



**PROJECT REPORT No. 151**

**ASSESSMENTS OF WHEAT  
GROWTH TO SUPPORT ITS  
PRODUCTION AND  
IMPROVEMENT (VOLUME II)**

**VOLUME II: How to Run a Reference Crop**

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**ASSESSMENTS OF WHEAT GROWTH  
TO SUPPORT ITS PRODUCTION  
AND IMPROVEMENT**

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**VOLUME II: How to Run a Reference Crop**

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## 1. INTRODUCTION

### 1.1. Why run a reference crop?

There is a strong belief that both seasons and sites confer distinct identities on crops. Certainly, for wheat in the UK, there is considerable variation in final performance between sites and years, and even more variation in the way crops progress from sowing to harvest, both through their changing form and their growth. This variation can have an important bearing on husbandry decisions. However, it is difficult to compare the current state of a crop with crops remembered from other sites or other seasons, since visual assessments of crop development and growth are inherently subjective and thus inaccurate. Ideally, specific steps need to be taken for development and growth to be accurately and comprehensively measured every year in every locality, and records kept for comparison with future crops so as to provide a sound basis for husbandry decisions. This ideal is far too laborious to be justified but, for a few *representative* crops, such records can still prove valuable. Crops measured in this way can provide data which will be referred to frequently; hence they can be called '**Reference Crops**'.

The concept of adjusting husbandry according to intelligence from Reference Crops will evolve slowly, according to experience. Whilst the cost of running a Reference Crop is significant, and the benefits remain unproven, some reference crops can be justified. Thus, in this Project, Reference Crops have been widely separated. Initially, differences due to season have been more obvious than those due to region (see Volume III, '*The Dataset*'). However, in time, the number of seasonal comparisons will become as great as the number of sites, and differences attributable to 'locality' will become more apparent. Thus the Reference Crop concept does show some initial return, but its eventual value particularly in showing site effects will not be realised for a number of years. Decisions to support one or more Reference Sites need to be taken with this long term view in mind.

The siting of Reference Crops is important. Clearly each Reference Crop can only be taken to *represent* those crops for which soil, weather and husbandry are thought sufficiently similar for effects on crop progress or performance to be minor. Initially the '*representativeness*' of sites will be a matter of degree, and so must be a matter of judgement. Eventually, the discrimination possible using Reference Crops will be known because it will depend on the precision of the most (economically) vital information. If this information proves very uncertain or of little value, few sites will be justifiable; if it is very precise and obviously leads to greater profit, it will be worth having sites at frequent distances throughout the wheat growing area. It may eventually prove worth representing several common husbandry conditions, for example first and second wheats, 'breadmaking' and 'feed' varieties, or early and late sowings.

Whilst Reference Crops are supported by the wheat industry as a whole (through the HGCA) it will be best to monitor crops which are widely spaced so that they can be taken to represent large areas. However, as the precision of the information



becomes better appreciated, and clearer patterns of variation are revealed, groups of farms (e.g. linked with farmers' discussion groups, marketing co-operatives, agricultural colleges, or large farming enterprises) may find it justifiable to support a Reference Crop to represent their own specific conditions.

Whatever the result of experience, there will inevitably be crops which Reference Crops cannot be taken to represent closely. For example, a wheat crop may be adjacent to a Reference Crop and on similar soil but sown at a markedly different time, or it may be at a distance and have a different level of soil fertility. It is unrealistic to expect that comprehensive measurements would be made on many crops. Our intention is that this field by field variation should be addressed using simpler observations, but observations which are compatible with those used on Reference Crops. Thus there is an accompanying section in this report giving methods by which field observations of development and growth might be made on any farm (see Volume I Part 2, "*Methods for In-Field Crop Assessment*").

## **1.2. Aims of this manual**

This manual provides a structured scheme of measurements to quantify the growth and development of a cereal crop. At present, workers use their own different schemes and this makes the results of experiments difficult to compare in a detailed and rigorous way. Adherence to methods intended to act as *standards*, such as those described here, will both make comparisons more objective and ensure that a full set of data are available; comprehensive data have much enhanced value for reference purposes.

If the procedures for running a Reference Crop are followed carefully they will provide high quality intelligence on the current state of a crop that can be compared directly with the data in Volume I Part 1, "*The Wheat Growth Digest*". For example, 'forwardness' or 'backwardness' of a crop, instead of being considered in vague these terms, may be quantified in terms of height, or canopy size, or growth stage, and more appropriate and confident adjustments may be made to crop management. Such data may also be used to predict further progress and performance that are likely for a crop. Methods for this are given in Volume I Part 3: "*Forecasting Crop Progress for Wheat*". Overall, we hope that this manual will become a standard tool for those involved in the cereal industry who wish to make assessments of crop development and growth.

The full set of measurements establish the dates of all stages in the development of the crop, quantify all aspects of growth throughout the season that relate to yield, such as changes in green area, leaf growth, plant height, nitrogen uptake and water soluble carbohydrates in the stems, as well as total dry weight. Supplementary measurements are made of soil mineral nitrogen, root depth and lodging, and records are kept of disease and pest incidence and treatment to allow comprehensive analysis of all influences on crop development and growth. The methods of measurement result from extensive experience of monitoring cereal crops; they have been refined by application to the 18 crops of Mercia winter wheat described in Volume III, "*The Dataset*".

Our intention has been to provide clear and comprehensive instructions. The result is a fairly large document; thus, the task of using the manual may seem rather daunting. However, if you read one section on how to collect the data for one 'period', and then try it out, you will see that it is not as difficult as it seems. In order to save time and effort it may be tempting to omit some of the measurements, but this is best avoided as it will reduce the comprehensiveness of the data, and cause the "*I wish we had measured that*" problem!

### **1.3. Organisation of this manual**

This manual is divided into four parts; the first part (including this section) describes the reasons for running a reference crop, the facilities required, including staffing information, objectives, definitions and methods used and the programme of records. The next part, starting with Section 4 describes how the site is selected, and how the crop is grown. Then Sections 5-9 describe in detail the records taken in each phase of the crops development. The final part, Section 10, describes essential calculations and data handling, and provides example data recording sheets. The appendices contain suggested plot layout plans, the soil testing scheme, and thermal time calculations.

Since several measurements (e.g. shoot numbers, dry weights, green area indices) are made throughout the life of the crop, this structure results in some repetition in sections 5-9, but it allows each section to be free standing and therefore easier to use in the field without repeated cross referencing.

