Animal &
Plant Health
Agency

United Kingdom Variety List Trials: Trial Procedures for Official Examination of Value for Cultivation and Use (VCU) Harvest 2023

Perennial, Italian and Hybrid Ryegrass, Timothy, Festulolium, Cocksfoot, Tall and Meadow Fescue

January 2023

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Section A – General information

A.1 Purpose

A.1.1 This document sets out the approved procedures to be used for growing trials, tests and assessments as required by the current **Protocol for Official Examination of Value for Cultivation and Use for Perennial, Italian and Hybrid Ryegrass, Timothy, Festulolium, Cocksfoot, Tall and Meadow Fescue**.

A.2 Scope

A.2.1 These procedures apply to all varieties of Perennial, Italian and Hybrid Ryegrass, Timothy, Festulolium, Cocksfoot, Tall and Meadow Fescue.

A.3 Responsibilities

A.3.1 **Procedures Development Group**

The Procedures Development Group is responsible for reviewing these procedures annually and making amendments for which it has responsibility, in accordance with the provisions of the VCU Protocol.

A.3.2 **Trials Organisers and Operators**

A.3.2.1 Trials Organisers

a. England & Wales
British Society of Plant Breeders Ltd (BSPB)
BSPB House
114 Lancaster Way Business Park
Ely
Cambs. Jeremy M: 07747 567351
CB6 3NX Louise M: 07917 046705
Email jeremy.widdowson@bspb.co.uk

b. Scotland
SASA
Roddinglaw Road
Edinburgh Tel No 0131 2448899
EH12 9FJ Fax No 0131 2448940
Email russell.thomson@sasa.gov.scot

A.3.2.2 NIAB is responsible for ensuring all operators have access to all VCU procedures and protocols. APHA are responsible for providing these procedures and protocols to Trial Organisers, or anyone on request. The Trials Organisers are responsible for ensuring all VCU Protocol and Procedures requirements are followed and liaison with all Operators carrying out trials for National List purposes, including supply of seed and data handling.

A.3.2.3 Data Handling Operator

The Data Handling Operator identified by the Trials Organisers is responsible for trial design and data validation in accordance with the VCU protocol and associated Procedures.

A.3.2.4 Growing Trial Operators, Seed Handling Operators, Pathology Trial Operator and Quality Testing Operators

The Trials Organisers are responsible for proposing potential Growing Trial Operators and Quality Testing Operators to carry out trials and tests as determined by the Procedures Development annual review in accordance with the **VCU Protocol**, and these **Procedures**. The Trials Organisers are also responsible for finding Seed Handling Operators who are able to carry out seed handling. Seed Handling Operators prepare trial seed for sowing on behalf of any Growing Trial Operator in accordance with the **VCU Protocol** and these **Procedures**. The Pathology Trial Operator is responsible for carrying out inoculated disease tests and collating natural infection data.

A.3.2.5 A list of all approved Organisers and Operators is shown in Appendix 1.

A.3.3 **VCU Protocol and Procedures non-compliance**

A.3.3.1 Where these procedures use the words “must” or “will” for any action then failure to carry out this action will result in non-compliance. Where the word “should” is used for any action then this is the method to be followed unless there are clear reasons not to, which can be justified by the operator as technically sound.

A.3.3.2 The Trials Organisers will forward any reports on **VCU Protocol** or **Procedures** non-compliance to APHA within 1 week of receipt. The Trials Organisers will obtain authorisation from APHA for any actions, including those necessary to remedy non-compliances, which are not within the requirements of the **VCU Protocol**. Such actions must be recorded as a non-compliance. Where emergency action is required and APHA staff are not available (e.g. evenings/weekends) the Trials Organiser should act but report this to APHA at the earliest opportunity. Where GMOs are concerned the arrangements are as detailed in section 3.4.

A.3.4 **Procedures for GM varieties**

A.3.4.1 The National Authorities and Trials Organiser will develop procedures for GM varieties if an application for a GM candidate variety is received.

A.3.4.2 **Procedures for candidates with endophytes**

A.3.4.2.1 Material containing more than 5% endophyte is not eligible for testing. The National Authorities and Trials Organiser will consider developing procedures for candidates with endophytes if an application for such a candidate is received.

A.3.4.2.2 Breeders must identify and alert the Trial Operators about varieties that are at risk of containing more than 5% endophytes.

A.3.5 **Handling of trial seed**

A.3.5.1 The Seed Handling Operator is responsible for organising the handling of seed of candidate varieties submitted by the applicant, and seed of control, or other reference varieties, in accordance with the requirements set out in these Procedures and the current VCU Protocol. The Trials Organiser will ensure that any seed treatments or additives are fit for the purpose. Seed treatment products are listed in Appendix 2.

A.3.6 **Dispatch of seed**

A.3.6.1 The Seed Handling Operator will arrange for seed to be dispatched by the agreed deadlines to the Growing Trial Operators, and, for authentication, to the DUS testing centres including, where appropriate, foreign testing authorities. Where seed will be kept until late summer for drilling the seed should be kept in cold storage to prevent deterioration.

A.3.7 **Monitoring of VCU Growing Trial Operators and Seed Handling Operators – documentation**

A.3.7.1 The appropriate Trials Organiser will take any necessary action to enforce deadline dates and quality standards for required documentation.

A.3.7.2 The Trials Organisers will ensure Growing Trial Operators and Seed Handling Operators have access to all current protocols and procedures relevant to them and that they are notified of any amendments.

A.3.8 **Seed quantities**

A.3.8.1 The Trials Organisers will determine the quantity of seed required for all VCU tests and trials in each annual series, including authentication, and will notify the applicant of quantities and delivery addresses.

A.3 9 **Labelling of seed**

A.3.9.1 The Trials Organisers are responsible for ensuring all seed is clearly labelled with variety name/breeders' reference, ploidy and AFP number.

A.3.10 **Seed quality**

A.3.10.1 Seed submitted for VCU testing must meet the standards for the final generation of seed given in the appropriate seed regulations, in respect of germination, analytical purity and content of other seeds and any other impurities.

A.4 Summary of growing trials, tests, and assessments procedures

A.4.1 The number of trials and site locations are as detailed in Appendix 4.

A.4.2 Control varieties are listed in Appendix 5.

A.4.3 The Trials Organisers are responsible for informing the Growing Trial Operators of the additional approved characters, which must be recorded as and when requested by applicants, and any samples that may be required for analysis.

A.4.4 Special Tests

An additional test for characters not specified in the procedures may be requested by the applicant. APHA is responsible for liaison with the Trials Organisers to produce a procedure for the conduct of a special test or trial. This procedure would require the approval of the National Authorities.

A.4.5 VCU trial assessments required

Bold = Obligatory *Italics = Additional if requested by the applicant (nonavailable at present)*

PRG (perennial ryegrass)

Type of character	Reference	Description of assessment
Yield	Section C	**Total dry matter yield under conservation management in the first harvest year.** **Total dry matter yield under simulated grazing management in the second harvest year.** **Total dry matter yield under conservation management in the third harvest year.**
Behaviour with respect to factors in the physical environment.	Section C	**Ground cover in the autumn of the second and third harvest years.** **Resistance to winter damage.**
Resistance to harmful organisms	Section D	**Mildew (%)** **Crown rust (%)** **Drechslera (%)** **Black stem rust (%)** **Rhynchosporium (%)** **RMV (%)** **Brown rust (%)** **BYDV (%)** **Bacterial wilt (%)** **Red thread (%)** **Snow mould (%)**
Quality characteristics	Section E	**First conservation cut digestibility in the first harvest year.** **Second conservation cut digestibility in the first harvest year.** **Mid-season digestibility in the second harvest year.**

IRG, HRG TF and FL (Italian ryegrass, hybrid ryegrass tall fescue and festulolium)

