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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

TECHNICAL WORKING PARTY ON AUTOMATION AND COMPUTER PROGRAMS

Twenty-Ninth Session
Geneva, June 7 to 10, 2011

ADDENDUM

TGP/8: TRIAL DESIGN AND TECHNIQUES USED IN THE EXAMINATION OF DISTINCTNESS, UNIFORMITY AND STABILITY

Document prepared by the Office of the Union

The Annexes to this document contain the following presentations given at the twenty-ninth session of the Technical Working Party on Automation and Computer Programs.

TABLE OF CONTENTS

[Annexes follow]

ANNEX I

Trial Design and Techniques used in the Examination of DUS
(Mr. Uwe Meyer, Germany, referring to Annex I to document TWC/29/14)

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TGP 8
TRIAL DESIGN AND TECHNIQUES USED IN THE EXAMINATION OF
DISTINCTNESS, UNIFORMITY AND STABILITY

ANNEX I
New Section 2 - Data to be recorded

TWC/29/14 (page 18 to 30)

Uwe Meyer UPOV – TWC/29 – Geneva, June 7 to 10, 2011 1

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History

Year/Location	Document	Title
1999/Turku	TWC/17/06	HANDLING OF VISUALLY ASSESSED CHARACTERISTICS
2000/Kyiv	TWC/18/09	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2001/Prague	TWC/19/10	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2002/Mexico	TGP/08.4 D1	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2003/Tjele	TGP/08.4 D2	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2004/Tsukuba	TGP/08.3 D3	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2005/Ottawa	TGP/8/1 D1	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2006/Nairobi	TGP/8/1 D4	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2007/Sibiu	TGP/8/1 D7	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2008/Jeju	TGP/8/1 D10	DATA TO BE RECORDED
2009/Alexandria	TGP/8/1 D13	under further development
2010/Angers	TGP/8/1 D15	under further development
2011/Geneva	TWC/29/14	TGP/8 New Section 2: Data to be recorded (New Draft)

Uwe Meyer UPOV – TWC/29 – Geneva June 7 to 10, 2011 2

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Authors of new draft

- Beate Rücker (TWA) and Uwe Meyer (TWC)
- With collaboration of
  - Andrea Menne (TWO)
  - Erik Schulte (TWF)

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Last changes

- Text was changed marginally
- Part of first introduction was cancelled
- Paragraphs and examples were rearranged
- Better wording to avoid confusion in case of qualitative data getting from quantitative characteristics
  - Ordinally scaled data instead of qualitative data
  - Metric data instead of quantitative data

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Structure of document

previously	Paragraph	updated
2.	Data to be recorded	2.
2.1	Introduction	2.1
2.2	Side-by-side visual comparison	cancelled
2.3	Notes/Single variety records	cancelled
2.4	Variety mean/Statistical analysis of group of plants	cancelled
2.5	Statistical analysis of individual plant data	cancelled
2.5.1	Introduction	cancelled
2.5.2	Different levels to look at a characteristic	
2.5.3	Types of expressions of characteristics	2.2
2.5.4	Types of scales of data	2.3
2.5.5	Scale levels for variety description	2.3.6
2.5.6	Relation between types of expression of characteristics and scale levels of data	2.3.7
2.5.7	Relation between method of observation of characteristics, scale levels of data and recommended statistical procedures	2.3.8
		2.4

Uwe Meyer UPOV – TWC/29 – Geneva June 7 to 10, 2011 5

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Disadvantages of new version

- In paragraph 2.3.2 (Data from qualitative characteristics) and 2.3.4 (Data from pseudo-qualitative characteristics) there are partly the same statements

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Advantages of new version

- Better to read and
- easier to understand for crop experts

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History

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2004/Tsukuba	TGP/08.3 D3	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2005/Ottawa	TGP/8/1 D1	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2006/Nairobi	TGP/8/1 D4	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2007/Sibiu	TGP/8/1 D7	TYPES OF CHARACTERISTICS AND THEIR SCALE LEVELS
2008/Jeju	TGP/8/1 D10	DATA TO BE RECORDED
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2011/Geneva	TWC/29/14	TGP/8 New Section 2: Data to be recorded (New draft)
2012/	TGP/8/2	TGP/8/2 New Section 2: Data to be recorded

[Annex II follows]

ANNEX II

Control of Variation due to different Observers
(Mr. Gerie van der Heijden, Netherlands, referring to Annex II to document TWC/29/14)

Control of variation due to different observers

Gerie van der Heijden & Henk Bonthuis
UPOV TWC, Geneva, June 7-10, 2011

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Variation is influenced by many factors

- Type of crop
- Type of characteristics
- Year / Location
- Trial design
- Crop management
- Method of measurement
- Observer
- ....