### **1.4. What is required to run a Reference Crop ?**

Apart from the usual requirements for cultivation and management of a wheat crop the following is needed:

#### **1.4.1. Time !**

The full set of measurements is comprehensive and, during the growing season, will take two to three working days per week to complete, including data processing and analysis. (This does not include chemical analysis, which may be done by outside laboratories). Obviously, savings in time may be made by omitting some of the measurements, or by reducing their frequency. If omissions are made, it must be accepted that comparability of the measurements will be restricted, and care will be needed to ensure that accuracy and precision of the methods are not compromised.

#### **1.4.2. For laying out the trial area:**

- i. An appropriate field site, which should represent the soil type of interest, is uniform over the whole trial area, to reduce variability between plots, and has a low nitrogen status. It should have no steep slopes or gradients in soil type or texture, and should be uniform with respect to previous cropping, manure use or experimental plots. Further details of the requirements are given in Section 4.1.

- ii. Approximately 28 quadrat frames (1.2 m x 0.6 m ) per plot. The quadrats should be placed in the crop prior to the first sampling in mid February, as described in Section 10.8.
- iii. Canes fitted with eye protectors for marking positions of sample areas.
- iv. Weed-wiper for 'burning out' paths.

**1.4.3. For collection of samples and field recording:**

The equipment needed for cutting and removing plants from the field prior to growth analysis is as follows :

- i. trowel.
- ii. sharp scissors.
- iii. secateurs.
- iv. polythene bags.
- v. clipboard.
- vi. ruler.
- vii. tape measure.
- viii. record sheets.
- ix. labels for identifying samples.
- x. split plastic drinking straws for collar labels (Section 7.2.2)
- xi. pre-prepared leaf number labels (made from about 0.5 mm diameter coloured sheathed single core wires in ten colours from insulated 'telephone' cable, with a 15 mm diameter loop formed by twisting the ends of an 8 cm length together).
- xii. a chilled cool box ('picnic box') for transfer from field to lab of the freshly-cut shoots for analysis of water soluble carbohydrates (anthesis onwards; Section 7.4).
- xiii. paper (potato) sacks, and linen bags for grain samples.

**1.4.4. For processing samples in the laboratory:**

- i. Appropriate large pan balances (up to about 2 kg by 0.1 g) with calibration weights, and more sensitive balances for smaller samples.
- ii. Forced circulation oven set at 102°C with gauze trays for drying grain and for stem samples for water soluble carbohydrate (WSC) analysis.
- iii. Forced circulation oven set at 80°C with trays for drying stem and leaf material for dry weight determinations.
- iv. Cool room or large refrigerator at 5°C for short term storage of samples.
- v. Freezer (-20°C) for soil samples.
- vi. Leaf area meter.
- vii. Bag sealer or ties for sealing bags containing dry samples that are awaiting analysis.
- viii. Grain counter (optional: this can be done by hand).
- ix. Gouge augers 40 mm, 30 mm and 20 mm in diameter from Eijkelkamp or EJH (see Section 5.8.1) for soil sampling to 90 cm depth.

#### **1.4.5. Analytical laboratory facilities**

You will need access to a suitable accredited laboratory for:

- i. Soil analyses.
- ii. Nitrogen analysis of straw, ears and grain.
- iii. Analysis of water soluble carbohydrate in stems.
- iv. Measurement of Hagberg falling number, specific weight and further (optional) quality characteristics of grain.

Before collecting samples it is important to establish the sample presentation requirements of any laboratory used.

#### **1.4.6. Meteorological Data**

Since one of the principal differences between sites and years is the weather, it is important to have a good source of meteorological data to accompany crop data. Values for daily maximum and minimum temperature, rainfall, run of wind, solar radiation (or sunshine hours) and wet and dry bulb (or some other measure that allows calculation of humidity or vapour pressure) are best recorded on site. Automatic weather recorders suitable for the purpose are now available at relatively modest cost but care should be taken to ensure that standard instrumentation is used and that it is properly maintained and calibrated. Alternatively, data may be available from another local source, or can be obtained via the Meteorological Office.

#### **1.4.7. Data processing**

The data from a reference crop is quite extensive so that the need for adequate office and computing facilities should not be underestimated. Most modern PCs with appropriate software, (e.g. spreadsheets) and back up arrangements should be adequate for the task, but enough time should be allowed for setting up the essential calculations. Examples of the calculations usually necessary are given in sections 10.2 and 10.3. Calculations should be made as soon as possible after data collection so that the growth of the crop can be monitored and any problems with the data investigated.

Organisation of data collected in the lab and field is important, and this is covered fully in Section 10.

#### **1.4.8. Reference Documents**

##### 1.4.8.1. Wheat Growth Stages

Tottman, D.R., illustrated by Broad, H. *The Decimal Code for the Growth Stages of Cereals with Illustrations*, in *Annals of Applied Biology* (1987) **110**, 441-454, also published as Occasional Publication No. 4 by BCPE Ltd. Publications Sales, Bear Farm, Binfield, BRACKNELL, Berkshire RG42 5QE; available from the publishers on receipt of a self addressed label and two first class stamps.

## *How to Run a Reference Crop*

### 1.4.8.2. Soil Survey Book.

Hodgson, J.M., (1985) *Soil Survey Field Handbook*, Technical Monograph No 5. Pub. Harpenden, available from The Soil Survey and Land Research Centre, Silsoe Campus, Silsoe, Bedford, MK45 4DT.

### 1.4.8.3.Disease Keys,

Anonymous, 1976. *Manual of plant growth stages and disease assessment keys*. Pub. Ministry of Agriculture, Fisheries and Food, Harpenden.

Anonymous, 1981. *Field Identification cards- crop diseases*. Pub by MAFF, Harpenden.

## **2. OBJECTIVES, DEFINITIONS AND METHODS OF MEASUREMENT**

### **2.1. Objectives**

#### **2.1.1. Crop Management**

To provide conditions in which winter wheat development and growth are not restricted by weeds, pests, diseases, or nutrient deficiencies, but are affected by ambient temperatures, and availability of sunlight and moisture. Crops should not be irrigated.

#### **2.1.2. Soil and root sampling**

To verify that nutritional conditions are unlikely to inhibit growth by assessing the initial nutrient status of the soil so that appropriate fertiliser can be added to provide for uninhibited crop growth, to record rooting depth of the crop and structure of the soil at anthesis, and to record the extent of uptake of available N by anthesis and by the end of the season.

#### **2.1.3. Plant development**

To provide a detailed record of plant development using the growth stages defined by Tottman (1987) see Section 1.4.8.1 and revised in Volume I Part 2, "*Methods for in-field crop assessment*", paying particular attention to node and leaf development.