Type of character	Reference	Description of assessment
Yield	Section C	**Total dry matter yield in sowing year.** **Total dry matter yield in the first harvest year.** **Total dry matter yield in the second harvest year.** **Total dry matter yield in the third harvest year for HRG Trials only.**
Behaviour with respect to factors in the physical environment.	Section C	**Ground cover in the sowing year (TF only).** **Ground cover in the autumn of the first and second harvest year and third harvest year for HRG only.** **Resistance to winter damage.**
Resistance to harmful organisms	Section D	**Mildew (%)** **Crown rust (%)** **Drechslera (%)** **Black stem rust (%)** **Rhynchosporium (%)** **Brown rust (%)** **RMV (%)** **BYDV (%)** **Bacterial wilt (%)** **Red thread (%)** **Snow mould (%)**
Quality characteristics	Section E	**First and second conservation cut digestibility in the first harvest year.**

TIM, CFT and MF (timothy, cocksfoot and meadow fescue)

Type of character	Reference	Description of assessment
Yield	Section C	**Total dry matter yield under conservation management in the first harvest year.** **Total dry matter yield under simulated grazing management in the second year.** **Total dry matter yield under conservation management in the third harvest year.**
Behaviour with respect to factors in the physical environment	Section C	**Ground cover in the autumn of the second and third harvest years.** **Resistance to winter damage.**
Resistance to harmful organisms	Section D	**Mildew (%).** **Black stem rust (%) (not meadow fescue)** **Yellow Rust (%) (Cocksfoot)** **Halo spot (%)** **Cladosporium (%)**
Quality characteristics	Section E	**First conservation cut digestibility in the first harvest year.** **Second conservation cut digestibility in the first harvest year.** **Mid-season digestibility in the second year.**

FESTULOLIUM (FL) and TALL FESCUE (TF)

NB: are tested according to the procedures for the ryegrass type which the applicant has selected

A.4.5.1 Further Measurements

The following must be measured or recorded in all trials, following procedures in Section C.

Sowing date
Establishment weakness
Ground cover in sowing Year
Ground cover in first harvest year
Harvest date
Pest damage (where present at a level which will affect results)
Plot size

Section B – Seed handling procedures

B.1 Responsibilities

B.1.1 Seed Handling Operator or Growing Trial Operators are responsible for carrying out the following seed handling procedures.

B.2 Seed handling procedures

B.2.1 Seed Handling Operator/Growing Trial Operators will receive a sowing list from the Trials Organiser.

B.2.2 Seed Handling Operator/Growing Trial Operators must record receipt of seed from applicants by checking it against the sowing list as it arrives. APHA should be notified of any damage to the packaging, loss of seed or certification problems that would affect the validation of the trials.

B.2.3 The Seed Handling Operator must retain the following:

- Ryegrasses and Festulolium - 30 g of untreated seed taken from the year 2 and subsequent seed submission
- Timothy - 20 g of untreated seed from every sample submitted

for authentication by the DUS test centre.

B.2.4 Cross contamination must be avoided by ensuring equipment is clean between weighing and treatments.

B.2.5 Each Seed Handling Operator must retain a 10 g sample of seed until one month after the end of the trial.

B.2.6 **Endophyte test**

Seed originating directly from New Zealand, Australia, South America and the USA will be routinely tested. Breeders must inform APHA if seed contains an endophyte. The Seed Handler must submit required seed for endophyte testing.

B.3 Authentication of seed stocks

B.3.1 Year 1 VCU and DUS submissions are taken from the single submitted seed stock. Year 2 and any further VCU seed submissions are authenticated by the DUS Test Centre according to the procedures set out in the appropriate DUS Protocol, except when there is 1 single seed submission or submissions from the same seed lot.

B.3.2 All samples must be kept under suitable conditions for the authentication procedures required and must be clearly labelled and sealed.

B.3.3 The Seed Handling Operator must send requested samples to the DUS test centre by the date specified by APHA.

B.3.4 If the level of uniformity recorded in DUS tests is not uniform (COYU) or VCU authentication of a candidate is negative the VCU tests will be considered invalid for that candidate in that season.

Section C – Growing trial procedures

C.1 Responsibilities

C.1.1 The Growing Trial Operators are responsible for conducting the trials according to these procedures.

C.2 Site suitability

C.2.1 The Growing Trial` Operator will be responsible for providing a suitable site, which meets the following criteria:

C.2.2 Soil type should be typical of those on which PRG, IRG, HRG, TIM, FL, CFT, TF and MF are grown locally. Soil fertility and texture should be uniform across the site. The soil should be sufficiently uniform to avoid variation in the growth of the trial.

C.2.3 Previous cropping must be appropriate for PRG, IRG, HRG, TIM, FL, CFT, TF and MF to be grown.

C.2.4 The trial should be sited away from trees, hedges, headlands and other features, which are likely to cause uneven growth or expose the trial to damage from pests.

C.2.5 The trial area should be cultivated in the direction of ploughing and should be sown across the direction of ploughing and cultivation such that each plot receives similar wheeling compaction. Cultivations should follow best practice.

C.3 Sowing the trial

C.3.1 **Plot size**

C.3.1.1 Plots must be drilled or broadcast to produce a minimum plot length of 4.5 m after cutting back. Minimum sown width is 0.9 m with a maximum unsown gap between plots of 0.5 m. Minimum harvest plot size is 6.5 m^2. The row number per plot should not be less than 10 rows for drilled plots. Two replicates will be sown.

C.3.2 **Plant population**

C.3.2.1 When sowing, self-cleaning type drills should be used at the following seed rates:

PRG	
Diploid candidates	25 kg/ha
Tetraploid candidates	37 kg/ha
IRG and HRG & FL	
Diploid candidates	33 kg/ha
Tetraploid candidates	50 kg/ha
TIM	16 kg/ha
MF	25 kg/ha
TF	50 kg/ha

C.3.3 **Trial layout**

C.3.3.1 The Trials Organisers following consultation with APHA, produce provisional sowing lists. The Trials Organisers will make final sowing lists available to Growing Trial Operators, along with the trial plans produced by the Data Handling Operator.

C.3.3.2 The trial must be sown according to the plan produced by the Data Handling Operators and may be an incomplete block design. In an incomplete block design, each replicate is split into a number of sub-blocks. Any splitting of replicates must be between sub-blocks and not through sub-blocks. Varieties can be moved within a sub-block but must not be moved from their sub-block. If plots are moved out of their original sub-block, they will have to be treated as missing plots. The Trials Organiser must be informed immediately if there are any departures from the original plan or if there are any other anomalies.

C.3.3.3 If there is a need to replace a planned variety e.g., if varieties are withdrawn, affected plots must be sown with any of the standard control varieties. Any such replacements must be agreed with the appropriate Trials Organiser. The control varieties are listed in Appendix 5.

C.3.4 **Sowing**

C.3.4.1 Care must be taken with drill settings and sowing speed to ensure uniform distribution of seed in each plot. It is also important to ensure that there is no carry over of seed between plots. Growing Trial Operators should inform the appropriate Trials Organiser as soon as it is apparent that the establishment of any plot has been unsuccessful.

C.3.4.2 Any missing rows or parts of rows or plot areas must be noted on the sowing plan and returned to the appropriate Trials Organiser so that a decision on the viability of these and adjacent plots can be made. It may sometimes be possible to patch in missing parts of rows without affecting the viability of the trial but this should only be done after consultation with the appropriate Trials Organiser if it is done after the sowing year.

C.3.5 **Confirmation of trial layout**

C.3.5.1 After the trial has been sown, the Growing Trial Operator must:

a) Confirm that the trial has been drilled or broadcast according to the plan and provide the sowing date, by returning site data 1 and associated trial sketch to the appropriate Data Handling Operator.

b) If any amendments to the plan have been made, return a hard copy of the plan to the appropriate Data Handling Operator with any amendments clearly indicated. Alternatively, amendments may be notified electronically with the agreement of the Data Handling Operator.

