>90	19
10	>95	20
10	>99	21
5	>90	1
5	>95	7
5	>99	13
3	>90	2
3	>95	8
3	>99	14
2	>90	3
2	>95	9
2	>99	15
1	>90	4
1	>95	10
1	>99	16
0.5	>90	5
0.5	>95	11
0.5	>99	17
0.1	>90	6
0.1	>95	12
0.1	>99	18

Table A.	Overview	of table an	nd figure	1 to 18.
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3.1.6.4 When using the tables the following procedure is suggested:

(a) Choose the relevant population standard.

(b) Write down the different relevant decision schemes (combinations of sample size and maximum number of off-types), with the probabilities of type I and type II errors read from the figures.

(c) Choose the decision scheme with the best balance between the probabilities of errors.

3.1.6.5 The use of the tables and figures is illustrated in the example section.

#### 3.1.7 Detailed description of the method for one single test

The mathematical calculations are based on the binomial distribution and it is common to use the following terms:

(a) The percentage of off-types to be accepted in a particular case is called the "population standard" and symbolized by the letter P.

(b) The "acceptance probability" is the probability of accepting a variety with P% of off-types. However, because the number of off-types is discrete, the actual probability of accepting a uniform variety varies with sample size but will always be greater than or equal to the "acceptance probability." The acceptance probability is usually denoted by  $100 - \alpha$ , where  $\alpha$  is the percent probability of rejecting a variety with P% of off-types (i.e. type I error probability). In practice, many varieties will have less than P% off-types and hence the type I error will in fact be less than  $\alpha$  for such varieties.

(c) The number of plants examined in a random sample is called the sample size and denoted by n.

(d) The maximum number of off-types tolerated in a random sample of size n is denoted by k.

(e) The probability of accepting a variety with more than P% off-types, say  $P_q$ % of off-types, is denoted by the letter  $\beta$  or by  $\beta_q$ .

(f) The mathematical formulae for calculating the probabilities are:

$$\alpha = 100 - 100 \sum_{i=0}^{k} {\binom{n}{i}} P^{i} (1 - P)^{n - i}$$
(1)  
$$\beta_{q} = 100 \sum_{i=0}^{k} {\binom{n}{i}} P_{q}^{i} (1 - P_{q})^{n - i}$$
(2)

P and  $P_{a}$  are expressed here as proportions, i.e. percents divided by 100.

#### 3.1.8 More than one single test (year)

3.1.8.1 Often a candidate variety is grown in two (or three years). The question then arises of how to combine the uniformity information from the individual years. Two methods will be described:

- (a) Make the decision after two (or three) years based on the total number of plants examined and the total number of off-types recorded. (A combined test).
- (b) Use the result of the first year to see if the data suggests a clear decision (reject or accept). If the decision is not clear then proceed with the second year and decide after the second year. (A two-stage test).

3.1.8.2. However, there are some alternatives (e.g. a decision may be made in each year and a final decision may be reached by rejecting the candidate variety if it shows too many off-types in both (or two out of three years)). Also there are complications when more than one single year test is done. It is therefore suggested that a statistician should be consulted when two (or more) year tests have to be used.

#### 3.1.9 Detailed description of the methods for more than one single test

#### Combined Test

3.1.9.1 The sample size in test i is  $n_i$ . So after the last test we have the total sample size  $n = \Sigma n_i$ . A decision scheme is set in exactly the same way as if this total sample size had been obtained in a single test. Thus, the total number of off-types recorded through the tests is compared with the maximum number of off-types allowed by the chosen decision scheme.

## Two-stage Test

3.1.9.2 The method for a two-year test may be described as follows: In the first year take a sample of size n. Reject the candidate variety if more than  $r_1$  off-types are recorded and accept the candidate variety if less than  $a_1$  off-types are recorded. Otherwise, proceed to the second year and take a sample of size n (as in the first year) and reject the candidate variety if the total number of off-types recorded in the two years' test is greater than r. Otherwise, accept the candidate variety. The final risks and the expected sample size in such a procedure may be calculated as follows:

$$\begin{aligned} \alpha &= P(K_{1} > r_{1}) + P(K_{1} + K_{2} > r \mid K_{1}) \\ &= P(K_{1} > r_{1}) + P(K_{2} > r - K_{1} \mid K_{1}) \end{aligned}$$

$$= \sum_{i=r_{1}+1}^{n} {\binom{n}{i}} P^{i} (1 - P)^{n - i} + \sum_{i=\alpha_{1}}^{r_{1}} {\binom{n}{i}} P^{i} (1 - P)^{n - i} \sum_{j=r - i + 1}^{n} {\binom{n}{i}} P^{j} (1 - P)^{n - j} \qquad (3)$$

$$\beta_{q} &= P(K_{1} < \alpha_{1}) + P(K_{1} + K_{2} \le r \mid K_{1}) \\ &= P(K_{1} < \alpha_{1}) + P(K_{2} \le r - K_{1} \mid K_{1}) \end{aligned}$$