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Variation caused by observers

- Especially important for visually assessed characteristics (QN/VG or QN/VS)
- Both variation and bias can play a role
- Important to know if an observed difference between Variety 1, observed by A and Variety 2, observed by B, is caused by differences between the varieties or between the observers

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Controlling the variation caused by observers

- Training
- Testing and calibration

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Training

- UPOV guidelines
- Calibration manuals, with detailed description of local situation
- Supervision and guidance by experienced observers

Testing the calibration

- After training, next step is to test the performance of the observers in a calibration experiment
- Especially true for inexperienced observers making visual observation
- They should pass a calibration test (also useful for experienced observers)

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Calibration experiment

- Involved multiple observers, measuring the same set of material
- Look at the differences between the observers

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MS characteristic

- Generally not necessary after proper instruction
- If needed, one can use approach of Bland and Altman (1986)
- Several steps

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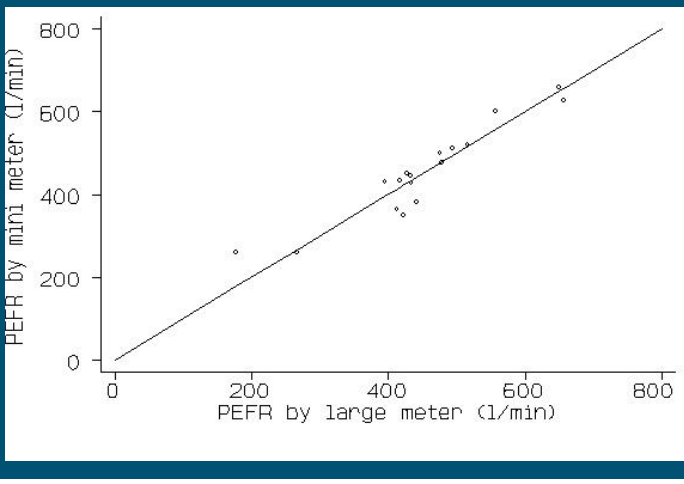
1. Scores in scatter plot, along with line y=x



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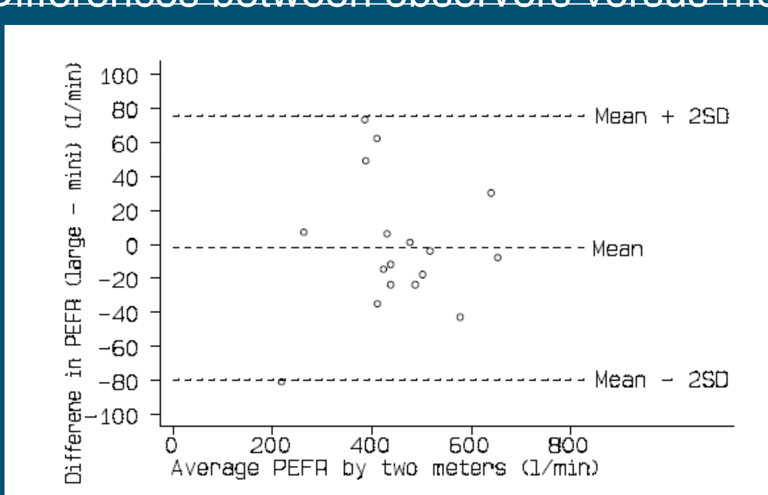
2 Differences between observers versus mean



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3 Tests

- One can use a paired t-test to test the difference between one observer versus another or versus the mean of the other observers.
- More complicated tests involve repeated measurements and study of variance components
- However, generally clear instruction and training suffices. In case of doubt, use calibration experiment as described

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Ordinal characteristics

- First construct contingency tables to look at the size of the problem. Ideally, only main diagonal is non-zero.
- Structural difference (bias) can be tested using Wilcoxon Matched Pair (Signed Rank) test
- Degree of agreement can be established with Cohen’s Kappa, which tries to correct for random agreement

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Different types of Cohen's Kappa

- Standard Cohen's Kappa only considers perfect agreement versus non-agreement between 2 observers (i.e. difference between 1 and 5 is same as difference between 1 and 2)
- Linear of quadratic weighted Kappa can be used for ordinal data, to take into account the degree of disagreement (i.e. difference between 1 and 5 is larger than difference between 1 and 2).
- Generalized kappa gives a single statistic for all observers simulaneously.

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Example

Variety	Observer 1	Observer 2	Observer 3
V1	1	1	1
V2	2	1	2
V3	2	2	2
V4	2	1	2
V5	2	1	2
V6	2	1	2
V7	2	2	2
V8	2	1	2
V9	2	1	2
V10	3	1	3
V11	3	1	3
V12	3	2	2
V13	4	5	4
V14	2	1	1
V15	2	1	2
V16	2	2	3
V17	5	4	5
V18	2	2	3
V19	1	1	1
V20	2	2	2
V21	2	1	2
V22	1	1	1
V23	6	3	6
V24	5	6	6
V25	2	1	2
V26	6	6	6
V27	2	6	2
V28	5	6	5
V29	6	6	5
V30	4	4	4

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Contingency table between O1 and O2

κ(O1,O2) = P(O1 and O2 agree) – P(e) / (1 – P(e))

P(agree) = (3+5+0+1+0+2)/30 = 11/30 ≈ 0.3667 (diagonal)

P(e) = (3/30).(15/30) + (17/30).(6/30) + (3/30).(1/30) + (1/30).(3/30) + (3/30).(0/30) + (3/30).(5/30) ≈ 0.1867. (pair-wise margins)

So κ(O1,O2) ≈ (0.3667-0.1867) / (1-0.1867) ≈ 0.22

O1\O2	1	2	3	4	5	6	Total
1	3	0	0	0	0	0	3
2	10	5	0	1	0	1	17
3	2	1	0	0	0	0	3
4	0	0	0	1	0	0	1
5	0	0	0	1	0	2	3
6	0	0	1	0	0	2	3
Tot	15	6	1	3	0	5	30

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Similarly between other pairs

- κ(O1,O2) ≈ 0.22
- κ(O1,O3) ≈ 0.72
- κ(O2,O3) ≈ 0.22

- Conclusion: Observer 1 and 3 are in good agreement. Observer 2 is clearly different from 1 and 3 and probably needs training.