C.4 Husbandry

C.4.1 **Agronomy**

Where not specified in these procedures' agronomy should follow best local practice, advisory and regulatory guidelines. Application of fertilisers and sprays should be uniform. It is normally best to apply these across the direction of the plots. Application wheeling's should not run through the harvested plot area.

C.4.2 **Fertiliser application**

Application of fertilisers should be uniform. It is normally best to apply these across the direction of the plots. It must take into account inherent fertility, previous cropping, winter rainfall and the best local practice. All fertiliser applications should take account of the AHDB Nutrient Management Guide (RB209), the corresponding advisory publications in England, Wales, Scotland and Northern Ireland and past trialling experience.

Details of fertiliser rates are given below:

C.4.2.1 **Nitrogen**

Application levels should seek to achieve optimum growing conditions in line with the official regional advisory publications and regulations. **Example application levels** used previously are as follows:

Series perennial ryegrass, timothy and meadow fescue

Sowing year:
At the discretion of the Growing Trial Operator to achieve optimum growing conditions, in compliance with the official advisory publications.

First harvest year	
Nine weeks prior to estimated date of first cut (optionally this application may be split with the second dose applied at least 1 week before the anticipated cut for each 10kgs).	60 to 100 kg/ha
After the first cut	90 kg/ha
After the second cut	90 kg/ha
After all further cuts except the last	35 kg/ha

Second harvest year	
In February or March	50 to 80 kg/ha
After all further cuts except the last	35 kg/ha

Third harvest year	
Nine weeks prior to estimated date of first cut (optionally this application may be split with the second dose applied at least 1 week before the anticipated cut for each 10kgs).	100 to 125 kg/ha
After the first cut	90 kg/ha
After the second cut	90 kg/ha
After all further cuts except the last	35 kg/ha

Series Italian and hybrid ryegrass, tall fescue and festulolium
Example application levels used previously are as follows:

Sowing year	
In seedbed prior to sowing	According to best local practice.
After all cuts except the last	35 kg/ha

In each harvest year	
In February or March	60 kg/ha
After the first cut	100 kg/ha
After the second cut	100 kg/ha
After the third cut	60 kg/ha
after all further cuts except the last	35 kg/ha

C.4.2.2 **Other elements**

Sulphate should be applied along with nitrogen applications at a rate between 20 and 40% of the N rate. Thus, for a nitrogen application of 100 kg N /ha, sulphate would be applied at between 20 and 40 kg/ha, as SO_3.

In addition to the above phosphate, potash, lime, etc. should be applied at the discretion of the Growing Trial Operator and in compliance with official regional advisory publications and regulations. In the sowing year Growing Trial Operators should note the necessity of adequate pH, phosphate, and potash for grass establishment.

C.4.3 **Herbicides**

Chemicals must not be used if there are any known varietal sensitivities. If in doubt, the appropriate Trials Organiser should be consulted. Application must be uniformly applied and should normally be across the direction of sowing.

C.4.4 **Growth regulators**

These must not be used.

C.4.5 **Pest and disease control**

C.4.5.1 **Pest control**

Frit fly, leatherjackets and wireworm are the most likely insect pests. During the sowing year they should be controlled by appropriate means if necessary but treatment should not be done in the harvest years without the permission of the appropriate Trials Organiser. Slugs can also damage the establishing trial and treatment with an approved molluscicide may be required in the sowing year. Treatment should not be done in the harvest years without the permission of the appropriate Trials Organiser. If necessary, approved means should be used to prevent or minimise damage by field mice, birds and other vertebrate pests. Control should be carried out throughout the trial period and not just in the sowing year.

C.4.5.2 **Disease control**

Disease control should only be undertaken after agreement with the Trials Organiser.

C.4.6 **Irrigation**

Irrigation will only be permitted to facilitate establishment. Permission from the Trials Organiser is not required to do this.

C.4.7 **Pathways**

A gap (pathway) is required at the end of each plot to allow access for harvesting and fertiliser application. It is usual to sow the pathways with a dense slower growing grass for ease of maintenance and to allow machinery to travel in wetter conditions.

C.5 Harvesting

C.5.1 **Series perennial ryegrass, timothy, and meadow fescue**

C.5.1.1 **Sowing year**

Plots to be topped over at the discretion of the Growing Trial Operator without weighing to produce a uniform dense sward by the end of the season.

C.5.1.2 **First and third harvest year**

A simulated conservation management comprising a maximum of five cuts per year. Cutting height as close as possible to 60 mm in the first three cuts, subsequent cuts should be as close as possible to 40 mm. After a mild winter, plots may be trimmed without weighing in the first half of February if required. The first conservation cut should be taken at or close to early ear emergence, with a target D value of 75. The 50% ear emergence of an early heading PRG reference variety may be used as a guideline for the commencement of the cutting programme (early PRG + TIM). Intermediate PRGs are usually cut 1 week later (about 25% ear emergence), and Late PRG a further week later (about 10% ear emergence). This interval may be shortened in warm conditions and extended in cooler conditions.

The cutting dates to use in each group are as follows:

First cut	as described above
Second cut	six weeks after the first cut
Third cut	six weeks after the second cut
Fourth cut	four to six weeks after the third cut

When the third cut is more than six weeks before the anticipated date of the final cut for the intermediate group, then an extra cut should be taken from all maturity groups.

The final cut **should** be taken in the middle to end of October, depending on location. All maturity groups to be cut at the same time.

C.5.1.3 **Second harvest year**

A frequent-cutting simulated grazing management applied at a cutting height as close as possible to 40 mm.

The first cut should target a plot yield of approximately 1-1.5 t/ha DM with not less than an estimated 500 kg/ha DM on most plots.

Subsequent cutting should be on an approximate three week cycle until after **1 July**, when cutting should be on a monthly cycle to the end of the growing season. Trials should be managed in order to provide a measure of performance during the four seasonal periods of 'spring', 'early summer', 'late summer' and 'autumn'.

If one maturity group is cut, then all groups should be cut except where all plots in a maturity group are below an estimated 300 g of fresh material.

C.5.1.4 **Excluded harvests**

If there is insufficient growth to comply with the 3 or 4 week cutting cycles, the decision to apply fertiliser is the responsibility of the trials co-ordinator who has the option to omit a fertiliser application if this is consistent with best practice.

C.5.2 **Series Italian and hybrid ryegrass and tall fescue**

C.5.2.1 **Sowing year**

At discretion of centre but the first cut must not exceed 4 t/ha dry matter yield. A maximum of five cuts should be taken.

Cutting heights as close as possible to:

First cut	60 mm
All subsequent cuts	40 mm

If only one cut then cutting height should be 40 mm

C.5.2.2 **All harvest years**

Five to seven cuts per year. Cutting heights should be as close as possible to the target given in the table below.

In order to take the first conservation, cut (cut 2) at a digestibility as close to 75D as possible the trial should be as close as possible to 5-10% ear emergence. The final cut **should** be taken in the middle to end of October.

The cutting dates to use are as follows:

Cut	Timing	Target height
1	At an estimated yield of 1100 kg/ha of dry matter but not later than six weeks before expected date of first conservation cut (cut 2)	40 mm
2	At 75D (see above)	60 mm
3	Five weeks after cut 2	60 mm
4	One calendar month after previous cut	40 mm
5	One calendar month after previous cut	40 mm
6	One calendar month after previous cut	40 mm
7	One calendar month after previous cut	40 mm

C.5.3 **Harvesting method**

C.5.3.1 Plots should be harvested using a specialist grass harvester with a reciprocating-knife cutter bar. The harvested herbage must be weighed either on-board or separately, using an electronic balance graduated to 0.1 kg. All harvested material must be removed from the plot after weighing.