$$= \sum_{i=0}^{\alpha_{1}-1} {\binom{n}{i}} P_{q}^{i} (1 - P_{q})^{n - i} + \sum_{i=\alpha_{1}}^{r_{1}} {\binom{n}{i}} P_{q}^{i} (1 - P_{q})^{n - i} \sum_{j=0}^{r - i} {\binom{n}{i}} P_{q}^{j} (1 - P_{q})^{n - j} \qquad (4)$$

$$n_{e} = n \left( 1 + \sum_{i=\alpha_{1}}^{r_{1}} {n \choose i} P^{i} (1 - P)^{n - i} \right)$$
(5)

where

P = population standard

 $\alpha$  = probability of actual type I error for P

 $\beta_q$  = probability of actual type II error for q P

 $n_e =$  expected sample size

r<sub>1</sub>, a<sub>1</sub> and r are decision-parameters

 $P_q = q$  times population standard = q P

 $K_1$  and  $K_2$  are the numbers of off-types found in years 1 and 2 respectively.

51. The decision parameters,  $a_1$ ,  $r_1$  and r, may be chosen according to the following criteria:

- (a)  $\alpha$  must be less than  $\alpha_0$ , where  $\alpha_0$  is the maximum type I error, i.e.  $\alpha_0$  is 100 minus the required acceptance probability
- (b)  $\beta_q$  (for q=5) should be as small as possible but not smaller than  $\alpha_0$
- (c) if  $\beta_q$  (for q=5) <  $\alpha_0 n_e$  should be as small as possible.

3.1.9.3 However, other strategies are available. No tables/figures are produced here as there may be several different decision schemes that satisfy a certain set of risks. It is suggested that a statistician should be consulted if a 2-stage test (or any other sequential tests) is required.

## <u>3.1.10 Sequential tests</u>

The two-stage test mentioned above is a type of sequential test where the result of the first stage determines whether the test needs to be continued for a second stage. Other types of sequential tests may also be applicable. It may be relevant to consider such tests when the practical work allows analyses of off-types to be carried out at certain stages of the examination. The decision schemes for such methods can be set up in many different ways

and it is suggested that a statistician should be consulted when sequential methods are to be used.

# 3.1.11 Note on type I and type II errors

3.1.11.1 We cannot in general obtain type I-errors that are nice pre-selected figures because the number of off-types is discrete. The scheme a of example 2 with 6 plants above showed that we could not obtain an  $\alpha$  of 10% - our actual  $\alpha$  became 0.6%. Changing the sample size will result in varying  $\alpha$  and  $\beta$  values. Figure 3 - as an example - shows that  $\alpha$  gets closer to its nominal values at certain sample sizes and that this is also the sample size where  $\beta$  is relatively small.

3.1.11.2 Larger sample sizes are generally beneficial. With same acceptance probability, a larger sample will tend to have proportionally less probability of type II errors. Small sample sizes result in high probabilities of accepting non-uniform varieties. The sample size should therefore be chosen to give an acceptably low level of type II errors. However small increases in the sample size may not always be advantageous. For instance, a sample size of five gives  $\alpha = 10\%$  and  $\beta_2 = 82\%$  whereas a sample size of six gives  $\alpha = 0.6\%$  and  $\beta_2 = 98\%$ . It appears that the sample sizes, which give  $\alpha$ -values in close agreement with the acceptance probability are the largest in the range of sample sizes with a specified maximum number of off-types. Thus, the smallest sample sizes in the range of sample sizes with a given maximum number of off-types should be avoided.

# 3.1.12 Definition of statistical terms and symbols

The statistical terms and symbols used have the following definitions:

*Population standard.* The percentage of off-types to be accepted if all the individuals of a variety could be examined. The population standard is fixed for the crop in question and is based on experience.

Acceptance probability. The probability of accepting a uniform variety with P% of off-types. Here P is population standard. However, note that the actual probability of accepting a uniform variety will always be greater than or equal to the acceptance probability in the heading of the table and figures. The probability of accepting a uniform variety and the probability of a type I error sum to 100%. For example, if the type I error probability is 4%, then the probability of accepting a uniform variety is 100 - 4 = 96%, see e.g. figure 1 for n=50). The type I error is indicated on the graph in the figures by the sawtooth peaks between 0 and the upper limit of type I error (for instance 10 on figure 1). The decision schemes are defined so that the actual probability of accepting a uniform variety is always greater than or equal to the acceptance probability in the heading of the table.

*Type I error*: The error of rejecting a uniform variety.

*Type II error*: The error of accepting a variety that is too non-uniform.

P Population standard

 $P_q$  The assumed true percentage of off-types in a non-uniform variety.  $P_q = q P$ .

In the present document q is equal to 2, 5 or 10. These are only 3 examples to help the visualization of type II errors. The actual percentage of off-types in a variety may take any value. For instance we may examine different varieties which in fact may have respectively 1.6%, 3.8%, 0.2%, ... of off-types.

