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A STUDY ON GRASS REFERENCE COLLECTIONS IN DIFFERENT LOCATIONS

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I Introduction

1.   DUS trials of grasses are very labor intensive and as a consequence very expensive. The number of reference varieties to be included in a DUS test for a single candidate is larger than in many other crops and due to the large variety x year interaction, the reference varieties have to be tested each year anew. Because of the outcrossing nature of the crop, 60 plants per variety are measured. UPOV members which conduct DUS trials of grass varieties have large reference collections. Countries such as Germany, the Netherlands and the United Kingdom each have a large reference collection in the field which are more or less repeats of each other. At a time where a concentration in the grass seed industry might lead to a lower number of applications for listing and plant breeders' rights and where governments tend to work more and more on a cost recovery basis, a revisiting of the use of reference collections in grass DUS testing would be worthwhile.

2.   This document reports on a project, the first aim of which was to consider the possibilities of combining the results of grass trials in 3 countries, Germany (DE), the Netherlands (NL) and the United Kingdom (GB), with respect to DUS testing.

3.   The second aim of the project was to study a scenario where, instead of having a large reference collection at each location, it would be possible to reduce the size of trials, for example by having a large reference collection in one location (the "Sun") to which "satellite" DUS centers were connected through core sets of varieties for "bridging" the data. Candidate varieties and these core sets would be tested at all locations, but the full reference collection would only be tested at one location.

4.   When testing different scenarios, such as the Sun and Satellites system, we do not only need to study the effects on testing for distinctness, but also have to take into account the effects on uniformity testing.

II Available data

5.   Measurements are available from forage and amenity grass DUS trials from 1993 to 2002 in Germany, the Netherlands and the United Kingdom. For forage grass, 7 characteristics were present for 81 varieties and for amenity grasses, 8 characteristics for 89 varieties. These characteristics had measurements for all three countries for at least 2 of the 10 years.

6.   In order to make it easier to obtain complete blocks of measurements, the data files were reduced to the following 5 characteristics:

Code Units Description
UP08 days Time of inflorescence emergence from 1 April (NL: G51 MED_DSD)
UP10 mm   Flag leaf: length (NL: G52 VLB_LEN)
UP11 mm   Flag leaf: width (NL: G53 VLB_BRE)
UP12 cm   Stem: length of longest stem (NL: G55 STE_LEN)
UP13 cm   Inflorescence: length (NL: G60 BLW_LEN)

7.   The UPOV Test Guidelines on Ryegrass (document TG/4/8) contains 23 characteristics of which 6 are asterisked. In practice, the number of characteristics used by each country differs. Germany uses 23 characteristics, of which 11 correspond to the UPOV Test Guidelines, the United Kingdom uses 15, of which 10 correspond to the UPOV Test Guidelines, and the Netherlands uses 16 of which 11 correspond to the UPOV Test Guidelines.

8.   This variation between countries might considerably complicate the introduction of a Sun and Satellites system.

9.   In order to be able to perform COYD analyses, first complete sets of varieties have been sought over a period of 3 years.

10.   In the years 1997, 1998 and 1999, data of amenity grass varieties were available for all 5 characteristics for 61 varieties in Germany, 59 varieties in the Netherlands and 69 varieties in the United Kingdom. The intersection of these 3 sets of varieties contained 40 varieties (i.e. 40 varieties were present in all countries in three years).

11.   In the years 1995, 1996 and 1997, data for forage grass varieties were available for all 5 characteristics for 50 varieties in Germany, 52 varieties in the Netherlands and 67 varieties in the United Kingdom. The intersection of these 3 sets of varieties contained 30 varieties.

III COYD analyses per country

12.   The first analysis was the COYD analysis for each country. Because all varieties in the dataset were already registered, there were in principle no candidate varieties. Therefore, we regarded each variety, successively, as a candidate variety and compared it with all other varieties. Thus, we counted the number of varieties declared distinct in each country. This number could be quite low as only a limited number (5) of characteristics was used. The number of distinct varieties per country is a good indicator of the discriminating power of each country.