Yield records should be transmitted electronically to the appropriate Data Handling Operator within seven working days of each cut.

C.5.4 **Samples**

Samples are required for dry matter determination and/or quality testing. Dry matter determination may be:

Either - by taking a sample of the fresh material at harvest and oven drying according to the procedure in 5.4.1.1.

Or – by NIR spectrometry on board the harvester. The NLSC is responsible for approving all equipment and calibrations. Prior to initial use of the calibration models and subsequently on an annual basis, a validation is carried out whereby a set of samples are analysed using the NIRS technique and the respective oven drying methodology (C.5.4.1.2). The results from the two techniques are analysed to ensure the accuracy of the NIRS calibration model.

C.5.4.1.1 Dry matter sample

A representative sample should be taken immediately from the cut herbage of each plot to assess dry matter content.

A fully representative sub-sample of fresh material is accurately weighed, or an accurately recorded catch weight taken and accurately weighed as soon as possible after the trial is harvested. The treatment of samples and the time interval between cutting and weighing should be such that there is no significant moisture loss between the weighing of the plot fresh yield and the accurate weighing of the fresh weight of the sample. The fresh sample is recorded to the nearest 1.0 g.

If the plot fresh yield is over 300 g then the sample should be a minimum of 300 g. If the whole plot fresh yield is less than 100 g then the yield should be recorded as zero and no sample should be taken. If the whole plot fresh yield is between 100 g and 300 g then use the whole plot yield as the dry matter sample.

The samples are placed in the drier which must be at a temperature of 104 ^{o}C with the air recirculator set in the range 80-100% recirculation in order to restore the temperature to 104 ^{o}C as rapidly as possible. When the temperature is restored to 104^{o}C the air regulator is set at 80% recirculation i.e. 20% fresh hot air. The regulator is critical for rapid drying. The samples are dried for such time as is necessary for complete drying. (usually 18 hours)

The dried sample is carefully removed from the drier and as soon as the sample is cool enough for accurate weighing. The dry weight is recorded to the nearest 0.1 g. When the dry weights are reported as a percentage, the fresh weight should be reported as 100.

C.5.4.1.2 Quality sample. (Cuts specified in E 2.1.2)
If dry matter determination is by oven drying, then this sample also forms the quality sample.

If dry matter determination is by NIR spectrometry on board the harvester, then a sample of the fresh herbage must be taken and dried as described in 5.4.1.1, without weighing, at the cuts specified in E 2.1.2.

C.5.4.2 Milled samples, for quality testing, should be forwarded to:

Quality Analysis Testing
NIAB
Park Farm
Villa Road
Impington
Histon
CB4 9NZ Tel: 01223 233258

It is important that samples are despatched as soon as possible after harvest. The trial Organiser should be notified of sample dispatch using appropriate means.

C.5.5 **Submission of data**

C.5.5.1 Appendix 6 lists the records, with deadlines, to be sent to the appropriate Data Handling Operator. Diary sheets and any other field records should be returned to the appropriate Trials Organiser immediately following the final cut of the season.

C.5.5.2 All plot records should be transmitted to the appropriate Data Handling Operator following the deadlines set out in Appendix 6. After scrutiny, copies of results will be sent to the Growing Trial Operator for action as agreed with the appropriate Trials Organiser.

C.6 Records

C.6.1 There are four components:

1. **Diary** Field notes of trial status.
2. **Site data part 1** Site details; including site sketch, map and location, previous cropping, soil analysis fertiliser applications
3. **Site data part 2** Details of agrochemical applications and irrigation.
4. **Plot records** Plot data.

C.6.1.1 An entry in the Diary sheet should be made for any observations relevant to variety performance

C.6.2 **Plot records**

C.6.2.1 Plot data may be recorded directly onto a data logger or recorded on paper then entered and validated onto a computer. A system of ensuring that data are recoverable, in the event of loss of original data, must be implemented, e.g. copy and safe storage. Whichever method is used, individual plot data will only be accepted at the appropriate Data Handling Operator in an approved format using the AFP number, variety name and units as listed in Sections C and D.

C.6.2.2 All observations should be checked at the time of recording to ensure that they lie within acceptable limits for the character recorded. Observations that have been identified as exceptional by the recorder should be identified with a note on the approved data file or hard copy medium describing the possible causes together with a recommendation for their exclusion or inclusion in the trial analysis.

C.6.2.3 Plot numbers on record sheets must correspond to the numbering on the field plan.

C.6.2.4 If a character is not recorded or is missing the Growing Trial Operator should indicate in the diary or on the recording sheet the reason why it has been excluded.

C.6.2.5 Where a plot record is missing the Growing Trial Operator should record this in any data file or hard copy medium as a symbol thereby indicating there is no recorded value associated with this plot.

C.6.2.6 Specific plot records must be made as counts or on the scales shown for each character. Only the character names as listed may be used.

C6.2.7 All records should be returned to the appropriate Data Handling Operator as soon as reasonably possible. Indicative deadlines are given in Appendix 6. All records must be returned by the final deadlines.

C.6.3 **Procedures for recording characters**

The following procedures must be followed for measuring all characters to be used in NL decision-making.

C.6.3.1 **GROUND COVER** **(OBLIGATORY)** **(%)**

Record in the autumn of the sowing year and once 7 to 14 days after cutting in September to early November of each harvest year. When scoring ground cover, assess the sown species in each plot by eye either as % ground cover or on a 1-9 scale where 9 is most cover. Determine the percentage ground cover of the highest and lowest eye score within each replicate using a point quadrat, 100 points per plot first strike. Ignore any weeds present in the plot. If preferred, it is permissible to use quadrats every plot.

C.6.3.2 **FRESH YIELD** **(OBLIGATORY)** **(kg)**

Record at each cut of the yield management to the protocol given in Section C.5 above. Enter the total harvested weight to the nearest 0.1kg in kg per plot and provide the harvested plot dimensions with the record. If the plot lengths or widths are not constant then these must also be entered as records to the nearest 0.1m

C.6.3.3 **DRY MATTER CONTENT** **(OBLIGATORY)** **(%)**

A detailed protocol for sampling for dry matter and assessment of dry matter content is given in section C.5.4.

C.6.3.4 **MILDEW (ALL CROPS)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.5 **RHYNCHOSPORIUM (NOT TIMOTHY)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.6 **CROWN RUST (NOT TIMOTHY)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.7 **DRECHSLERA (NOT TIMOTHY)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.8 **RMV (NOT TIMOTHY)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.9 **BROWN RUST (NOT TIMOTHY)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.10 **BYDV (NOT TIMOTHY)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.11 **STEM RUST** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.12 **HALO SPOT (TIMOTHY ONLY)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.13 **CLADOSPORIUM (TIMOTHY ONLY)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.14 **RED THREAD (NOT TIMOTHY)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.15 **SNOW MOULD (NOT TIMOTHY)** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.16 **BACTERIAL WILT** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.17 **MASTIGOSPORIUM LEAF FLECK (CFT ONLY) (OBLIGATORY if present) (%)**

Record as described in Section D

C.6.3.18 **YELLOW RUST (CFT ONLY) (OBLIGATORY if present) (%)**

Record as described in Section D

C.6.3.19 **RESISTANCE TO WINTER DAMAGE (ALL CROPS) (OBLIGATORY if present) (1-9)**

Record any winter damage in the spring or after any particularly cold spell. Record only if significant damage (score of 6 or below) is seen on the most affected variety on the scale:

1. Total loss of plant
2. Very severe leaf damage, up to 75% loss of plant
3. Very severe leaf damage, up to 50% loss of plant
4. Severe leaf damage, up to 25% loss of plant estimated
5. Severe leaf damage, and slight loss of plant
6. Severe leaf tipping
7. Moderate leaf tipping
8. Slight to very slight leaf tipping
9. No damage

Damage is frequently not apparent until several days after the end of a cold period. Also describe the damage seen on the most severely affected plot.