#### **2.1.4. Crop sampling**

To provide a frequent record of growth in total dry matter (DM) and concurrent changes in DM of crop components, together with changes in expansion of the crop's green canopy (green area index), its N uptake and changes in water soluble carbohydrate in its stems.

#### **2.1.5. Harvesting**

To provide the basis for estimating and explaining commercial crop productivity at harvest by measuring both the grain yield and quality of the crop by combine harvesting, and by obtaining compatible results from hand-sampling.

#### **2.1.6. Data analysis**

To provide procedures for rapid checking and computation of results and for reporting in time to influence husbandry decisions.

## 2.2. Definitions

### 2.2.1. Plant

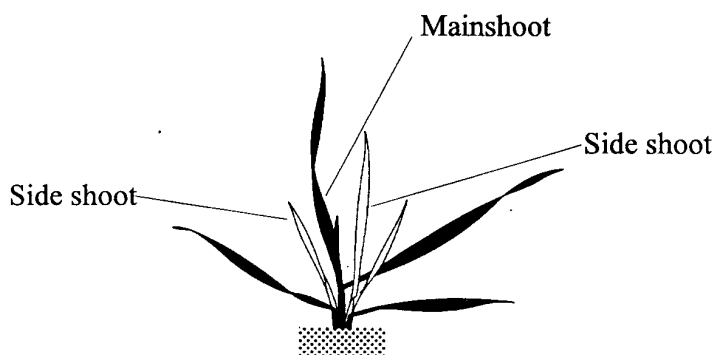
A plant consists of all shoots and roots arising from a single seed.

### 2.2.2. Shoots and mainshoots

#### Shoot

A shoot is counted when its prophyll or first leaf has emerged by 1 cm from its subtending leaf sheath. When counting total shoot number, include both side shoots arising through the process of tillering as well as mainshoots (Fig. 2.1)

#### Mainshoot (or mainstem)



*Figure 2.1 The winter wheat plant at GS 22; the main shoot is shown in black and the side shoots in white*

This is the first shoot arising from the seed. Detailed records of leaf number and developmental stage are made on mainshoots.

#### 2.2.2.1. Distinction between potentially fertile shoots and infertile shoots

The aim of this distinction is to separate those shoots which are likely to produce grain ('potentially fertile') from those that are unlikely to produce grain ('infertile'). When new shoots are first detected it is unlikely that their eventual fate can be predicted, and thus all new shoots are considered to be potentially fertile. However, as soon as the development of a shoot falls significantly behind that of its neighbours it becomes more likely to die before producing grain. The criteria used to distinguish the potentially infertile shoots vary as the plant grows and are given in Sections 6 to 9 below. For example in young plants before stem extension starts if the youngest expanding leaf is dying, and there is no evidence of overall frost or spray damage on similar leaves, then the shoot is likely to be dying. However later in the life of a plant, at the grain filling stage, death of leaves is a less important criterion providing grain filling continues normally. If in doubt it is probably better to record a shoot as

potentially fertile, as the differences are likely to be greater, and therefore the decision easier, in subsequent weeks.

### **2.2.3. Detectable nodes**

Node 1 (the basal node) is counted as detectable when the internode length is more than 1 cm long. If the basal internode does not grow to 1 cm, the next node is counted as node 1 when its internode reaches 1 cm. Subsequent nodes are counted when their internodes are greater than 2 cm.

### **2.2.4. Leaf size**

A leaf is defined for the purposes of length and area measurement as the lamina (between the ligule and the tip) or, if the ligule has not emerged, the partially emerged portion of the lamina. Leaf *sheaths* are left attached to the stem.

### **2.2.5. Stem Area**

Is the projected area of the stem with its surrounding leaf sheaths.

## **2.3. Methods**

### **2.3.1. Sub-sampling**

It is important that all of the plants within a quadrat are harvested. Large samples are taken (0.72 m<sup>2</sup>) to even out row to row and plant to plant variation. The whole sample is then weighed fresh and split into a number of sub-samples for growth analysis, nitrogen analysis, etc. The fresh weights must then be re-recorded immediately, before storage or further analysis. Since these fresh weights are used in calculations on all results derived from the sub-samples it is important that they are recorded as quickly and carefully as possible. If the crop is wet when sampled, or if the plants are washed to remove soil it may be difficult to remove all surface water. This will give rise to some uncertainty in partitioning subsamples on the basis of fresh weights. Thus for wet plants any remainder from the quadrat sample after removing subsamples should also be dried and weighed (section 2.3.2). This will allow a check to be made of the partitioning of subsamples on the basis of dry weight

When sub-sampling, spread out the plant material on a bench and select about 5 bunches of shoots at random to comprise about 50% by fresh weight (Fwt). Spread out the 50% sample and in a similar way, randomly select about 50% by Fwt. Keep this 25% sub-sample (SS1) for growth analysis. Again, take a further 15% sub-sample (SS2) for N% determination. SS2 should be the larger of (approximately) 500g Fwt (200g after all green tissue is lost) or 15% of the total Fwt. Further sub-samples are taken as directed in subsequent Sections.

It is important not to take sub-samples which have larger or smaller shoots than average. This must be checked regularly by calculating the ratio of fresh weight to shoot number for each sub-sample i.e. fresh weight per shoot (g). Experience has shown that this ratio should not differ by more than 2-3% between sub-samples.



However it is quite easy to take sub-samples which differ by as much as 10%. Sample again if necessary. Repeat this procedure for the first few weeks of the season until you are confident that your sub-samples are not biased.

### **2.3.2. Oven drying**

The aim is to reduce the moisture content of all plant material to zero before recording dry weight, as any water present will cause large errors. Weight losses by respiration will also be minimised if the plant material is raised to 80°C as soon as practicable after collection. Generally samples should be dried in a forced draught oven in metal trays or paper bags at 80°C; drying for 16 hours may be sufficient early in the season using lightly loaded ovens but drying for 40 hours may be necessary later in the season or with heavily loaded ovens. Whichever period of drying is used it is important to check initially and periodically that the sample has reached constant weight by removing it from the oven, cooling for 1 minute in a dry room, and weighing accurately. Then replace the sample in the oven, remove after 2 hours, cool and re-weigh as before. If there is evidence of water loss between the two weighings, repeat the process until an adequate drying period is established. It is also important to remove dried samples from the oven before adding fresh material.