- n Sample size
- k Maximum number of off-types allowed
- α Probability of type I error
- $\beta$  Probability of type II error

#### 3.2.13 Tables and figures

Table and figure 1:

Population Standard = 5% Acceptance Probability ≥90% n=sample size, k=maximum number of off-types

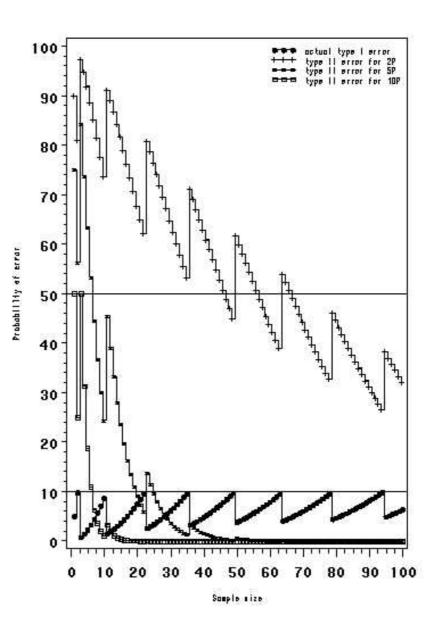


Table and figure 2:

#### Population Standard = 3% Acceptance Probability ≥90% n=sample size, k=maximum number of off-types

748to $777$ $29$ $778$ to $806$ $30$ $807$ to $836$ $31$ $837$ to $865$ $32$ $866$ to $995$ $33$ $896$ to $925$ $34$ $926$ to $955$ $35$ $956$ to $984$ $36$ $985$ to $1014$ $37$ $1015$ to $1044$ $38$ $1045$ to $1074$ $39$ $1075$ to $1104$ $40$ $1105$ to $1134$ $41$ $1135$ to $1164$ $42$ $1165$ to $1225$ $44$ $1226$ to $1225$ $45$ $1266$ to $1285$ $46$ $1286$ to $1315$ $47$ $1316$ to $1346$ $48$ $1347$ to $1376$ $49$ $1377$ to $1406$ $50$	1 4 18 38 59 82 106 131 157 183 209 236 263 290 318 346 374 402 430 458 487 516 4573 602 631 660 690 719		3 17 37 58 81 105 130 156 182 208 235 262 289 317 345 373 401 429 457 486 515 543 572 601 630 659 689 718 747	k 0 1 2 3 4 5 6 7 8 9 10 11 12 3 4 5 6 7 8 9 10 11 12 3 14 15 16 17 18 9 20 21 22 3 24 25 26 27 28
1407         to         1437         51           1438         to         1467         52           1468         to         1498         53	748 778 807 837 866 926 925 1015 1045 1045 1075 1105 1105 1105 1135 1165 1226 1256 1316 1347 1377 1407 1438	to t	777 806 836 895 925 955 984 1014 1044 1074 1104 1104 1104 1104 110	29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 9 50 51 52

#### Table and figure 3:

Population Standard = 2% Acceptance Probability ≥90% n=sample size, k=maximum number of off-types

#### Table and figure 4:

#### Population Standard =1% Acceptance Probability ≥90% n=sample size, k=maximum number of off-types

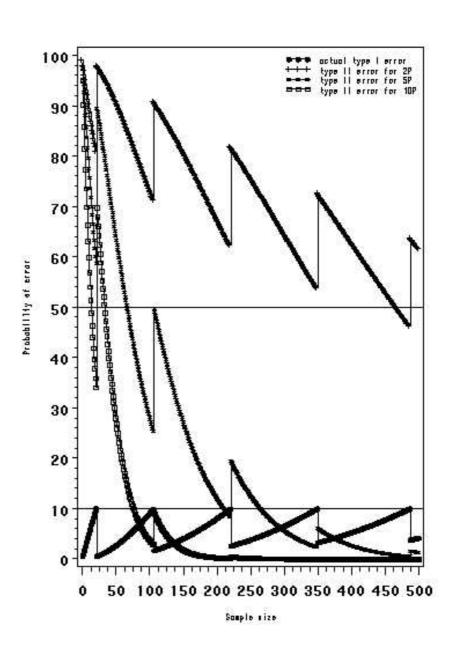
	n		k
1	to	10	0
11	to	53	1
54	to	110	2
111	to	175	3
176	to	244	4
245	to	316	5
317	to	390	6
391	to	466	7
467	to	544	8
545	to	623	9
624	to	703	10
704	to	784	11
785	to	866	12
867	to	948	13
949	to	1031	14
1032	to	1115	15
1116	to	1199	16
1200	to	1284	17
1285	to	1369	18
1370	to	1454	19
1455	to	1540	20
1541	to	1626	21
1627	to	1713	22
1714	to	1799	23
1800	to	1887	24
1888	to	1974	25
1975	to	2061	26
2062	to	2149	27
2150	to	2237	28
2238	to	2325	29
2326	to	2414	30
2415	to	2502	31
2503	to	2591	32
2592	to	2680	33
2681	to	2769	34
2770	to	2858	35
2859	to	2948	36
2949	to	3000	37

10.000

#### Table and figure 5:

Population Standard = .5% Acceptance Probability ≥90% n=sample size, k=maximum number of off-types