13.   To compare the results between the 3 countries, the common 40 varieties in the years 1997, 1998 and 1998 were taken for amenity grass and the common 30 varieties in the years 1995, 1996 and 1997 for forage grass. Table 1 below shows the calculated 1% lsd values and the ranges (difference between maximum and minimum of the variety means) per country.

Table 1: 1% lsd values and ranges per country

AMENITY	40 varieties in the period 1997-1998-1999				
	1% lsd values				
	UP08	UP10	UP11	UP12	UP13
DE	1.72	30.9	1.04	4.68	2.56
NL	2.54	24.0	0.31	7.13	1.79
GB	2.14	13.6	0.22	4.15	1.04
	ranges				
DE	29.9	87.1	2.25	21.1	6.41
NL	29.5	92.4	1.49	32.5	6.85
GB	29.4	103.9	1.43	21.1	9.13
FORAGE	30 varieties in the period 1995-1996-1997				
	1% lsd values				
	UP08	UP10	UP11	UP12	UP13
DE	3.91	29.9	0.63	6.79	4.63
NL	3.79	30.8	0.61	6.42	2.63
GB	4.00	20.6	0.47	4.69	1.56
	ranges				
DE	39.5	109.9	2.30	33.1	13.59
NL	41.1	99.8	2.45	27.3	9.46
GB	58.1	99.0	1.75	23.6	9.24

14.   In Germany, the Netherlands and the United Kingdom there were, respectively, 5, 3 and 17 varieties distinct for amenity grass and 1, 2 and 6 varieties distinct for forage grass. These are shown in Table 2. The United Kingdom has a substantially higher number of distinct varieties than Germany and the Netherlands for both types of grasses and hence the United Kingdom seems to have a higher discriminating power than the other two countries. This has been known for a long time and is the result of a considerably reduced variety x year interaction as shown by the 1% lsd values.   The climate in Northern Ireland (United Kingdom) is rather stable and does not contain periods with extreme weather conditions (temperature or drought), thereby reducing the variety x year interaction.

15.   It is interesting to see how much we can gain by combining the data in a very straightforward way by looking at the combination of the three countries, i.e. by looking at the number of varieties distinct in at least one of the three countries. For amenity grass, 19 varieties are distinct in at least one of the three countries. For forage grass, 7 varieties are distinct in at least one of the three countries.

Table 2: Distinct varieties (x) in the 3 countries. The varieties which are not distinct in any of the three countries with these five characteristics are not listed in this table (21 for amenity and 23 for forage). In total 19 amenity and 7 forage varieties are distinct in at least one of the three countries.

AMENITY Varieties	Country			FORAGE Varieties	Country		
	DE	NL	GB		DE	NL	GB
AMADEUS			x	BAREZANE			x
BARBALL			x	BARYLOU	x	x	x
BARCLAY	x	x		FRANCES			x
BARCREDO			x	JUBILAR			x
BARLUXE	x		x	PERAMO			x
BARRAGE			x	RESPECT		x	
BARSPORTIVO	x			VERITAS			x
BOLOGNA			x				
DALI			x				
EDEN		x	x				
FRAGMENT	x	x	x				
GERONA			x				
LEONARDO			x				
MANHATTAN			x				
MARIETTA			x				
MONTREUX			x				
RENOIR	x		x				
REPELL			x				
SENSATION			x				
Total distinct varieties	5	3	17	Total distinct varieties	1	2	6

IV REML analyses with all data together

16.   We performed a REML analysis where we combined all the data from all three countries in a single analysis and treated country as a factor.

17.   First, this REML analysis is performed with all main effects and interaction terms in the random model to estimate the variance components of the random terms. For amenity grass, data of the 40 common varieties in the period 1997-1998-1999 were used. For forage grass, data of the 30 common varieties in the period 1995-1996-1997 were used. In Table 3 the estimated variance components of the random terms are given for each characteristic.