C.6.3.20 **Site factors**

Any factors which may have affected the yield of the trial or individual plots must be noted and accompany the yield data.

Records for other scores, including pests or diseases not specified in the procedures, may be recorded as plants affected on a 1 to 9 scale, and reported with definitions for each rating on the 1 to 9 scales.

C.6.3.21 **Trial inspection**

All trials will be inspected by the Trial Inspection and Technical Validation Operator and, in some cases, it may be necessary to visit on more than one occasion.

The requirements of Growing Trial Operators in respect of inspections are to:

1. Give inspectors reasonable access to trials
2. Provide the inspector with information (for example pesticide sprays applied etc) at the time of inspection if requested.
3. Co-operate with the inspector in making any non-routine assessments required to establish the validity of the trial (for example population counts).
4. Carry out any action agreed in consultation with the inspector. In particular it is important that any requirement to shorten plots is undertaken and that missing values are returned for any plots excluded from the trial.

C.6.3.22 **Establishment weakness**

Visual assessment of trial establishment to be undertaken between six weeks after sowing and prior to secondary tiller development.

Records of density (of seedlings) to be made where the complete sward establishment of all plots is in doubt. Specifically, where differences in density of seedlings exist between plots of the same ploidy, or seedling density of the total trial is lower than normal. Seedling density 0-9 high, to be scored as a matter of urgency and communicated to the Trials Organiser for agreed remedial action and dissemination to all test centres, if appropriate.

The Trials Organiser can require control plots to be over-sown to ensure a complete establishment, but individual candidate variety weaknesses will not be compensated. Corrective actions can be taken on all plots where site weaknesses have affected the trial, either partially or as a whole. Where satisfactory resolution is not achieved, then abandonment of the trial will be endorsed by the NLSC.

Section D – Disease testing procedures

D.1 Assessment of natural infection

Recording of disease on the growing trials is the responsibility of the Growing Trial Operator at the appropriate site.

D.1.1 **Diseases recorded**

D.1.1.1 No inoculated disease tests are carried out routinely.

D.1.1.2 All disease assessments must be sent to the Data Handling Operator as soon as they are made.

D.1.2 **Naturally occurring diseases in VCU growing trials**

D.1.2.1 Foliar disease should be recorded when the level of infection on the most affected variety is over 5% of the leaf area. Percentage leaf area infected on the plot as a whole should be recorded using the key below as a guide.

D.1.2.2 Other pathogens should be recorded when more than 5% of the plot area is affected. The percentage of the area infected in each plot should be recorded.

D.1.2.3 If disease infection persists, successive records should be made throughout the season.

D.1.2.4 **Diseases recorded**

Perennial, Italian and Hybrid Ryegrass, Tall Fescue and Festulolium can be affected by a number of pathogens which can affect yield and quality. The most likely diseases to be encountered are mildew (*Erysiphe graminis*), crown rust (*Puccinia coronata*), brown rust (*Puccinia recondita ssp lolii*), stem rust (*Puccinia graminis*), *Drechslera* leaf spot (*D. siccans*), *Drechslera* net blotch (*D. andersenii*), *Rhynchosporium spp*, red thread (*Laetisaria fuciformis*), snow mould (*Fusarium nivale*), bacterial wilt (*Xanthomonas campestris pv. graminis*), ryegrass mosaic virus and barley yellow dwarf virus.
For Timothy, the most likely diseases to be encountered are mildew (*Erysiphe graminis)*, stem rust (*Puccinia graminis*), Halo spot (*Selenophoma donacis*) and Cladosporium leaf spot (*Cladosporium phlei)*.
For Cocksfoot, the most likely diseases to be encountered are mildew (Erysiphe graminis), yellow rust (Puccinia striiformis) and Mastigosporium leaf fleck (Mastigosporium spp)

Relative importance of ryegrass diseases and optimum time for recording are shown in the table. Assessments should be made just before the plots are cut.

N/A	PRG and TF	IRG and HRG	TIM	CFT	FL	Time
Mildew	***	****	**	**	**	May to August
Crown rust	****	**	n/a	n/a	**	August to October
Brown rust	**	***	n/a	n/a	n/a	April to June
Stem rust	*	*	*	n/a	*	September to October
Cladosporium	n/a	n/a	**	n/a	n/a	July to September
Drechslera leaf spot	****	**	n/a	n/a	*	January to March and August to September
Drechslera net blotch	****	**	n/a	n/a	*	January to March and August to September
Halo spot	n/a	n/a	**	n/a	n/a	N/A
Rhynchosporium spp	**	****	n/a	n/a	n/a	March to May
Red thread	**	*	n/a	n/a	n/a	N/A
Snow mould	***	**	n/a	n/a	n/a	N/A
Bacterial wilt	*	**	n/a	n/a	n/a	N/A
Ryegrass mosaic virus	**	****	n/a	n/a	n/a	June to July
Barley yellow dwarf virus	***	***	n/a	n/a	n/a	May to June
Mastigosporium leaf fleck	n/a	n/a	n/a	***	n/a	N/A
Yellow rust	n/a	n/a	n/a	***	n/a	N/A

**** common and often reduces yield or quality
*** frequent and may reduce yield or quality
** frequent, but has only a small effect on yield or quality
* Occasional, but has only a small effect on yield or quality

Section E – Quality testing procedures

E.1 Responsibilities

E.1.1 The Quality Testing Operator appointed by the Trials Organiser is responsible for conducting the approved quality tests according to these procedures.

E.2 Quality assessment methodology for obligatory and additional tests

E.2.1 **Preparation of samples prior to quality analysis**

E.2.1.1 Although in some instances all of the sampling and weighing of fresh material may be carried out in the field, it is acceptable for samples to be brought to the laboratory for weighing. If the latter option is followed the representative sample is immediately sealed in a 500-gauge polythene bag and kept out of direct sunlight and as cool as possible until transported to the laboratory. Each sample is identified with a label.

E.2.1.2 Dried material from the following cuts should be retained for digestibility analysis. Instructions for milling these samples are given below. Samples from each replicate should be bulked for each variety and milled following oven drying. Samples to be despatched to the Quality Testing Operator for analysis:

PRG TIM CFT and MF trials
Cut 1 and cut 2 in the first harvest year should be used for quality testing. Cut 6 in the second harvest year. However, if there is less than 1 tonne/ha DM present on most plots, then cut 7, or exceptionally cut 8, may be used for quality testing instead. The Quality Testing Operator should be informed which cut is used.

IRG, HRG and TF trials
Cuts 2 and 3 of the first harvest year should be used for quality testing.

E.2.2 **Quality tests**

E.2.2.1 Milling of dried samples for further quality analysis

1. The dry matter samples (Section C.5.4) from both replicate plots must be combined and a representative sample taken for milling (sufficient to provide 150 ml of milled material for analysis).
2. The mill must be a hammer mill fitted with a screen with 1.0 mm apertures. Screens must be checked for wear of the inside surface at regular intervals. Frequent use causes the circular 1.0 mm hole to elongate, and when the elongation reaches 1.2 mm the screen must be changed.
3 Samples for milling must be absolutely dry. This can be achieved either by milling immediately after weighing out of the dryer or by re-heating dried samples to 104 °C for 1 hour before milling.
4 The mill must be thoroughly clean before use.
5 The mill must be at maximum speed before the sample is introduced gradually to prevent the mill labouring.
6 All of the sample must be removed from the receptacle and thoroughly mixed. Care must be taken at all stages to prevent the loss of fine powder which is a critical part of the milled sample.
7 After mixing, a representative sub-sample should be taken in the following manner:
(a) If less than 150 ml of milled sample, all of it should be placed in the sample tubs.
(b) If more than 150 ml of milled sample, the tub should be filled with a fully representative sub-sample that has been fully mixed before placing in the tub.
8. The sample tub must be sealed with a close-fitting lid and labelled with information in an approved format.
9. The milled samples must be sent to the laboratory for analysis immediately and by 15 September at the latest, with appropriate identification documentation.