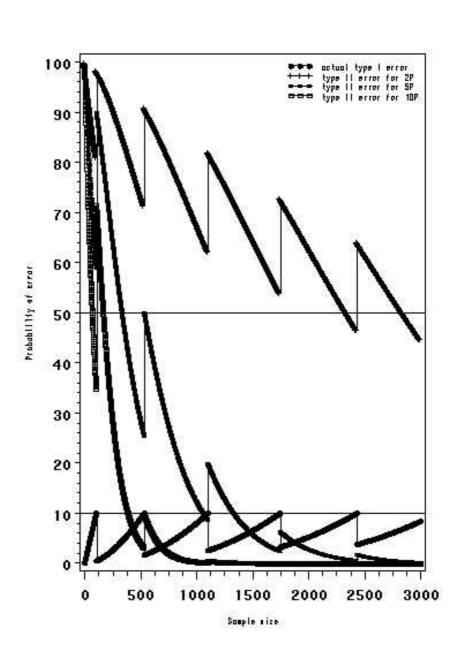
	n		k
1	n to	21	0
22	to	106	1
107	to	220	2
221	to	349	3
350	to	487	4
488	to	631	5
632	to	780	6
781	to	932	7
933	to	1087	8
1088	to	1245	9
1246	to	1405	10
1406	to	1567	11
1568	to	1730	12
1731	to	1895	13
1896	to	2061	14
2062	to	2228	15
2229	to	2397	16
2398	to	2566	17
2567	to	2736	18
2737	to	2907	19
2908	to	3000	20



#### Table and figure 6:

Population Standard = .1% Acceptance Probability ≥90% n=sample size, k=maximum number of off-types

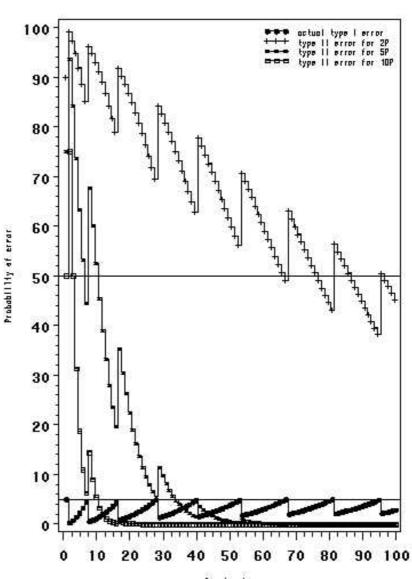
	n		k
1	to	105	0
106	to	532	1
533	to	1102	2
1103	to	1745	3
1746	to	2433	4
2434	to	3000	5



#### Table and figure 7:

#### Population Standard = 5% Acceptance Probability ≥95% n=sample size, k=maximum number of off-types

	n		k
1	to	1	0
2	to	7	1
8	to	16	2
17	to	28	3
29	to	40	4
41	to	53	5
54	to	67	6
68	to	81	7
82	to	95	8
96	to	110	9
111	to	125	10
126	to	140	11
141	to	155	12
156	to	171	13
172	to	187	14
188	to	203	15
204	to	219	16
220	to	235	17
236	to	251	18
252	to	268	19
269	to	284	20
285	to	300	21
301	to	317	22
318	to	334	23
335	to	351	24
352	to	367	25
368	to	384	26
385	to	401	27
402	to	418	28
419	to	435	29
436	to	452	30
453	to	469	31
470	to	487	32
488	to	504	33
505	to	521	34
522	to	538	35
539	to	556	36
557	to	573	37
574	to	590	38
591	to	608	39
609	to	625	40
626	to	643	41
644	to	660	42
661	to	678	43
679	to	696	44
697	to	713	45
714	to	731	46
732	to	748	47
749	to	766	48
767	to	784	49
785	to	802	50
803	to	819	51
820	to	837	52
838	to	855	53
856	to	873	54
874	to	891	55
892	to	909	56
910	to	926	57
927	to	944	58
945	to	962	59
963	to	980	60
981	to	998	61
/01	10	//0	01

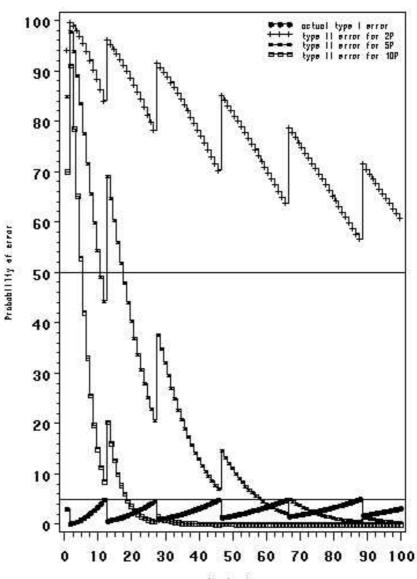


Sample size

## Table and figure 8:

Population Standard = 3% Acceptance Probability ≥95% n=sample size, k=maximum number of off-types