### **2.3.3. Preparation for chemical analysis**

This generally requires samples dried at 80°C for 40 hours, but exceptionally water soluble carbohydrate analyses require 'flash' drying at 102°C for 2 hours. Before drying samples, the analytical laboratory should be consulted about sample presentation as, for example, ADAS laboratories require samples cut into lengths less than 10 cm before drying (except for analysis of water soluble carbohydrates where sap loss would result from cutting, so that bending of stems is more appropriate). It is only possible to take a representative subsample for analysis if the whole tissue to be analysed is presented rather than the material dissected for growth analysis. Since drying prevents any changes in chemical composition, dried samples may be stored in sealed, labelled polythene bags until analysis.

### **2.3.4. Assessments of Plant Development**

Stage of plant development (Growth Stage, GS) and numbers of leaves emerged and expanding will be recorded weekly on 10 marked mainstems in a spare quadrat in each replicate. The procedure for preparing marked plants is described in Section 5.7. Assessments of the number of nodes detectable on the mainstem will also be made in the laboratory on whole plants (i.e. with roots attached) taken at random from just outside the quadrat.

Additionally, around the times of ear emergence (GS 59) and anthesis (GS 61) ten marked shoots in another quadrat in each replicate will be visited three times per week (on Monday, Wednesday and Friday) and plants tagged with split plastic drinking straws as they reach GS 59 and 61 respectively. When 50% of shoots have reached these stages, the date should be recorded.

### **2.3.5. Soil and Root Sampling**

Methods for checks on soil nutrients are described in Section 5.8 and methods for description of the soil structure and texture are described in Sections 8.9 and 8.10. The rooting assessment is carried out at the same time as soil sampling, and is described in Section 8.10.

### **2.3.6. Using analytical equipment**

Always check the zero and calibration of balances and do not operate on unstable surfaces or in draughts. Ensure leaf area meters are kept clean and are frequently calibrated, using shapes similar to the leaves to be measured.

### 3. PROGRAMME OF RECORDS

The records collected depend on the time of year and the development of the crop. A summary of the records taken in each period is given below, starting with Period 1 (Section 3.1) which runs from before sowing until detailed growth measurements start in mid -February.

#### Timing of growth recording

Generally detailed growth recordings are made weekly throughout the growing season until two weeks after harvest, however early in the season (for the first four samples) when growth is slow the measurements are less frequent, at the times specified below

#### **Sample number**

1	about 14 th February
2	4 weeks after sample 1
3	2 weeks after sample 2
4	2 weeks after sample 3
5	1 week after sample 4

The records collected change with the major stages of crop development and the locations of the summaries and detailed descriptions for each phase are given below:

<b>Period</b>		<b>Summary of records</b>	<b>detailed description</b>
<i>No.</i>	<i>Description</i>	<i>section</i>	<i>section</i>
1	Before sowing to mid February	3.1	5
2	Mid Feb to ear emergence	3.2	6
3	Ear emergence to Anthesis	3.3	7
4	Anthesis to 30% grain moisture	3.4	8
5	Grain moisture 30% to two weeks after combining including harvest	3.5	9

### **3.1. Period 1 - Before sowing to mid-February**

- i. Topsoil samples should be taken before sowing for analyses of pH, P, K, Mg, % organic matter and N to check for nutrient status (Section 4.1.1).
- ii. The sowing date should be recorded; preferably it should be between 27 September and 5 October.
- iii. Assessments of plant emergence should be made within a few days of sowing (Sections 5.1 and 5.2).
- iv. There should be a fortnightly assessment of winter damage (Section 5.5), and crop health check (Section 5.4).
- v. Identification of leaf numbers on marked plants (Section 5.7)
- vi. Placement of quadrats in the crop after plant emergence (Section 5.6).
- vii. Deep soil samples should be taken between mid January and mid February for mineral N determination (Section 5.8.1).

### **3.2. Period 2 - Mid-February until ear emergence.**

At each sequential harvest the following data will be collected and samples taken for:

- i. Assessment of crop health (Section 6.1).
- ii. Measurement of leaf growth, plant height and assessment of plant development and recording of date of ear emergence (Section 6.2).
- iii. Samples for growth analysis (Section 6.3, 6.5 and 6.6)
- iv. Measurement of plant numbers on first 3 sampling dates (Section 6.3)
- v. Samples for analysis of water soluble carbohydrates in stems from GS 32 (Section 6.4).
- vi. Samples for analysis of nitrogen in above-ground plant parts (Section 6.7).

### **3.3. Period 3 - Ear emergence until anthesis.**

At each sequential harvest the following data will be collected and samples taken for:

- i. Assessment of crop health (Section 7.1).
- ii. Assessment of plant development including accurate recording of date of anthesis (Section 7.2).
- iii. Samples for growth analysis (straw & ears separately- Sections 7.3, 7.5 and 7.6)
- iv. Samples for determination of water soluble carbohydrates in stems (Section 7.4).
- v. Samples for determination of nitrogen concentration in ears and straw (Section 7.7).

### **3.4. Period 4 - Anthesis to grain moisture content of 30%.**

As soon after anthesis as possible

- i. Collect deep soil samples (but not top soil samples) for soil mineral N determination (Section 8.9).
- ii. Dig a soil pit and record made soil texture and root abundance (Section 8.10).

At each sequential harvest the following data will be collected and samples taken for:

- i. Assessment of crop health (Section 8.1, ).
- ii. Assessment of plant development and height (Section 8.2)
- iii. Samples for growth analysis (straw & ears separately Sections 8.3, 8.6 and 8.7).
- iv. Samples for determination of water soluble carbohydrates of stems (Section 8.4.)
- v. Grain samples from 8 ears per plot from 2 weeks after anthesis (8.5)
- vi. Samples for determination of nitrogen concentration in ears and straw (Section 8.8).

### **3.5. Period 5 - Grain moisture 30% until two weeks after combining - including sampling on the day of combine harvesting**

During this period the date of combine harvesting will be kept under review (section 9.2), but weekly sampling and recording continues for:

- i. Yield component analysis (Section 9.9) :
  - a) Ear number/m<sup>2</sup>,
  - b) Grain number/ear,
  - c) Mean grain size,
  - d) Total crop above ground biomass,
  - e) Grain DM,
  - f) Chaff DM,
  - g) Straw DM,
  - h) Total N offtake,
  - i) N in grain,
  - j) N in chaff,
  - k) N in straw,
- ii. Nitrogen harvest index (NHI),
- iii. Progress of grain ripening (Section 9.7).
- iv. Hagberg falling number of grain (Section 9.9).
- v. Water soluble carbohydrates in stems (Section 9.6).
- vi. Crop health (Section 9.3).
- vii. Lodging scores (Section 9.3)
- viii. Plant development (Section 9.4).