Table 3: Variance components per characteristic

AMENITY					
	UP08	UP10	UP11	UP12	UP13
Variety	33.39	59.6	0.041	8.89	0.69
Year	0.00	295.8	0.000	2.66	0.56
Country	0.00	574.7	0.629	32.34	9.08
Variety.Year	0.06	46.6	0.003	0.49	0.04
Variety.Country	1.06	29.5	0.007	6.56	0.45
Year.Country	6.27	42.8	0.027	6.85	0.74
Variety.Year.Country	0.94	76.5	0.085	5.96	0.74
FORAGE					
	UP08	UP10	UP11	UP12	UP13
Variety	92.32	88.3	0.043	8.32	1.74
Year	15.81	211.9	0.000	7.21	0.00
Country	0.00	213.6	0.354	0.64	6.54
Variety.Year	2.34	82.8	0.016	0.33	0.32
Variety.Country	4.15	37.7	0.005	2.24	0.00
Year.Country	6.68	134.0	0.138	28.84	1.20
Variety.Year.Country	0.88	77.3	0.054	7.37	1.83

18. The calculation of the 1% lsd values per country is based on the variance component "Variety.Year". The "Variety.Year" variance component in Table 3 is, broadly speaking, the average of the "Variety.Year" variance components (the residual variance) of the COYD per country.

19. A way to test the variety means in an analysis with all data together is to ignore all interactions with "Variety" as separate terms in the model so that the Residual term becomes the sum of the 2-factor-interactions "Variety.Year" and "Variety.Country" plus the 3-factor-interaction "Variety.Year.Country". Then the model can be written as:

$$\text{Characteristic}_{ijk} = \text{OverallMean} + \text{Variety}_i + \text{Year}_j * \text{Country}_k + \text{Res}_{ijk} \quad\quad (1)$$

where i=1…40, j=1…3 (1997,1998,1999) and k=1…3 (DE,NL,GB) for amenity
where i=1…30, j=1…3 (1995,1996,1997) and k=1…3 (DE,NL,GB) for forage

20. From the residual variance component of model (1) the 1% lsd values were calculated and are shown in Table 4. For reasons of comparison, the 1% lsd values per country from Table 1 have also been added to this table.

Table 4: 1% lsd values per country and over all countries

AMENITY	40 varieties in the period 1997-1998-1999				
	UP08	UP10	UP11	UP12	UP13
DE	1.72	30.9	1.04	4.68	2.56
NL	2.54	24.0	0.31	7.13	1.79
GB	2.14	13.6	0.22	4.15	1.04
All countries	1.63	14.1	0.37	4.10	1.28
FORAGE	30 varieties in the period 1995-1996-1997				
	UP08	UP10	UP11	UP12	UP13
DE	3.91	29.9	0.63	6.79	4.63
NL	3.79	30.8	0.61	6.42	2.63
GB	4.00	20.6	0.47	4.69	1.56
All countries	2.94	15.9	0.32	3.73	1.76

21.   The REML analysis with model (1) can be viewed as an alternative for the COYD analysis if data of more than one country are combined together. The 1% lsd values are then the yardsticks for comparing the estimated Variety means from this model.

22.   For amenity grass, the 1% lsd values of the United Kingdom were for most of the characteristics much smaller than those of Germany and the Netherlands. Taking the data together gives only smaller 1% lsd values for UP08 and UP12 in comparison with those of the United Kingdom.

23.   For forage grass the 1% lsd values of the United Kingdom are also for most of the characteristics much smaller than those of Germany and the Netherlands. Combining the data gives smaller 1% lsd values (except for UP13) in comparison with the data of the United Kingdom alone. This means that, in principle, for forage grass, a higher number of distinct varieties can be expected by combining data for all countries than for the countries Germany, the Netherlands and the United Kingdom separately.

24.   Table 5 shows the distinct varieties using the overall 1% lsd values (column DE+NL+GB). The distinct varieties of Table 2 are added to this table to make the comparisons easier. For amenity grass, the number of distinct varieties was 10 instead of 5, 3 and 17 for, respectively, Germany, the Netherlands and the United Kingdom separately. Nine of the 10 belong to the set of 19 varieties listed in Table 2. Only the variety "PRESTER" is new here. For forage grass, the number of distinct varieties was 8 instead of 1, 2 and 6 for, respectively, Germany, the Netherlands and the United Kingdom separately. Five of the 8 belong to the set of 7 varieties listed in Table 2. The varieties "BARLET", "SAMBIN" and "YORK" are new, and "PERAMO" and "FRANCES" (distinct in the United Kingdom) were not distinct when the data of all countries were combined.