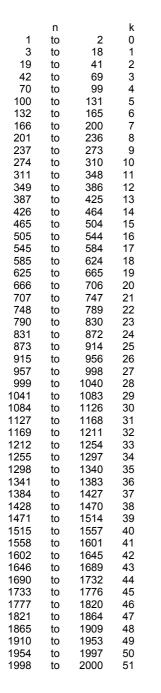
$\begin{array}{c} 28 & \text{to} \\ 47 & \text{to} \\ 89 & \text{to} \\ 111 \\ 135 & \text{to} \\ 233 \\ 259 \\ 285 \\ 311 \\ 338 \\ 418 \\ 445 \\ 473 \\ 500 \\ 528 \\ 553 \\ 391 \\ 418 \\ 445 \\ 473 \\ 500 \\ 528 \\ 555 \\ 5831 \\ 639 \\ 667 \\ 696 \\ 445 \\ 100 \\ 724 \\ 752 \\ 101 \\ 1041 \\ 1070 \\ 1099 \\ 982 \\ 1011 \\ 1041 \\ 1070 \\ 1099 \\ 1128 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1245 \\ 1041 \\ 1070 \\ 1099 \\ 1128 \\ 1051 \\ 1041 \\ 1070 \\ 1099 \\ 1128 \\ 1041 \\ 1041 \\ 1070 \\ 1033 \\ 1041 $	$\begin{array}{c} 110\\ 134\\ 158\\ 182\\ 207\\ 232\\ 258\\ 284\\ 310\\ 337\\ 363\\ 390\\ 417\\ 444\\ 472\\ 499\\ 527\\ 554\\ 582\\ 610\\ 638\\ 666\\ 723\\ 751\\ 780\\ 809\\ 837\\ 866\\ 895\\ 924\\ 952\\ 981\\ 1010\\ 1040\\ 1069\\ 837\\ 866\\ 895\\ 924\\ 952\\ 981\\ 1010\\ 1040\\ 1069\\ 837\\ 866\\ 895\\ 924\\ 952\\ 981\\ 1010\\ 1040\\ 1069\\ 837\\ 866\\ 895\\ 924\\ 952\\ 981\\ 1010\\ 1040\\ 1069\\ 1156\\ 1186\\ 1215\\ 1244\\ 1303\\ 1362\\ 1422\\ 1451\\ 1511\\ 1541\\ 1570\\ 1600\\ 1630\\ 1600\\ 1630\\ 1600\\ 1630\\ 1600\\ 1630\\ 1600\\ 1630\\ 1600\\ 1720\\ 1750\\ 1810\\ 1840\\ 1870\\ 1810\\ 1$	$\begin{array}{c} 6\\ 7\\ 8\\ 9\\ 10112314151677822222222222222222222222222222222222$
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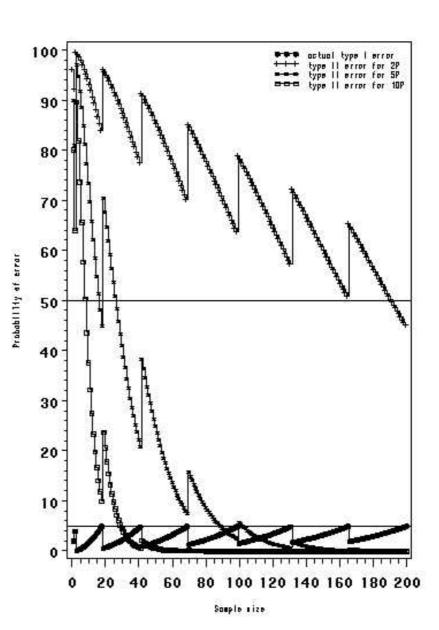


Somple size

#### Table and figure 9:

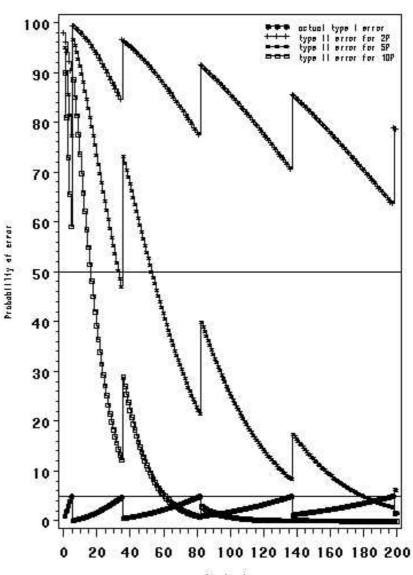
Population Standard = 2% Acceptance Probability ≥95% n=sample size, k=maximum number of off-types





## Table and figure 10:

### Population Standard = 1% Acceptance Probability ≥95% n=sample size, k=maximum number of off-types

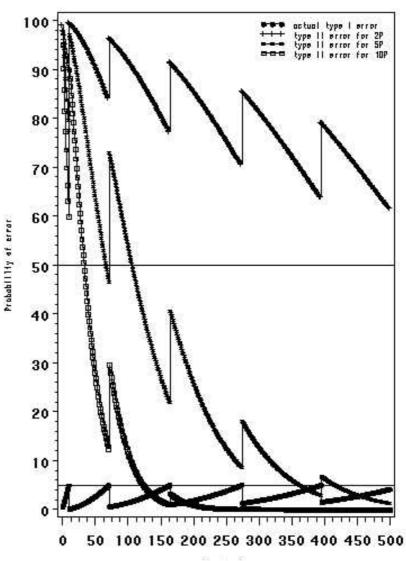


Sample size

## Table and figure 11:

Population Standard = .5% Acceptance Probability ≥95% n=sample size, k=maximum number of off-types