### *How to Run a Reference Crop*

Combine harvesting will be carried out when grain moisture content has fallen below 20%, see section 9.2 for details. Grain will be weighed on the combine and samples taken for:

- i. moisture content,
- ii. mean grain weight,
- iii. grain nitrogen concentration,

Deep soil samples (but not top soil samples) should be taken at combine-harvesting for soil mineral N determination (Section 9.10.4).

## **4. CROP HUSBANDRY AND SITE LAYOUT**

### **4.1. Site selection and checking**

As already mentioned (Section 1.4.2) an appropriate field site representing the soil of interest should be selected. It should have no steep slopes, no variation in soil type or soil texture. The whole site should not cross previous crop boundaries, or comprise areas dissimilar in any way such as may result from previous experimental plots or differences in any other factors. A Reference Crop best follows oil seed rape with less than 200 kg per hectare N applied. The site should have a relatively low N status; in particular there should be no grass grown in the last five years or animal manures used in the last three years and the land should have been cropped, not 'set-aside' or fallow. Ideally the previous crop should not have been a legume and should have received less than 200 kg per hectare N (linseed less than 100 kg per hectare N) particularly if it was oilseed rape. The site should also be one with a low risk of take all, and thus the previous crop should not be wheat but, for example, oil seed rape, oats, linseed, potatoes or sugar beet.

The topsoil nutrient status of the selected site should then be checked well before sowing, as described below, and any problems rectified or a new site selected and the topsoil check repeated.

If the site is in an area subject to minor nutrient deficiencies, analysis of plant material collected after emergence (Section 5.9) should be carried out to allow diagnosis and correction of any deficiencies.

#### **4.1.1. Topsoil nutrient status**

Using a soil auger or cheese corer, take 0-15 cm soil samples at 5 points in the expected position of each sample plot (see Appendix 1) and bulk these to make 3 samples for topsoil nutrient analysis. Place the soil in a polythene bag, label, seal and submit the samples to an accredited analytical laboratory for analysis of pH, P, K, Mg, CaCO<sub>3</sub> (%), organic matter (%OM) and total organic nitrogen (%N). Record the results on the 'Soil Analysis' sheet (Section 10.4/13)

### **4.2. Plot layout and size**

The basic plan is to set up plots of a minimum size 4 m × 24 m, with alternating plots for combine harvesting and growth analysis. The example plans (Appendix 1) may be modified as required for the shape of an area available, as long as plots for combine harvesting are at least 1 m wider than the combine harvester used. The plots for growth analysis are harvested sequentially using quadrats (1.2 m × 0.6 m), laid out as shown in the plan, and specified in detail in Section 5.6. Quadrat samples must be taken from at least 3 different plots to provide estimates of the error of measurements and, where resources permit, increased replication may be necessary to reduce the error. However, no more than 5 replicates should be used, as extra plots cover a greater area and therefore tend to increase variation.

The plan also provides spare quadrat areas in case of crop damage or a long season.

#### **4.3. Pathways and access for tractor operations**

It is preferable that plots are combined across the direction of the rows. Thus the plots will be marked out after drilling. This is best achieved by 'burning out' paths in the young crop using a weed-wiper. There should be an access pathway between all plots (along the 24 m sides), but the width of this pathway is not critical. Spraying and spreading operations should take place at right angles to the long side of the plots. If the sprayer cannot span the whole plot (by passing by one end and then the other, depending on the width of the booms being used) there will need to be one set of tramline wheelings crossing each plot (see Appendix 1). Quadrats should not be positioned within 0.75 m of a wheeling. When the combine plots are to be harvested, the tramlines should be cut out so that they do not form part of the harvested area. This may leave two or even three lengths of crop per plot. These lengths should be measured accurately before combining, as should the width of the combine's cutter bar.

#### **4.4. Choice of variety**

Choice of variety should be co-ordinated with other Reference Crops. The choice must be of a variety which is currently "Recommended" or "Provisionally Recommended" for general use, and which either is likely to be "Recommended" for several years, or which has been the subject of previous Reference Crops, so as to maximise reliable comparisons between sites and seasons. It will be an advantage to choose a variety for which there is a quantity of available data such as those for Mercia in Volume I Part 1, "*The Wheat Growth Digest*".

#### **4.5. Seed**

Since the aim is to compare between sites and seasons, seed should be obtained from a single reputable supplier and farm saved seed should not be used. Seed should be dressed with a good general purpose fungicidal seed treatment (e.g. fenpiclonil or bitertanol + fuberidazole), but chemicals known to have a direct affect on crop growth should be avoided (e.g. triadimenol + fuberidazole).

#### **4.6. Sowing**

The crop should be drilled between 27 September and 5 October into a good seed bed. Sowing should only be delayed after 5 October if seedbed conditions are judged likely to result in less than 50% plant establishment.

The aim should be to establish 275 plants per m<sup>2</sup> in the spring, in rows 12.5 cm apart. If establishment is poor (with significant parts less than 50 plants per m<sup>2</sup>), then the crop is unsuitable as a reference crop.



#### **4.7. Weed control**

Aim to keep the crop free of weed competition; the treatments required will depend on the weed species present at each site. This aim should include the avoidance of over-winter competition; use of autumn residuals should be planned wherever possible. Ensure that a full record of all weed control measures is included in the site report (Section 10.5/1).

#### **4.8. Disease control**

Aim to keep the crop disease free using a full prophylactic fungicide programme. However, avoid chemicals which may scorch the crop. A suitable programme is as follows (compulsory spray timings in **bold**, otherwise sprays are optional) :

November - if mildew or yellow rust developing	Folicur (0.5 l/ha plus Corbel (0.5 l/ha)
February - if mildew or yellow rust developing	Folicur (0.5 l/ha plus Corbel (0.5 l/ha)
<b>GS 31/32</b>	Sportak Delta (1.25 l/ha) + Patrol (0.5 l/ha)
<b>GS 39</b>	Opus Team (1.5 l/ha)
<b>GS 59</b>	Folicur (0.5 l/ha) (+ Patrol @ 0.5 l/ha if mildew active )

(This is slightly modified from the programme used for variety testing)

NB. Prior to application check product labels for changes in approval. Ensure that a full record of all disease treatments is included in the site report (Section 10.5/1).