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References

- **Cohen, J. (1960)** A coefficient of agreement for nominal scales. *Educational and Psychological Measurement* **20:** 37-46.
- **Cohen, J. (1968)** Weighted kappa: Nominal scale agreement provision for scaled disagreement or partial credit. *Psychological Bulletin*, **70**(4): 213-220.
- **Bland, J. M. Altman D. G. (1986)** Statistical methods for assessing agreement between two methods of clinical measurement, *Lancet:* 307–310.

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Thank you for your attention

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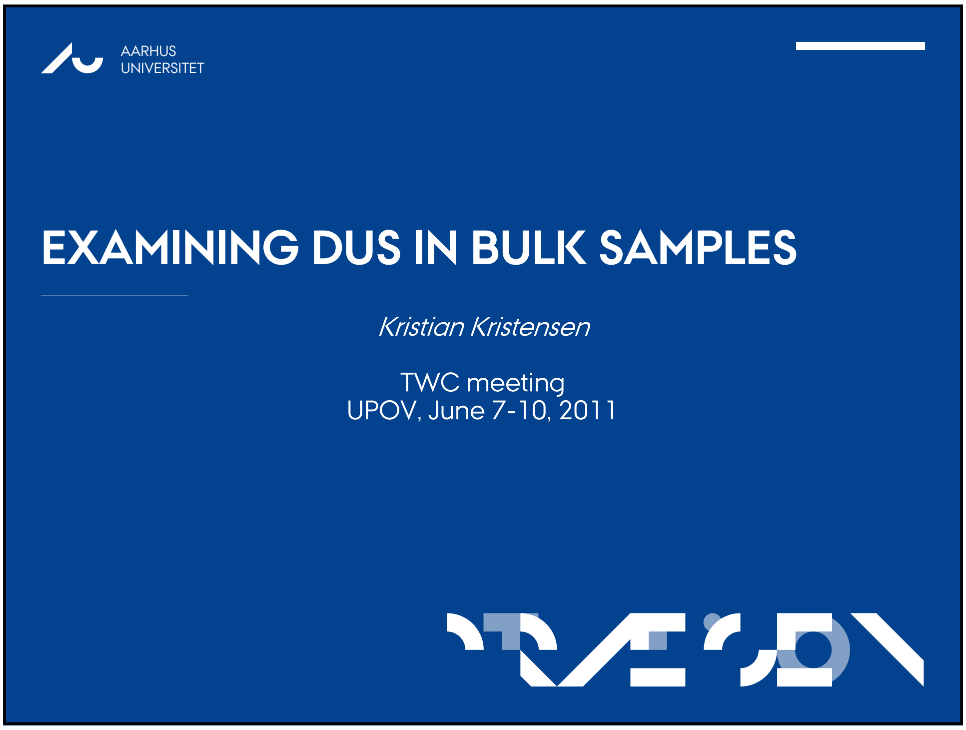
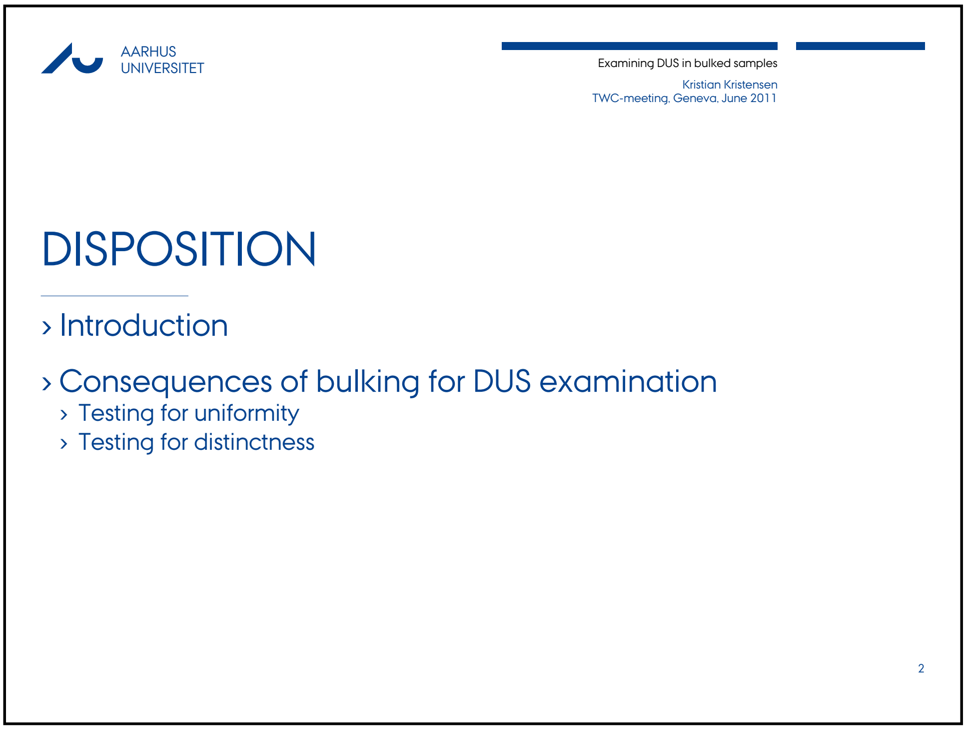
[Annex III follows]