1 11 72 165 275 396 524 659 798 941 1087 1236 1387 1541 1696 1852 2010 2170 2330 2492 2654	n to	10 71 164 274 395 523 658 797 940 1086 1235 1386 1540 1695 1851 2009 2169 2329 2491 2653 2814	k 0 1 2 3 3 4 5 6 7 8 9 10 11 123 14 15 16 17 18 19 20
2818	to	2981	21
2982	to	3000	22

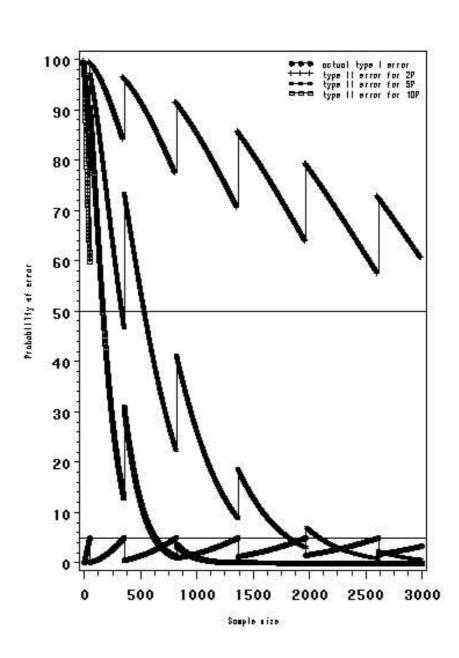


Somple size

## Table and figure 12:

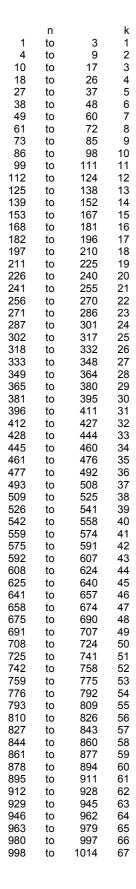
Population Standard = .1% Acceptance Probability ≥95% n=sample size, k=maximum number off-types

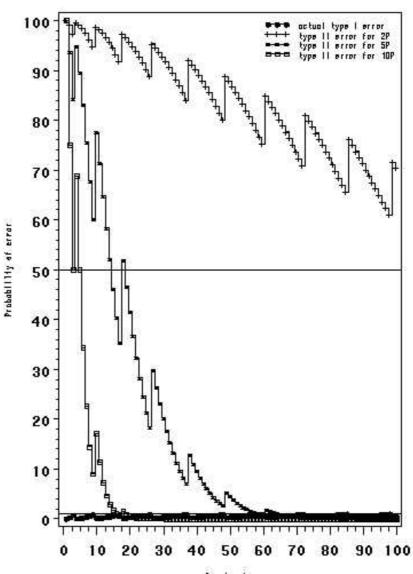
	n		k
1	to	51	0
52	to	355	1
356	to	818	2
819	to	1367	3
1368	to	1971	4
1972	to	2614	5
2615	to	3000	6



#### Table and figure 13:

Population Standard = 5% Acceptance Probability ≥99% n=sample size, k=maximum number of off-types



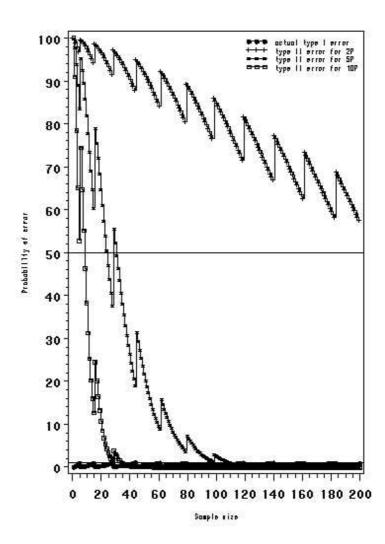


Sample size

## Table and figure 14:

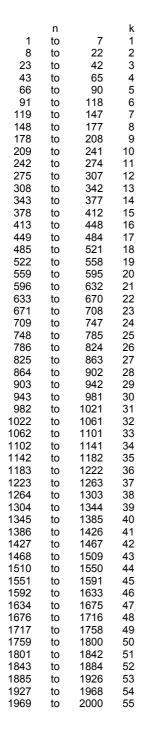
Population Standard = 3% Acceptance Probability ≥99% n=sample size, k=maximum number of off-types