#### **4.9. Pest control**

The aim is to keep the crop free of pests; appropriate preventative measures should be taken to deal with any problems likely to be encountered at the site. For example preventive application of slug pellets (e.g. methiocarb) may be required post-drilling or pre-emergence in high-risk fields i.e. wet weather, heavy soil, cloddy seedbeds and history of slug problems.

## How to Run a Reference Crop

To avoid aphid transmission of BYDV apply a synthetic pyrethroid (e.g. cypermethrin, deltamethrin) at full label rate in last week of October or first week of November.

Other appropriate treatments should be applied to avoid any problems forecast (by pest forecasting services such as ADAS Crop Action Reports) in a particular season. It is essential to deal quickly and effectively with any problems revealed by the crop health checks (e.g. aphids in June or July). A full record of all pest treatments must be included in the site report.

### 4.10. Growth regulators

The aim is to avoid lodging; therefore a full plant growth regulator programme should be used. For example, apply chlormequat as a split application, 2/3 rate at the end of tillering and 1/3 rate at GS 31 followed by Terpal at 1/2 rate at GS 37-39.

NB. Prior to application check product labels for changes in approval and ensure that a full record of all growth regulator applications is included in the site report.

### 4.11. Nitrogen fertiliser

Nitrogen should be applied as ammonium nitrate granules, so as to provide a total supply from soil and fertiliser of about 300 kg/ha N. To assess the N supply from the soil, mineral nitrogen should be measured to 90 cm depth (see Section 5.8.1) during January or early February and an estimate made of N in the crop at the same time. If crop measurements are not available, an estimate of crop N can be made using the following table :

<i>Plant density</i>		<i>Plant Growth</i>					
		seedling	up to 3 leaves	2 to 3 shoots	4 to 6 shoots	more than 6 shoots	
<i>Description</i>	<i>plants/m<sup>2</sup></i>	GS	<10	10-13,20	21-22	23-25	>25
		<i>crop N (kg/ha)</i>					
Sparse	50-149		0	0	5	20	20
Moderate	150-249		0	5	5	20	35
Dense	250-350		0	5	20	35	50

## How to Run a Reference Crop

As soon as results are available from the laboratory the 'soil plus crop N' should be subtracted from 300 to give the fertiliser N requirement in kg/ha. The fertiliser should be split as follows, always keeping intervals of at least 10 days between applications. Ensure that a full record of dates and amounts of fertiliser applied is included in the site report (Section 10.4/1).

<i>Soil plus crop N (kg/ha)*</i>	<i>Fertiliser N requirement (kg/ha)</i>	<i>N application</i>		
		<i>First</i>	<i>Second</i>	<i>Third</i>
		<i>timing</i>		
		From mid February to mid March	From GS 30 to GS 31 but not before mid March	At GS 31 but not after 7 May
		<i>amount (kg/ha)</i>		
160-200	100-140	0	40	rest
100-160	140-200	40	½ rest	½ rest
<100	200-300	60	½ rest	½ rest

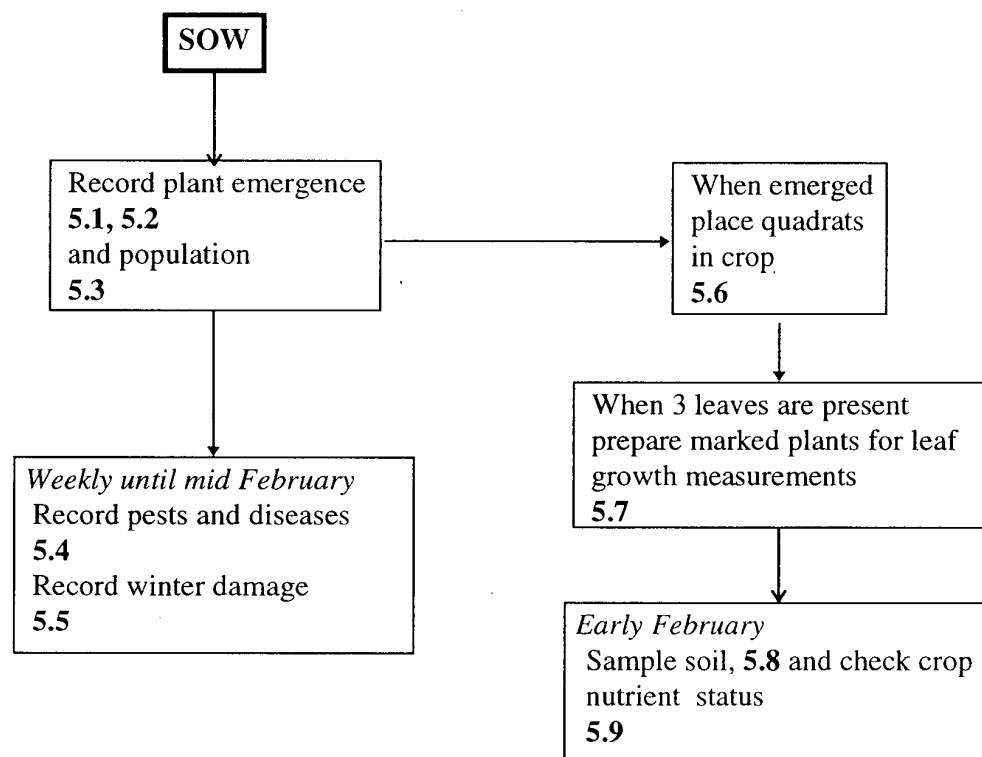
\* Sites with more than 200 kg/ha soil N supply are likely to be unrepresentative of surrounding crops and should be re-selected.

### 4.12. Irrigation

Crops should not be irrigated, even if this is local practice.

## 5. PERIOD 1 - SOWING UNTIL MID FEBRUARY

Flow chart of the recording and sampling strategy



### 5.1. Date of first plant emergence

After drilling, assess on a daily basis until first coleoptiles or leaf tips are observed above ground.

### 5.2. Date of 50% and full plant emergence

On each plot, mark out five lengths of row, each 1.0 m in length. Count, and record on the 'Crop emergence record' sheet (Section 10.4/3), the number of emerged plants in each of these lengths of row once every two days until a constant plant number is achieved. Be careful not to count volunteer cereals or grass weeds. The date of full emergence is the first date on which the constant record was achieved and 50% emergence the date when half this number was recorded. Interpolate between assessment dates as necessary.