ANNEX III

Examing DUS in Bulk Samples
(Mr. Kristian Kristensen, Denmark, referring Annex VI to document TWC/29/14)





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Examining DUS in bulked samples

Kristian Kristensen
TWC-meeting, Geneva, June 2011

INTRODUCTION

- Definition
  - the process of merging some or all individual plants before recording the characteristics
- Intensity of bulking, e.g.
  - Merging pairs of plants
  - Merging more plants within a plot
  - Merging all plants of a variety (across blocks)
- Reasons for bulking
  - Characteristics which are expensive to record
    - Content of potassium in beet roots
  - Characteristics which are difficult to record
    - Weight of individual seeds, e.g. in peas and beans
    - Content of potassium in sugar beets
- Bulking has some consequences for DUS testing

3

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Examining DUS in bulked samples

Kristian Kristensen
TWC-meeting, Geneva, June 2011

TESTING FOR UNIFORMITY

- Bulking can be very serious for uniformity
- Off-types may be completely masked if bulking is performed
- COYU
  - Bulking will decrease the power of the tests because
    - Partially masking varieties with large standard deviations
    - Loss of degrees of freedom for calculating SD and thus log SD
    - A 3-4 times larger heterogeneity may be necessary to retain the present power with intensive bulking within each block
    - Impossible to perform if all plants within a block is bulked – except in some special and rare cases
  - Bulking across plots
    - is expected to (partially) mask any differences in heterogeneity if the plot to plot variation is notable
    - May invalidate the assumptions for the analysis

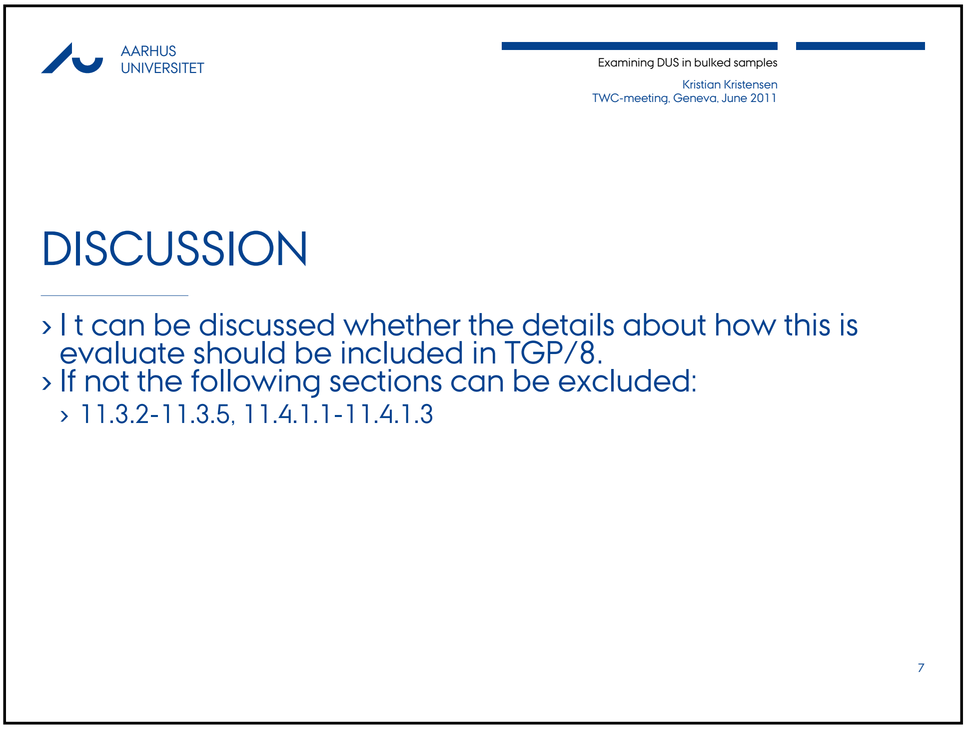
4

Testing for distinctness

- The effect is considered to be small if bulking is done
  - Within plots for COYD and for the 2 times 1% method
  - Within years for COYD
  - Because these methods are based on the means so only the measurement error are decreased – and this error is most often much smaller than the variation cased by other sources (such as soil and climate variability)

GENERAL

- If bulking is necessary
  - Bulk only moderate if uniformity has to be tested
  - Always ensure that equal amount of material from all plants



[Annex IV follows]

ANNEX IV

Example illustrating how Variety Descriptions are developed in Herbage crops (Mr. Vincent Gensollen, France, referring to Annex VIII to document TWC/29/14)