TWC/28/31
page 8

Table 5: Distinct varieties (x) per country and overall (DE+NL+GB)

AMENITY Varieties	Country			DE+NL+GB	FORAGE Varieties	Country			DE+NL+GB
	DE	NL	GB			DE	NL	GB	
AMADEUS			x	x	BAREZANE			x	x
BARBALL			x		BARLET				x
BARCLAY	x	x		x	BARYLOU	x	x	x	x
BARCREDO			x		FRANCES			x	
BARLUXE	x		x	x	JUBILAR			x	x
BARRAGE			x	x	PERAMO			x	
BARSPORTIVO	x				RESPECT		x		x
BOLOGNA			x		SAMBIN				x
DALI			x	x	VERITAS			x	x
EDEN		x	x	x	YORK				x
FRAGMENT	x	x	x	x					
GERONA			x	x					
LEONARDO			x						
MANHATTAN			x						
MARIETTA			x						
MONTREUX			x						
PRESTER				x					
RENOIR	x		x	x					
REPELL			x						
SENSATION			x						
Total distinct varieties	5	3	17	10	Total distinct varieties	1	2	6	8

25. Thus, for amenity grass, the analysis with only the United Kingdom data gives far more distinct varieties (17) than the analysis with the data of all countries together (10).

26. For forage grass, a comparable number of distinct varieties was found for every analysis: 6 when only using the United Kingdom data, 7 when the individual results per country were combined and 8 for the overall analysis.

V An experimental approach to examine variety distinctness

27. An experimental approach for declaring varieties distinct with data from more than one country is to consider the characteristics for each country independently from the same characteristics in another country. This means that 2 varieties which do not differ in a characteristic in one country, but differ in another country with respect to the same characteristic, are considered different. In this way, the number of characteristics is expanded from 5 to 15 (5 chars x 3 countries).

28. The rationale of this approach is that the characteristic is expressed differently in every country and hence can be regarded as a different characteristic, although there may be correlation, in a similar way to length of the seedling being used in addition to the length of the mature plant. Of course it would require a uniformity test for each characteristic in each country.

29.   For amenity grass, carrying out such an analysis gave 26 distinct varieties; the set of 19 distinct varieties listed in Table 2, plus 7 other varieties.

30.   For forage grass, carrying out such an analysis gave 8 distinct varieties; the set of 7 distinct varieties listed in Table 2, plus the variety "SAMBIN".

31.   Especially for amenity grass, the number of distinct varieties was much larger in comparison with the methods in Section III (COYD analyses per country) and Section IV (REML analyses with all data together). However, this approach is highly debatable.

VI. Discussion and conclusions

32.   The intention has been to investigate a Sun and Satellites system and to see what the consequences would be if at one location (the so-called "Sun"), all varieties were tested and at other locations (the Satellites) a restricted number of varieties were tested. With respect to the Sun, the United Kingdom would be the most suitable country based on the 5 characteristics chosen here. As can be seen from Table 1, the other countries have comparable 1% lsd values only with respect to "Time of inflorescence emergence" (UP08).

33.   The idea of the Sun and Satellites system is based on the expectation that combining data from different countries would improve the discrimination. However, the proposal in Section IV (REML analyses with all data together) to use model (1) for calculation of the 1% lsd values shows that the numbers of distinct amenity varieties was even less than when only the data of the United Kingdom were used. For forage grass, the number of distinct varieties is only marginally higher than that for the United Kingdom alone. It can be concluded that using all data from the Satellites does not, or only marginally, improve the discrimination between varieties, due to the large interaction between varieties and location. Reducing the reference collection at the satellites would create incomplete datasets and increase the variance and hence further reduce the discriminating power. Therefore, no further efforts have been made to investigate what the results would be if only a limited number of varieties were available in the other countries (Satellites).

34.   An experimental approach (described in Section V) for determining distinct varieties increased the number of distinct varieties. For amenity grass, the number of distinct varieties was much larger (26) in comparison with the number of distinct varieties in the United Kingdom (17) and, for forage grass, a little larger (8 in comparison to 6). The assumption for this approach is that the same characteristic in a different country is regarded as a different characteristic. This approach is rather debatable.

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