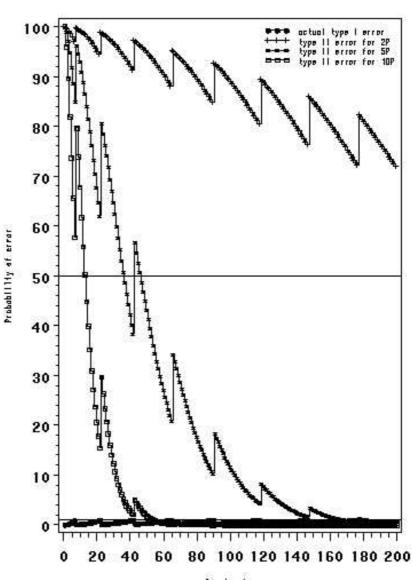
$\begin{array}{c}1\\6\\16\\29\\45\\62\\80\\99\\120\\141\\162\\184\\207\\230\\253\\277\\301\\325\\349\\374\\449\\475\\552\\578\\604\\630\\657\\683\\710\\737\\764\\790\\817\\845\\872\\996\\954\\981\\1009\\1036\\1064\\1092\\1120\\1147\\1175\\1203\\1231\end{array}$	n to	$\begin{array}{c}5\\15\\28\\44\\61\\79\\98\\119\\140\\161\\183\\206\\229\\252\\276\\300\\324\\348\\373\\98\\423\\448\\479\\5551\\577\\603\\629\\656\\2709\\656\\2709\\656\\2709\\656\\2709\\816\\441\\898\\5551\\1003\\1091\\1146\\1174\\12030\\1091\\1119\\1146\\12230\\1258\end{array}$	$\begin{smallmatrix} k & 1 & 2 \\ 3 & 4 & 5 & 6 \\ 7 & 8 & 9 \\ 1011 & 121 & 141 \\ 1161 & 171 & 192 \\ 212 & 223 & 226 \\ 222 & 229 & 301 \\ 313 & 333 & 356 \\ 313 & 339 & 414 \\ 414 & 444 \\ 445 & 447 \\ 415 & 446 \\ 416 & 41$
1092 1120 1147 1175	to to to	1119 1146 1174 1202	47 48 49 50



#### Table and figure 15:

Population Standard = 2% Acceptance Probability ≥99% n=sample size, k=maximum number of off-types

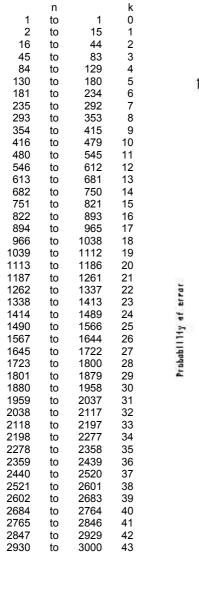


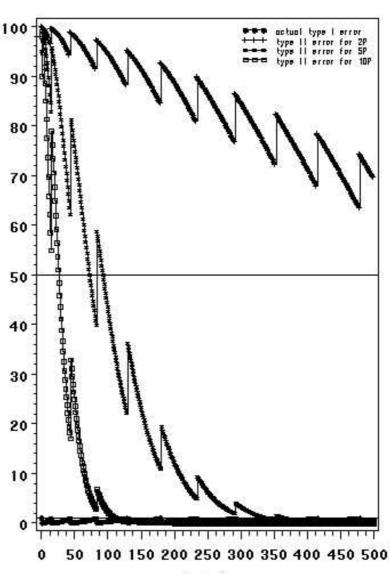


Somple size

#### Table and figure 16:

Population Standard = 1% Acceptance Probability ≥99% n=sample size, k=maximum number of off-types

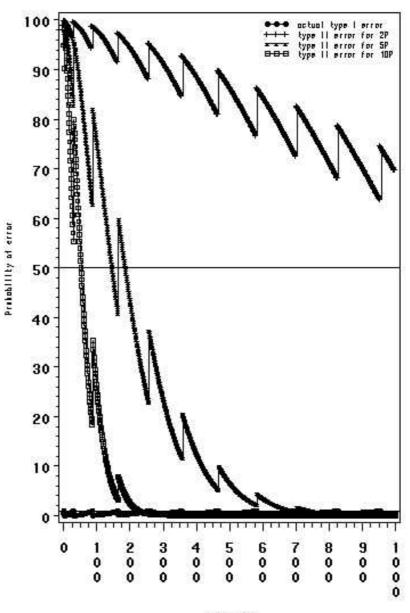




Somple size

## Table and figure 17:

Population Standard = .5% Acceptance Probability ≥99% n=sample size, k=maximum number of off-types

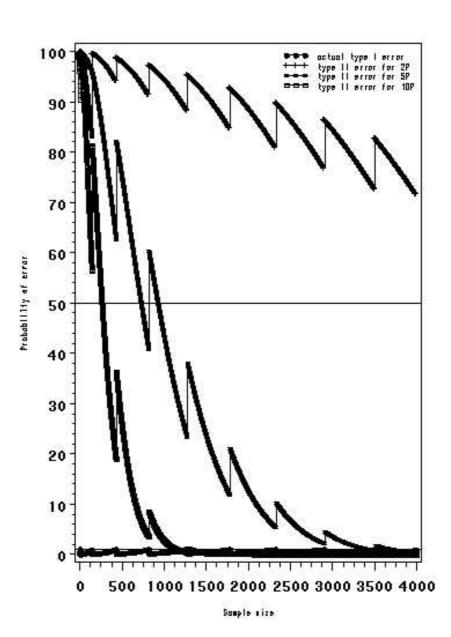


Sample elze

## Table and figure 18:

Population Standard = .1% Acceptance Probability ≥99% n=sample size, k=maximum number of off-types