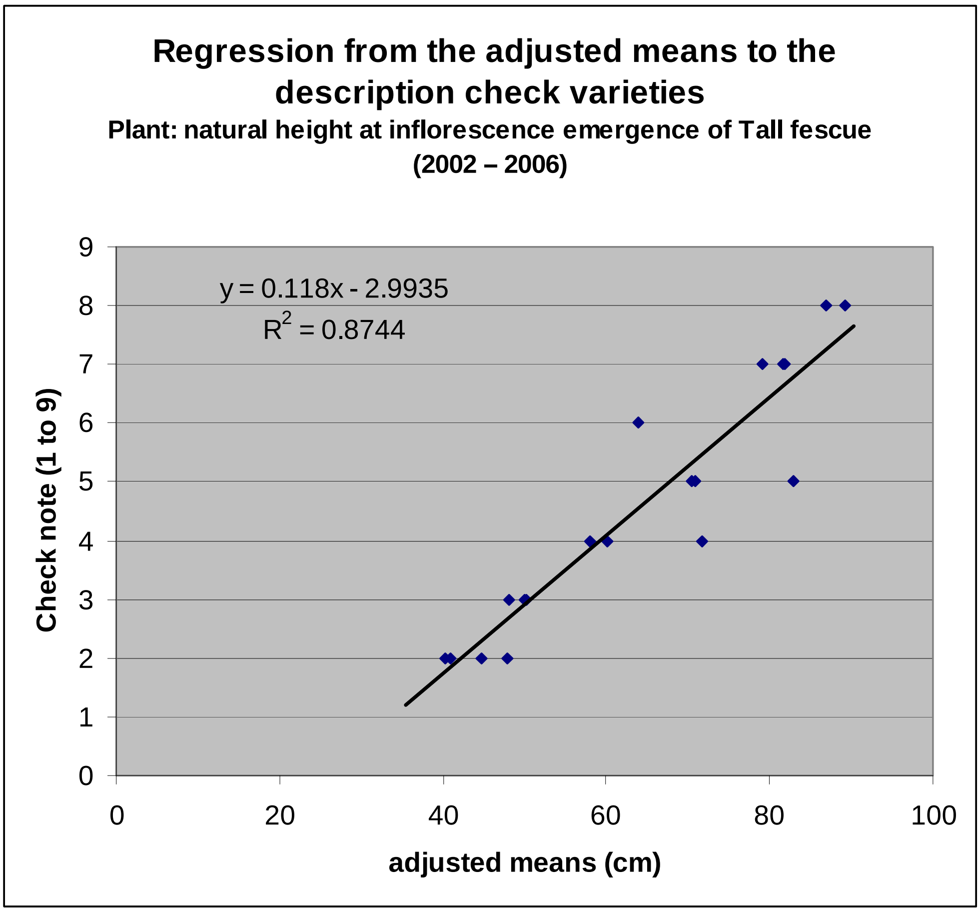
GUIDE LINE TG/39/8 MEADOW FESCUE, TALL FESCUE, CHARACTERISTIC N°10 "PLANT: NATURAL HEIGHT AT INFLORESCENCE EMERGENCE" FOR TALL FESCUE VARIETIES

The data of this characteristic come from measurements on Single plant (MS) in spaced plant trials (A). In that case, the Combined Over Years Distinctness (COYD) analysis provides adjusted means of the reference varieties and the candidate varieties.

For the purpose of the description, we transform the adjusted means to notes. We use a linear regression from the adjusted means to "description check varieties". The description check varieties are already well described example varieties (i.e. example varieties of the UPOV guide line or national example varieties).

The graph below shows the regression from the adjusted means to the description note. In this case 4 varieties had been described with the note 2, 2 varieties with note 3…

FIG. 1: LINEAR REGRESSION FROM THE ADJUSTED MEAN TO THE DESCRIPTION CHECK VARIETY



Regression square (R^2) = 0.8744.
The regression is valid if $R^2 > 0.6$.

Predicted note = 0.118 x adjusted mean - 2.9935.

From the equation above, we can compute the description note.

TAB 3: ADJUSTED MEAN AND DESCRIPTION NOTE FOR THE CHARACTERISTIC NATURAL HEIGHT AT INFLORESCENCE EMERGENCE OF TALL FESCUE VARIETIES.