### **5.3. Calculation of row width and plant population**

After full emergence, in the centre of each of the quadrat replicate strips (①, ②, and ③ in Appendix 1), measure the distance between the first and the eleventh in a series of adjacent rows which does not include a junction between drill bouts. The row width is a tenth of this distance. Where the row width was measured count the number of plants either side of a 0.5 m stick placed between alternate rows and sum the values to give the number of plants present in a 5m row length. Calculate the plant population as follows:

$$\text{Plants / m}^2 = \frac{\text{Total number of plants in 5 m length of row}}{5 \times \text{mean row width in m}}$$

### **5.4. Crop health check**

The health of the crop should be checked and recorded (on the 'Crop health record Section 10.4/2) fortnightly in winter by examination in the field. A record should be made under each heading, even if there is no problem. Where a problem is identified this should be quantified by scoring, and the values of the scoring system should be defined (see the example for winter damage above). Any leaf or stem affected by disease should be identified (paying particular attention to the lowest leaf), and appropriate keys (see Section 1.4.8.3) used to assess the percentage of the area affected. Any pest attacks, (e.g. grazing by slugs or aphid infestation), mechanical damage (e.g. trampling), lodging (scored using Section 10.4/2) or symptoms of nutrient deficiency should also be recorded.

Records should be made of any remedial treatments carried out. If there are any major crop health problems the suitability of the crop as a Reference Crop should be re-considered.

### **5.5. Winter damage**

Crops may be damaged by prolonged severe cold weather or after a sharp frost following a period of mild weather. After such an event, a period of 7-8 days should be allowed for symptoms to develop before winter damage is scored using the following scale:

1. No damage.
2. Leaf tip damage
3. Severe leaf damage, up to 25% loss of leaf area.
4. Very severe leaf damage, up to 50% loss of leaf area.
5. Very severe leaf damage, up to 75% loss of leaf area.
6. Plant or all leaves dead.

The percentage of the crop in each category should be recorded as well as the crop growth stage at time of assessment.

## **5.6. Preparation of sample areas**

To avoid local bias in the selection of samples, sample from predetermined areas by placing quadrats in the field prior to the start of sampling. Furthermore, to avoid systematic bias, sample each plot by starting at a different point along its length. The starting point and the direction to be taken through the plot is shown on the trial plan (Appendix 1).

Each quadrat should be  $0.72 \text{ m}^2$  ( $1.2 \text{ m} \times 0.6 \text{ m}$ ) oriented so that one row of plants passes through diagonal corners of the quadrat. There should be at least 0.5 m between adjacent quadrats. Quadrats should be at least 0.75 m from ends and edges of plots and from any tramlines.

If it is thought at any stage that a particular quadrat is not representative of the plot, record the precise reason for discarding it and estimate the proportion of the growth analysis and combine plots likewise affected. Move on to the next quadrat. If there are not enough quadrats within the quadrat replicate area, (for example in a long season or if there is crop damage in a number of quadrats) quadrats can be taken from adjacent spare plots designated for quadrat sampling

Any quadrat position unlikely to be required in a normal season, such as quadrat 28 on the plan (Appendix 1), should be reserved for measurement of leaf growth and recording of dates of ear emergence and anthesis on marked shoots (see Sections 6.2 & 7.2).

## **5.7. Preparation of marked plants**

As soon as emergence is complete, identify and label with plastic labels 20 plants per replicate in a spare quadrat position (e.g. 28) for leaf growth and leaf number recording. Appearance of successive leaves on the main shoots of these plants will need to be recorded between emergence and the commencement of leaf extension measurements in mid-February.

If a record is not taken overwinter then it will be difficult to

- 1) Identify correctly the main shoot.
- 2) Count accurately the total number of leaves on the plant, as older leaves die and drop off or decay, and may confound the records of total leaf number.

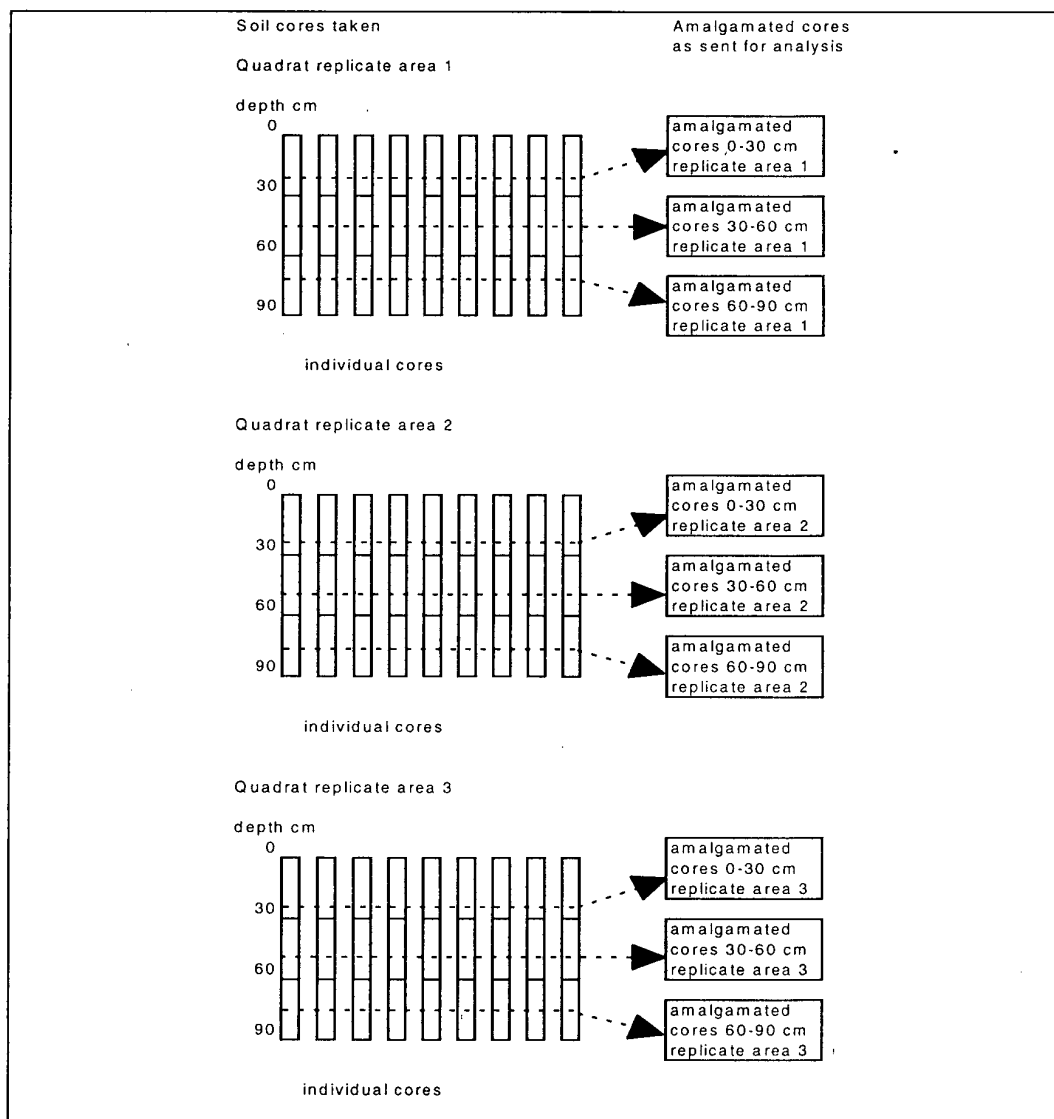
At least every fortnight until mid-February, examine the marked plants, and label every new fully expanded leaf with a length of coloured insulated copper wire prepared as described in Section 1.4.3, placed around the stem under the leaf and above the next older fully expanded leaf. Take care to exclude tillers from the ring, and to record the sequence of colours for the wire rings. Note that the first wire ring should be applied between the first and second true leaves, care being taken to distinguish the first leaf from the coleoptile (seed leaf).