E.2.2.2 Digestibility analysis

The Dry Organic Matter Digestibility (DOMD or D-value) of all the samples taken for quality must be determined according to an agreed protocol.

The samples are milled using a Foss Tecator 1093 Cyclotec sample mill fitted with a 1 mm screen. The screen and grinding ring should be inspected for wear frequently and replaced at appropriate intervals, at least annually. It is important that all samples are milled through a **single** Cyclotec mill to maintain the precision of the analysis.

The milled samples are scanned and the spectral data stored using a FOSS NIR systems 5000N scanning instrument or equivalent. A ‘Digestibility Analysis (de Boever)’ calibration, (supplied by Departement Qualite des Productions Agricoles, Belguim) is used to convert the spectral data for each sample to D-value units. The calibration has been shown to relate NIRS spectra to D-values as assessed by wet chemistry techniques eg (pepsin cellulase method). The NIRS calibration models are maintained and validated on an annual basis, whereby a set of control samples are analysed using the NIRS technique and the respective laboratory methodology. A comparison of the results from the two techniques are compared to ensure the accuracy of the NIRS calibration model.

Inconsistent or apparently anomalous results must be repeated. The final data values must be sent to the Data Handling Operator in an approved form. The laboratory must be prepared to immediately undertake any repeat analyses requested by the Data Handling Operator, which may be individual varieties or even whole trials.

The results for the first year first conservation cut of herbage samples must be available to the Testing Authority by 1 February and for the first year second conservation cut by 1 May. The results from the second year 1 August simulated grazing cut and second year first and second conservation cuts of Italian and hybrid ryegrass and tall fescue must be available by 15 January.

Section F – Trial design and data handling procedures

F.1 Plan validation and storage

F.1.1 After the trial has been sown, the Growing Trial Operator must:

a) Confirm that the trial has been sown according to plan and provide the sowing date, by returning site data 1 and associated trial sketch to the appropriate Data Handling Operator.

b) If any amendments to the plan have been made, return a hard copy of the plan to the appropriate Data Handling Operator with any amendments clearly indicated. Alternatively, amendments may be notified electronically with the agreement of the Data Handling Operator.

F.1.2 The Data Handling Operator will check these for statistical validity and, once this has been done, will load the plan on the database.

F.2 Data recording

F.2.1 Data are recorded using the methods and characters given in Sections C, D and E.

F.2.2 Site information is recorded for each trial including, for example, data on previous cropping, seed rates, soil details and fertiliser applications.

F.2.3 Details of any agrochemical applications are also recorded and retained by the Growing Trial Operator.

F.3 Other tests and trials

F.3.1 Any additional or alternative designs required for the assessment of additional VCU characters not detailed in Appendix 3 of the **VCU TRIAL PROTOCOL** for Perennial, Italian and Hybrid Ryegrasses, Timothy, Festulolium, Cocksfoot, Tall and Meadow Fescue will be added to these **Procedures** as and when approved by the NLSC.

Appendix 1 – Approved Trial Organisers/ Operators for perennial, Italian and hybrid ryegrass, timothy, cocksfoot, tall and meadow fescue and festulolium

Activity	Organisers/Operators responsible
Trial Design and Data Handling Operator	NIAB for England & Wales BioSS for Scotland AFBI for Northern Ireland
VCU Trials Organiser	BSPB for England, Wales & Northern Ireland BSPB & SASA for Scotland
Growing Trial Operator	DLF Seeds Ltd for England DSV UK for England NIAB for England IBERS for Wales SRUC for Scotland SASA for Scotland AFBI for Northern Ireland
Seed Handling Operator	NIAB
Pathology Trial Operator	NIAB Barenbrug
Trial Inspection Operator	NIAB and BSPB for England & Wales SASA and BSPB for Scotland AFBI and BSPB for Northern Ireland
Technical Validation Operator	NIAB for England & Wales BioSS for Scotland AFBI for Northern Ireland
Quality Testing Operator	NIAB

Data Review and Standard Setting Operator	NIAB

Appendix 2 – Seed treatment products for use on VL trials

None

Appendix 3 – Seed dispatch deadlines

VCU seed must be delivered to the Seed Handling Operator by 5th February.

Appendix 4 - VCU growing trials

Not all trials are grown at all sites in each year. The Trials Organiser will provide details.

Four different trial series are grown as follows:

Trial series	Species which may be included at the request of the applicant
PRG, CFT Alternating conservation and simulated grazing over three harvest years	Perennial ryegrass Hybrid ryegrass Festulolium Cocksfoot
IRG, TF and FL Combined conservation and simulated grazing over two harvest years	Italian ryegrass Tall fescue Festulolium Hybrid ryegrass
HRG Combined conservation and simulated grazing over three harvest years	Hybrid ryegrass Italian ryegrass Tall fescue Festulolium
TIM and MF Alternating conservation and simulated grazing over three harvest years	Timothy Meadow fescue

Hybrid ryegrasses and festulolium should only be tested in up to two of the three testing systems – PRG and HRG or HRG and IRG.

A brief description of the trials series are as follows:

PRG, CFT

Number of sowing years:	2
Number of harvest years	3 harvest years for first sowing and 2 harvest year for second sowing
Number of trial sites:	6
Number of replicates	2 in each trial
Heading groups	Early, Intermediate and Late heading varieties are grown in separate trials and cut on different dates under conservation management.
Ploidy groups	Diploid and tetraploid varieties of each heading group are grown in the same trial but candidates are compared with the appropriate control variety. See Appendix 5
Trial regimes	Top plots as required without weighing in sowing year. Conservation management in first harvest year. Simulated grazing management in second harvest year. Conservation management in third harvest year.
Number of control varieties	Two for each ploidy within each heading group plus a hybrid if required.

The applicant must allocate varieties to a specific maturity class. The heading group classification used to achieve this is given in Appendix 5

Where there are no candidates in a PRG maturity Group a control will be sown in the adjacent group.

IRG, TF and FL

IRG, TF and FL	
Number of sowing years:	2
Number of harvest years	Sowing year plus 2 harvest years
Number of trial sites:	3 + 3 additional sites
Number of replicates	2 in each trial
Heading groups	All candidates are considered in a single group
Ploidy groups	Diploid and tetraploid varieties are grown in the same trial but candidates are compared with the appropriate control variety. See Appendix 5
Trial regimes	As detailed in Section C.5. Maximum of 5 cuts in sowing year. Combined management in first and second harvest year.
Number of control varieties	One for each ploidy, plus hybrid control if required.

HRG

HRG	
Number of sowing years:	2
Number of harvest years	3 harvest years for first sowing and 2 harvest years for second sowing
Number of trial sites:	3 + 3 additional sites
Number of replicates	2 in each trial
Heading groups	All candidates are considered in a single group
Ploidy groups	Diploid and tetraploid varieties are grown in the same trial but candidates are compared with the appropriate control variety. See Appendix 5.
Trial regimes	As detailed in Section C.5. Maximum 5 cuts in sowing year. Combined management in first, second and third harvest year.