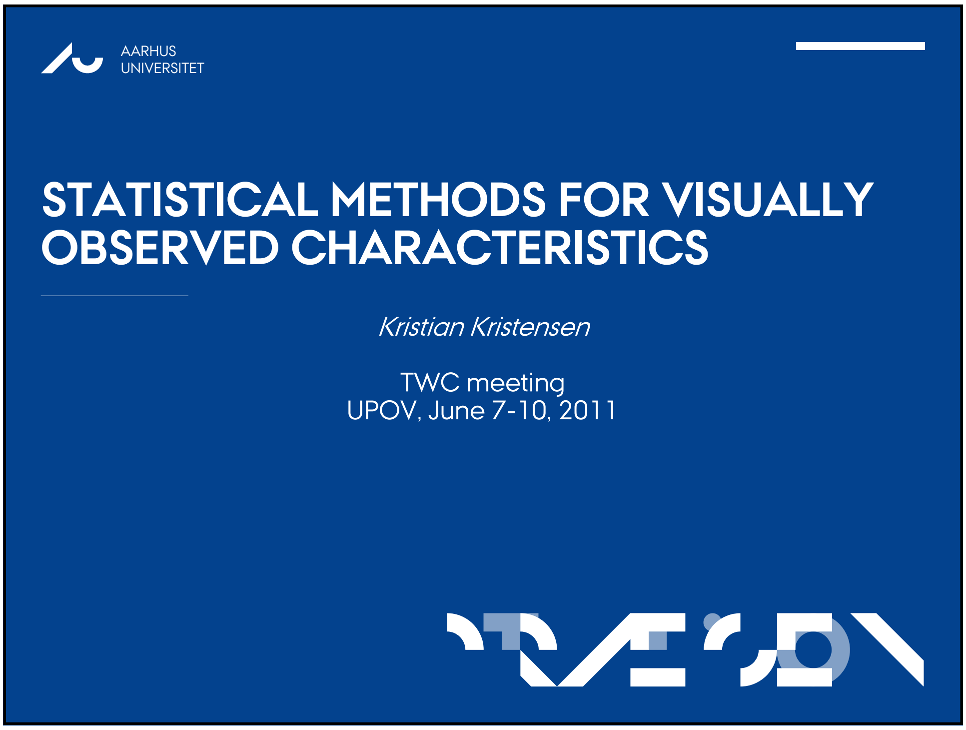
Variety name	Adjusted mean (cm)	Check description note	Predicted note	Description note
C1	35.50	.	1.19423	1
BONAPARTE	44.71	2	2.28068	2
ELDORADO	47.90	2	2.65699	3
C2	48.15	.	2.68648	3
MONTSERRAT	48.15	3	2.68648	3
MURRAY	50.29	3	2.93893	3
C3	52.78	.	3.23266	3
TOMAHAWK	54.80	.	3.47095	3
BORNEO	58.11	4	3.86141	4
C4	58.94	.	3.95932	4
BARDAVINCI	60.28	.	4.11739	4
VILLAGEOISE	62.07	.	4.32855	4
C5	62.13	.	4.33563	4
DANIELLE	63.97	6	4.55268	5
DIVYNA	64.54	.	4.61992	5
C6	69.54	.	5.20975	5
GARDIAN	70.55	5	5.32889	5
EMERAUDE	70.91	5	5.37136	5
CENTURION	71.81	4	5.47753	5
SZARVASI 56	73.18	.	5.63914	6
BARCEL	79.41	.	6.37406	6
DULCIA	81.63	7	6.63594	7
LUNIBELLE	81.85	7	6.66190	7
C7	86.57	.	7.21869	7
BARIANE	87.02	8	7.27177	7
C8	87.44	.	7.32132	7
APRILIA	89.28	8	7.53837	8
C9	89.65	.	7.58202	8
FLEXY	90.31	.	7.65988	8

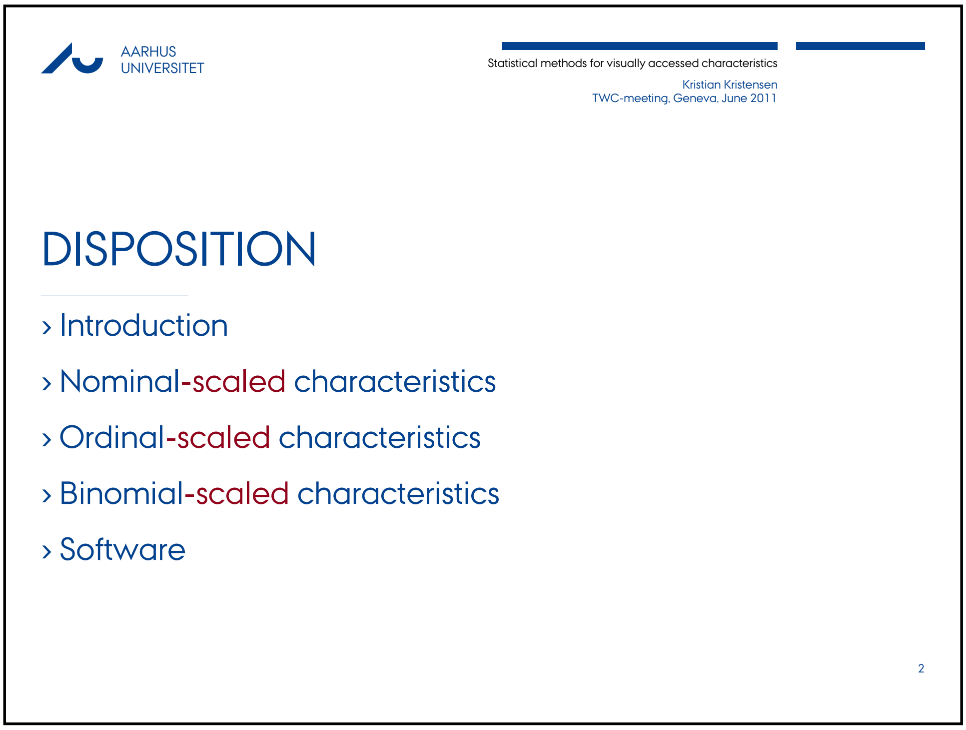
This example illustrates a simple way to obtain coherent notes with computations that can be performed without the need of a statistical package.