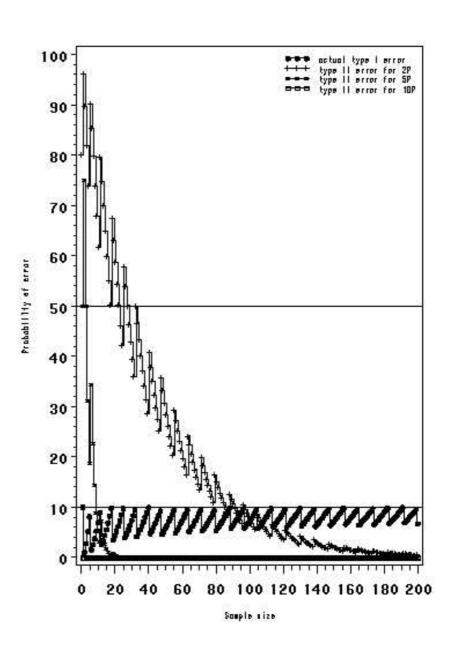
	n		k
1	to	10	0
11	to	148	1
149	to	436	2
437	to	824	3
825	to	1280	4
1281	to	1786	5
1787	to	2332	6
2333	to	2908	7
2909	to	3000	8



## Table and figure 19:

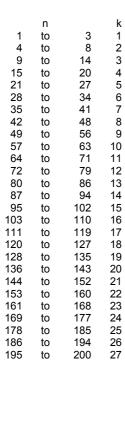
### Population Standard = 10% Acceptance Probability ≥ 90% n=sample size, k=maximum number of off-types

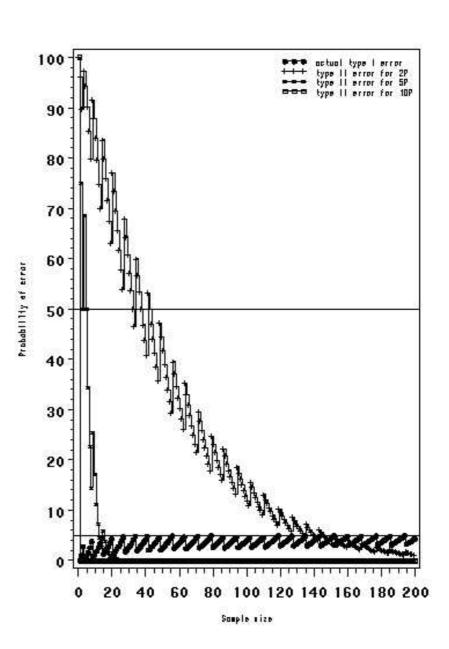
$\begin{array}{c}1\\2\\6\\12\\19\\26\\33\\41\\48\\56\\64\\72\\80\\97\\105\\114\\122\\131\\148\\157\\165\\174\\183\\200\end{array}$	n to	$\begin{array}{c}1\\5\\11\\18\\25\\32\\40\\47\\55\\63\\71\\79\\88\\96\\104\\113\\121\\130\\138\\147\\156\\164\\173\\182\\191\\199\\200\end{array}$	k 0 1 2 3 4 5 6 7 8 9 10 11 22 3 4 4 5 6 7 8 9 10 11 12 13 14 15 16 17 8 19 20 21 22 3 24 25 26



#### Table and figure 20:

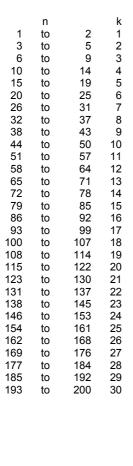
### Population Standard = 10% Acceptance Probability ≥ 95% n=sample size, k=maximum number of off-types

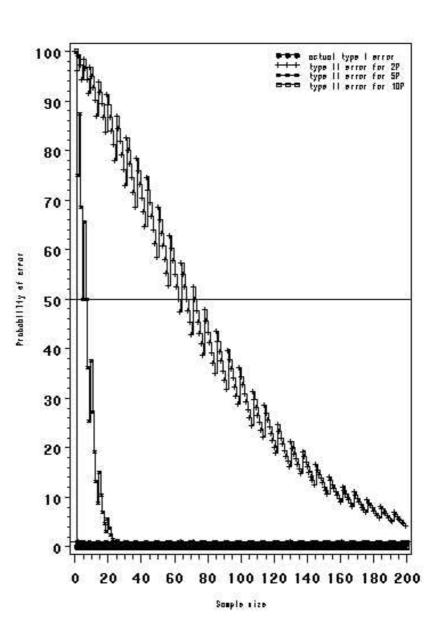




#### Table and figure 21 :

Population Standard = 10% Acceptance Probability ≥ 99% n=sample size, k=maximum number of off-types





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