HRG	
Number of control varieties	One for diploid, two for tetraploid

TIM and MF

TIM and MF	
Number of sowing years:	2
Number of harvest years	3 harvest years for first sowing and 2 harvest years for second sowing
Number of trial sites:	3
Number of replicates	2 in each trial
Heading groups	Early, intermediate and late heading groups are all grown in one trial
Ploidy groups	One group – all hexaploid
Trial regimes	Top plots as required without weighing in sowing year Conservation management in first harvest year Simulated grazing management in second harvest year Conservation management in third harvest year
Number of control varieties	2 – Early and intermediate varieties used as controls for all heading groups

The applicant must allocate varieties to a specific maturity class. The heading group classification used to achieve this is given in Appendix 5

Appendix 5 – Control varieties for VCU assessments

Species	Control Varieties
Early Perennial Ryegrass	Genesis (D) Glasker (D) Abertorch (T)
Intermediate Perennial Ryegrass	Abermagic (D) Boyne (D) NL2 & Galgorm (D) NL1 Glasker (Early) (D) Drumbo (Late) (D) Dunluce (T) Seagoe (T) Abertorch (Early) (T) Abergain (Late) (T)

Species	Control Varieties
Late Perennial Ryegrass	Aberchoice (D) Drumbo (D) Abermagic (inter) (D) Abergain (T) Meiduno (T) Dunluce (Inter) (T)
Italian Ryegrass	Alamo (D) Muriello (D) Hunter (T)
Hybrid Ryegrass	Aberecho (T) Astoncrusader (T) Pirol (D)
Timothy	Motim Comer
Cocksfoot	Sparta Lidacta
Meadow Fescue	Merifest
Tall Fescue	Dulcia

Maturity class

The applicant must allocate varieties to the correct heading group for perennial ryegrass and Timothy trials. The heading date of each candidate is checked as part of DUS testing procedures and candidates will have to re-start VCU tests and trials if they head more than four days outside the allocated group.

The heading group classification used is as follows:

PRG

PRG	
Early heading group	Heading date earlier than or equal to Lilora.
Intermediate heading group	Heading date later than Lilora but earlier than Barplus.
Late heading group	Heading date equal to or later than Barplus.

Timothy

Timothy	
Early heading group	Heading date earlier than Motim
Late heading group	Heading date equal to or later than Motim.

Appendix 6 – Dates for submission of records and samples

To Data Handling Operator

Record	Latest date of receipt
Site data part 1 (incl. site sketch)	Within 2 weeks of sowing the trial
Site data part 2	Annually by end of November
Yield records	Electronically to the appropriate Data Handling Operator within seven working days of each cut.
Plot records (in approved electronic format)	Annually by end of November

Plot samples to Quality Testing Operator

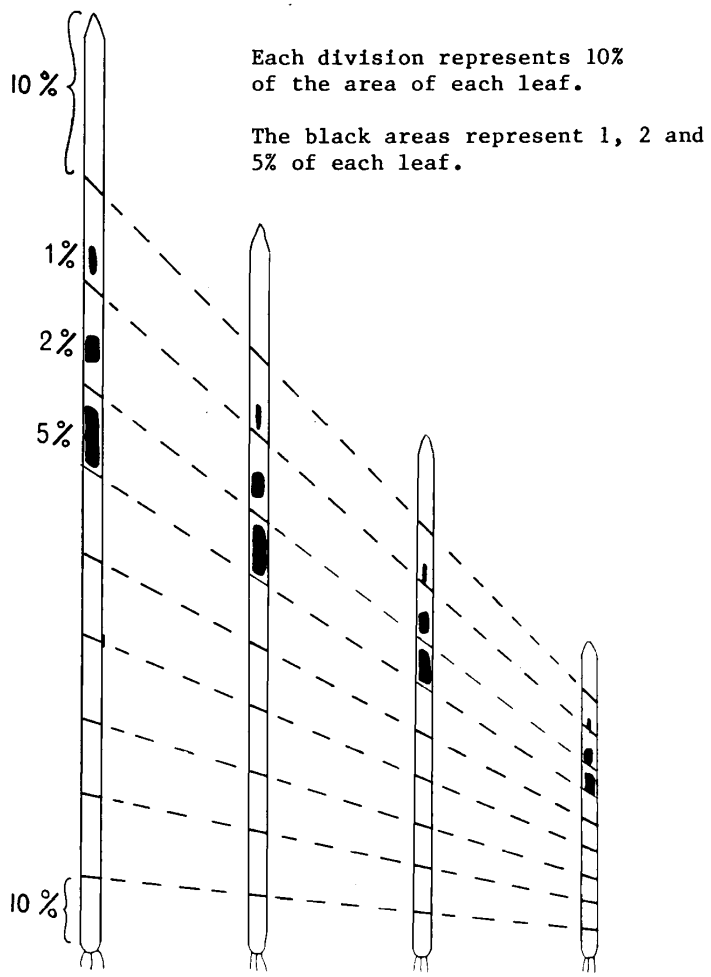
Quality testing – samples to be coarse milled	To Quality Testing Operator by 15 September
Quality testing – samples to be fine milled	To Quality Testing Operator by 15 September

Appendix 7 – Recording Methods and Ryegrass Leaf Area Guide

Leaf diseases

1. Select 4 points per plot. Part the foliage to expose all leaves.
2. At each point, estimate the % leaves showing disease symptoms.
3. For Mildew, Crown Rust, Dreschlera or Rhynchosporium, estimate the average % infection on the diseased leaves only, using the drawings below.
   For Ryegrass Mosaic Virus, classify the type of infection on the infected leaves only as **Slight**, **Moderate** or **Severe** using the definitions below.
4. Use the tables below to calculate the average % infection per plot or the 0-100 disease index as appropriate.

Ryegrass leaf area guide



1 Each division represents 10% of the area of each leaf. The black areas represent 1, 2 and 5% of each leaf.

Disease % key for mildew, rusts, *Drechslera* and *Rhynchosporium*

Average % infection on diseased leaves only

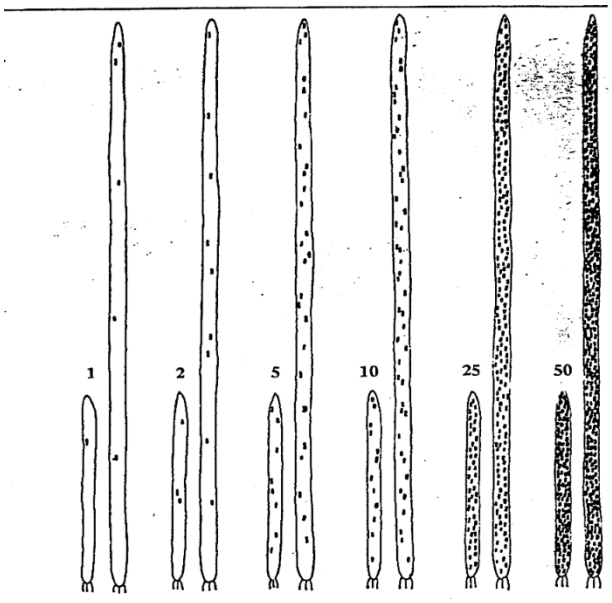
% leaves with infection	0%	1%	5%	10%	25%	50%	75%	100%
0	0	0	0	0	0	0	0	0
1	0	0.01	0.05	0.1	0.3	0.5	0.8	1
5	0	0.05	0.3	0.5	1	3	4	5
10	0	0.1	0.5	1	3	5	8	10
25	0	0.3	1	3	6	13	19	25
50	0	0.5	3	5	13	25	38	50
75	0	0.8	4	8	19	38	56	75
100	0	1	5	10	25	50	75	100

Disease index key for ryegrass mosaic virus

Slight Leaves green with mosaic of pale green streaks when held up to light.
Moderate Leaves green but with pronounced chlorotic streaks.
Severe Leaves and sheaths showing dark brown necrotic streaks over entire area.

% leaves with infection	Slight	Moderate	Severe
0	0	0	0
1	0.3	0.7	1
5	2	3	5
10	3	7	10
25	8	17	25
50	17	33	50
75	25	50	75
100	33	67	100

Ryegrass leaf spot – Drechslera siccans (Drechsl) Shoemaker



Percentage of leaf area affected

Appendix 8 – Perennial ryegrass varieties submitted into wrong maturity group in VCU trials

The VCU maturity grouping of candidates is reviewed in November following each year of testing and the final decision on VCU maturity grouping is taken in November after the VCU trials have been completed, prior to the VCU decision meeting of the following February.