[Annex V follows]

ANNEX V

Statistical Methods for Visually Observed Characteristics
(Mr. Kristian Kristensen, Denmark, referring to Annex X to document TWC/29/14)





INTRODUCTION

- For normally distributed characteristics
  - 2 times 1% method - variety×year interaction not taken directly into account
  - COYD which take into account many sources of variation and thereby help insuring that the decisions will be consistent over time
- For visually accessed characteristics
  - χ^2- test in contingency table is probably the most frequent applied method
    - Does only take the random variation caused by sampling into account and thus not any addition sources of variation (such as variation caused by soil and climate variation)
    - Does not take any ordering of the notes into account
- A COYD method for such characteristics are not been described

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Statistical methods for visually accessed characteristics
Kristian Kristensen
TWC-meeting, Geneva, June 2011

NOMINAL CHRACTERISTICS

- Requirements for application
  - Nominal characteristics (the notes can't be ordered)
  - Variation from plant to plant (all plants of a variety don't get the same note)
  - The observations is made over at least 2 years or growing cycles
  - At least 20 degrees of freedom to estimate the variety-by-year interaction
  - The expected number of plants
    - Should be at least 1 for all variety/note combination
    - Should be at least 5 for most variety/note combination
    - (as for the χ^2-test)

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NOMINAL CHRACTERISTICS

- The analyses involve the following steps
  - Calculate the number of plants in each note for each combination of variety and year (i.e. a 3-way table)
  - Estimate the parameters of the model described in the technical section using an appropriate software
  - Compare the candidates to the other varieties
  - Check if the variety-by-year interaction for distinct pairs are considerably larger than the average of all variety pairs

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NOMINAL CHARACTERISTICS

- Example of data
- Hypocotyl colours for some sugar beets varieties

Variety	Colour							
	1 Green		2 White		3-5 Red[1]		7 Orange	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
A	30	21	9	1	15	25	46	53
B	5	9	9	5	48	46	38	40
C	0	3	17	12	31	35	52	50
D	1	0	7	8	71	77	21	15
E	0	3	5	0	80	72	20	25
F	30	28	0	4	30	30	40	38
G	33	25	12	2	16	24	39	49
H	72	76	2	4	3	2	23	18
I	3	2	4	2	37	29	56	67
J	82	82	2	0	7	5	9	13
K	52	7	16	33	0	44	32	16
L	50	37	17	9	5	12	28	42
M	0	0	12	2	58	56	30	42
N	0	0	9	8	74	69	17	23
O	0	0	12	10	58	65	30	25
P	25	22	0	10	17	11	58	57
Q	0	0	0	10	65	64	35	26
R	0	0	0	0	75	55	25	45
S	0	0	6	1	53	61	41	38
T	83	92	5	1	3	1	9	6
U	54	30	12	13	3	4	31	53
V	0	0	6	18	71	63	23	19

NOMINAL CHRACTERISTICS

- The method is based on a generalised linear mixed model
  - The notes can't be ordered
  - A generalised logit is used to describe the parameters of the multinomial distribution as a sum of 4 terms
    - A term for each of the (first) n-1 notes, μ_i
    - A term for each combination of note and variety, β_{ij}
    - A term for each combination of note and year, δ_{ik}
    - A random term for each combination of the (first) n-1 notes, varieties and years, E_{ijk}
  - The method is expected to yield fewer significant pairs of varieties, but to better insure consistent decisions over future years than the χ^2-test

NOMINAL CHRACTERISTICS- Results

Variety	Candidate A			
	F	$P_{dif.}$	F_3	P_{F3}
A	-	-	-	-
B	2.34	0.1157	0.50	0.6855
C	5.70	0.0062	0.57	0.5829
D	6.29	0.0033	0.50	0.6485
E	5.40	0.0063	0.41	0.6601
F	0.52	0.6757	1.20	0.2671
G	0.16	0.9224	0.01	0.9976
H	6.91	0.0036	0.94	0.4998
I	5.44	0.0073	0.24	0.7018
J	10.36	0.0004	0.19	0.8365
K	2.19	0.1361	3.17	0.0405
L	2.02	0.1621	0.11	0.9719
P	0.21	0.8896	1.79	0.0934
T	13.62	<.0001	0.65	0.7695
U	2.34	0.1202	0.52	0.7387

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Statistical methods for visually accessed characteristics

Kristian Kristensen
TWC-meeting, Geneva, June 2011

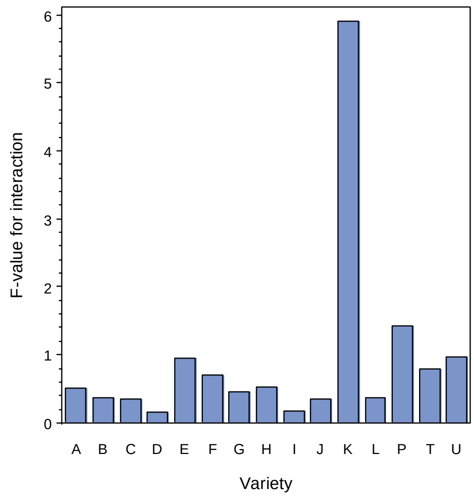
NOMINAL CHARACTERISTICS- Results

F_4-values

Variety by year interaction for each variety

Variety K: much larger interaction than the other varieties.