The following actions are required for candidates where the mean date of ear emergence recorded in DUS testing falls outside the VCU maturity group they were submitted into.

Rules for actions

Stage of testing	Maturity relative to submitted VCU group	Notification
After 1st year of testing	Less than 1 day outside group	No action
After 1st year of testing	More than 1 day outside group	Notify applicant of implications
After 2nd year of testing	Outside the group by less than or equal to 4 days	If not informed after 1st year, notify applicant of implications
After 2nd year of testing	Outside the group by more than 4 days	Notify applicant of implications and indicate that this candidate is currently at risk of not being considered for VCU

Implications for the VCU decision:

At the time of the final VCU decision taking meeting, if the candidate is:

- less than or equal to four days outside the VCU maturity group to which it was submitted - the VCU performance data for the candidate will be transferred to the correct maturity group and its VCU considered against the standards for that maturity group.

- more than four days outside the VCU maturity group to which it was submitted - the VCU performance data for the candidate will not be transferred to the correct group and its VCU will not be considered. To be considered for listing the VCU testing must be re-started and this will incur full trial fees.

Notes:

The VCU maturity of candidates is determined in spaced plant trials by comparison with delineating varieties as listed in Appendix 5.

VCU maturity grouping and the DUS description of maturity may not necessarily match. This is because the VCU system has three maturity groups (early, intermediate and late) and the DUS system has five descriptive groups (very early, early, medium, late and very late).

Appendix 9: Guidance on Applications to Use Haldrup Plot Harvester On-board NIRS to Assess the Dry Matter Content of Herbage Varieties in UK National List Trials

Introduction

Traditionally, dry matter (DM) content has been assessed by oven drying a representative aliquot (normally 300 - 700 g) of freshly-harvested plot material. Fresh samples are weighed then placed in a pre-heated forced draught oven at 104 °C and samples are dried until all the moisture is removed (typically 18 - 24 h).

Modern Haldrup plot harvesters are now typically supplied with on-board NIRS systems. With these on-board NIRS systems, forage passing through the sample chamber is scanned using electromagnetic radiation from the near infra-red region. Reflected sample spectra are used to develop calibrations against laboratory determined oven DM content which may then be used to predict DM content during routine harvesting. Samples used in the calibrations would typically cover multiple harvest years, cuts and trials. If multiple plot harvesters are being used on the same site, it is considered to be good practice that the data from each machine contributes to the calibrations.

Minimum standards

To be considered any calibration must meet the following minimum standards:-

- The calibration should only be used to predict DM content of species included in the calibration.
- The calibration should be based on data from multiple cuts, harvest years and trials.
- Maximum SEP(C) (standard error of prediction (corrected)) ≤ 1.75 (please specify actual SEP(C)).
- For validation purposes a minimum of either 10 % of samples or 4 samples, whichever is the greater, must be taken at random from each harvest of each trial. These samples should be taken inside the Haldrup cab at the same time as the sample is scanned and the sample ID recorded and DM content determined by oven drying. NIR predicted values should be reported. Alternative methods for validation will be considered by the NLSC on a case-by-case basis.
- A method of identifying odd spectra must be fully documented see example below

- Under the following circumstances NIRS predicted values must not be used:
    a. Any spectrum giving, e.g. Mahalanobis distance (M-value) above 2.0 (please specify actual M-value or equivalent).
    b. Predicted DM content is outside the DM range covered by the calibration.
    c. Any spectrum that is clearly unusual.
    d. Atypical samples e.g. herbage with high surface moisture levels. Ideally such material should not be harvested but if this is unavoidable then all spectra should be supported by DM determination by oven drying.

In any of the above circumstances DM content should if possible be determined by oven drying of a sample of the chopped forage ejected from the Haldrup and the sample IDs should be recorded.

- Validation data and data from a) in the bullet above – be aware that the M value may indicate an outlier due to sample abnormality, system error etc. and b) in the bullet above should be made available to expand the calibration.
- When submitting data to the data handling operator and PVS/APHA it must be made clear whether the DM% was determined by the oven drying method or predicted using NIRS specifying the calibration used.

Following initial approval an annual declaration must be made prior to the commencement of each cutting season. This should confirm that the calibration still meets or exceeds the original standards at the time of initial approval. This may be subject to Audit during Official Supervision Visits by the National Authorities. Applications and declarations must be made to PVS at:

Plant Variety Rights Office for the UK
APHA
Eastbrook
Shaftesbury Road
Cambridge
CB2 8DR

Telephone:- 02080 265930
Email:- pvs.helpdesk@apha.gov.uk

Application

Please provide the following details in your application:

General details of calibration

- The forage species you are seeking approval to use NIRS with.
- The person/company responsible for developing, supplying and maintaining the calibration, including a version or release number.
- Make and model of the on-board NIRS instrument(s) used to scan calibration samples.
- Software used to capture and store spectra.
- Spectral wavelength range (e.g. 950 - 1690; 2 nm).
- Details of the maintenance schedules for the on board NIRS.

Specific details of calibration

- Identity of the species, number of samples of each species included in the calibration set.
- Geographical location (sites) of samples included in the calibration set and the trial management practices (i.e. the number of cuts per year, harvest year).
- Time period over which calibration samples were scanned on a location and species basis.
- Make and model of the on-board NIRS instrument to be used for DM prediction, and the number of contributing machines if the scanner make and model are the same.
- Software settings e.g. black and white references, wavelength standards and checks, number of scans averaged for each sample during scanning.
- Software used to develop and run the calibration.

- Details of the oven drying method (drying temperature, weight of fresh sample used (minimum and maximum), number of replicates, duration) used for calibration samples.
- Details of the calibration development
    - Spectral transformation
    - Regression method
    - Mathematical treatment of Spectra
    - Number of terms
- Calibration statistics
    - Number of samples in the calibration set
    - DM content (%) covered by the calibration set (min, max, SD (standard deviation))
    - Cross validation statistics (SEC, SECV (standard error corrected variance), r2 (coefficient of determination))

Routine scanning during harvesting (required for all calibrations)

- Provide a description of the process you employ to collect the scans using NIRS on-board
- Detail your Quality Assurance processes undertaken on a daily/monthly/annual basis.
- Describe the safeguards you have in place to ensure that any data collected is fit for purpose e.g. real time visual checks on spectra, e.g. predicted DM content, Mahalanobis distances, low/high intensity warnings etc.

Routine validation process (required for all calibrations)

- Number of spectra examined and the statistical methodology used to compare the predicted and observed DM values
- Details of the oven drying method (drying temperature, weight of fresh sample used (minimum and maximum), number of replicates, duration) used for validation samples.
- Validation statistics
    - Correlation coefficient
    - SEP(C)
    - MSPE (mean squared prediction error) and components (bias, slope and random)
    - X-Y plot of NIRS predicted versus observed oven DM content
- Tolerances for validation statistics
- Procedure to be followed when the validation statistics are out of tolerance

Calibration equation maintenance program (required for all calibrations)

How often will the calibration performance be monitored other than the checks performed during routine scanning?

How frequently do you intend to expand/update the calibration routinely? If a high (please specify) proportion of scans are giving a high (please specify) e.g. Mahalanobis distance what action will be taken. (E.g. will the equation be updated more frequently?).

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Any enquiries regarding this publication should be sent to us at

webmaster@apha.gov.uk

www.gov.uk/apha

The Animal and Plant Health Agency (APHA) is an executive agency of the Department for Environment, Food & Rural Affairs, and also works on behalf of the Scottish Government and Welsh Government.