Should be considered to be excluded from the analyses



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Statistical methods for visually accessed characteristics

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TWC-meeting, Geneva, June 2011

ORDINAL CHRACTERISTICS

- Requirements for application
  - The notes are ordered
  - Variation from plant to plant (all plants of a variety don't get the same note)
  - The observations is made over at least 2 years or growing cycles
  - At least 20 degrees of freedom to estimate the variety-by-year interaction
  - The distribution of the data should be unimodal
  - Zero observations are allowed for each variety if they occur at one or both ends of the ordered scale
  - The number of plants for each variety should be at least 5 times the number of different notes

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Statistical methods for visually accessed characteristics

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TWC-meeting, Geneva, June 2011

ORDINAL CHRACTERISTICS

- The analyses involve the following steps
  - Calculate the number of plants in each note for each combination of variety and year (i.e. a 3-way table)
  - Estimate the parameters of the model described in the technical section using an appropriate software
  - Compare the candidates to the other varieties
  - Check if the variety-by-year interaction for distinct pairs are considerably larger than the average of all variety pairs

  - (As for nominal characteristics, but the formulas are slightly different)

11

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Statistical methods for visually accessed characteristics

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TWC-meeting, Geneva, June 2011

ORDINAL CHRACTERISTICS

Anthocyanin coloration on winter wheat coleoptiles

Variety	Note									
	1 absent or very weak		3 weak		5 medium		7 strong		9 very strong	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
A	98	86	1	3	0	0	0	0	0	0
B	4	14	14	65	178	20	0	0	0	0
C	6	0	32	6	56	83	0	4	0	0
D	1	4	5	13	75	82	17	1	1	0
E	84	62	106	19	3	0	0	0	0	0
F	96	100	4	0	0	0	0	0	0	0
G	96	100	4	0	0	0	0	0	0	0
H	77	84	23	16	0	0	0	0	0	0
I	8	4	15	16	55	69	4	1	0	0
J	95	93	3	0	2	0	0	0	0	0

12

ORDINAL CHRACTERISTICS

- The method is based on a generalised linear mixed model
  - The data are multinomial distributed
  - A cumulative logit is used to describe the parameters of the multinomial distribution as a sum of 4 terms
    - A term for each of the first n-1 notes, μ_i
    - A term for each variety, β_j
    - A term for each year, δ_k
    - A random term for each combination of, varieties and years, E_{jk}
  - The method uses fewer parameters than for nominal characteristics
  - The method utilises the ordering of the notes
  - The method may yield more or fewer significant pairs of varieties than the χ^2-test
  - It is supposed to better insure consistent decisions over future years

ORDINAL CHRACTERISTICS- Results

Anthocyanin coloration on winter wheat coleoptiles

Variety	Candidate A			
	Difference	$P_{Difference}$	F_3	P_{F3}
A	-	-	-	-
B	7.06	0.0009	2.47	0.1503
C	8.11	0.0004	0.38	0.5548
D	9.33	0.0001	1.42	0.2644
E	3.33	0.0471	0.67	0.4353
F	-0.61	0.7152	1.56	0.2425
G	-0.61	0.7152	1.56	0.2425
H	2.41	0.1319	0.21	0.6612
I	7.77	0.0005	0.03	0.8561
J	-0.40	0.8088	1.68	0.2273

BINOMINAL CHARACTERISTICS

- Requirements for application
  - Only two different notes (Multinomial characteristics with only two notes is called binomial characteristics)
  - A note is given to each individual plant
  - Variation from plant to plant (all plants of a variety don't get the same note)
  - The observations is made over at least 2 years or growing cycles
  - At least 20 degrees of freedom to estimate the variety-by-year interaction
  - The expected number of plants
    - Should be at least 1 for all variety/note combination
    - Should be at least 5 for most variety/note combination

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BINOMINAL CHRACTERISTICS

- The analyses involve the following steps
  - Calculate the number of plants in each note for each combination of variety and year
  - Estimate the parameters of the model described in the technical section for either nominal or ordinal characteristics or similar software
  - Compare the candidates to the other varieties
  - Check if the variety-by-year interaction for distinct pairs are considerably larger than the average of all variety pairs
- The method is expected to yield fewer significant pairs of varieties, but to better insure consistent decisions over future years than the χ^2-test
- As there are only two notes there will be no distinction between nominal and ordinal scale.
- Both methods mentioned above for multinomial characteristics reduces to the same method if only two notes are used

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General for all three types of data

› Software
- › The procedure GLIMMIX of SAS can be used to
  - › Estimate the parameters of the models (using different estimation methods)
    - › In the examples a pseudo "REML-estimation method" were used.
  - › Compare pairs of varieties and test if they are significant different
    - › The denominator degree of freedom was calculated using the Kenward-Roger method
- › The procedure IML (and/or data step facilities of SAS) can be used to calculate the F_3-and F_4-values

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General for all three types of data

› Software
- › Similar software may be available in other software packages.
- › Some programmes use "ML-estimation" in stead of "REML-estimation" which may yield (more) biased estimates of variances.
- › Programmes may use different methods, WARLD, Likelihood-ratio, χ^2, F-tests with different methods for calculating dnumerator degrees of freedom

› More information on the methods may be found in the literature
- › 4 references in the paperversion. Others may be relevant also, e.g.
- › Bolker et al. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology and Evolution..**24**, 127-135

[End of Annex V and of document]