## 5.8. Soil and plant sampling & analysis

Topsoil samples should be taken before sowing (Section 4.1.1), and deep samples for mineral N analysis should be taken before mid-February.

### 5.8.1. Soil mineral nitrogen

Using 40 mm, 30 mm and 20 mm diameter gouge augers from Eijkelkamp (Van Walt Lt., Haslemere, Surrey) or EJJ Laboratories (Brund, DK7700, Thisted, Denmark), sample the soil from 0-30 cm, 30-60 cm and 60-90 cm depths respectively at 9 positions in each of the quadrat plots, but avoiding the quadrat sampling positions. Bulk the soil to give one sample from each of the three depths in each of the three plots (as shown in the diagram below). The samples should be stored frozen at -18°C and submitted to an appropriate analytical laboratory for analysis of percentage dry matter, nitrate-N and ammonium-N (mg/kg).



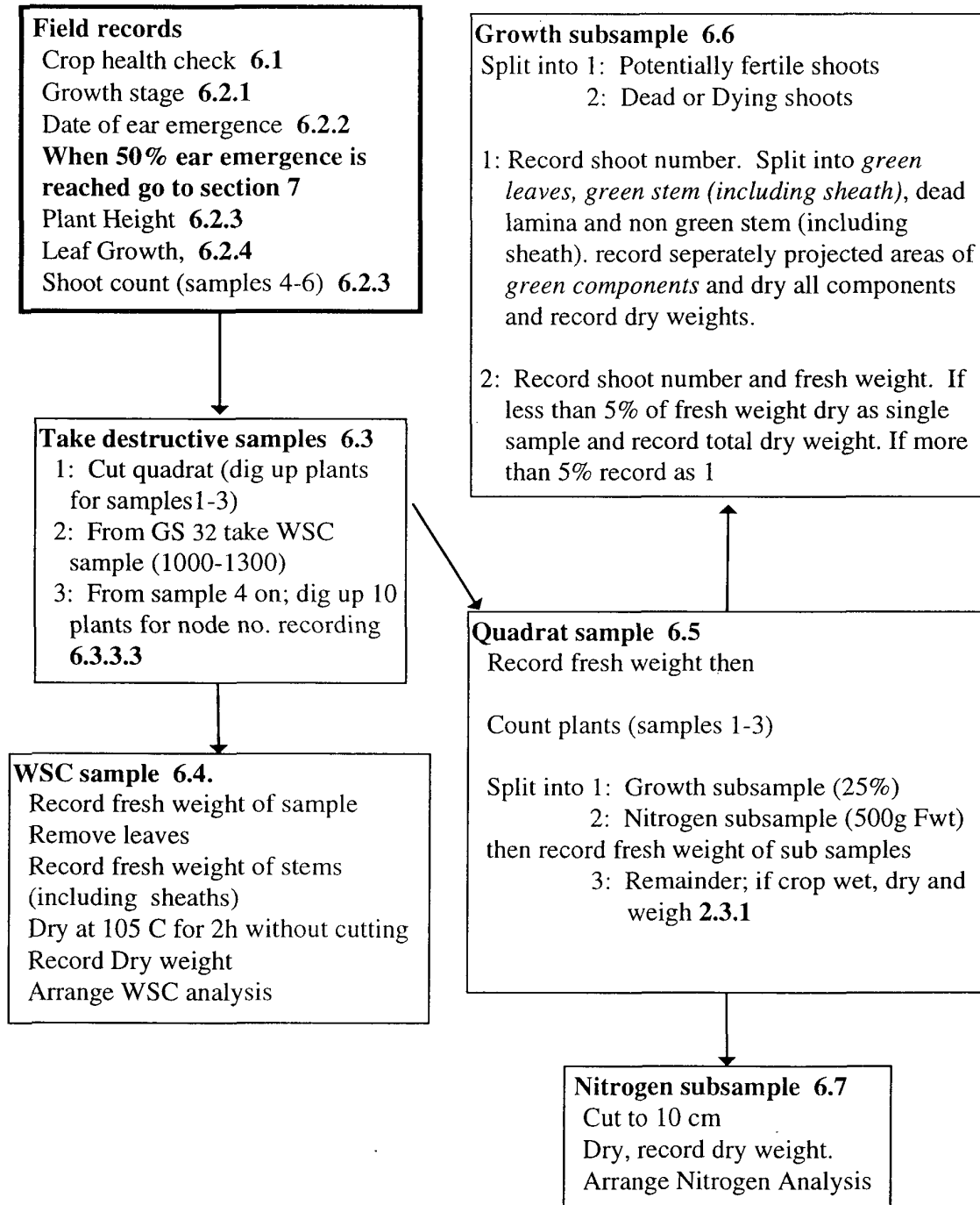
## **5.9. Plant nutrient status**

Sampling procedures for nitrogen analysis are described under later Sections (e.g. 6.7). For assessment of other elements in plant material (e.g. for crops which may be prone to manganese deficiency because they are on sandy soils, soils with high organic matter content, or high pH soils), take quadrat samples from sufficient plots to give a sample of 100g Fwt (approximately 10g DM) of above-ground plant material. If plant and soil sampling cannot be carried out on the same day, aim to carry out both within the same week. Wash in the laboratory to remove soil then dry for at least 48h at 80°C. Submit in sealed bags to an accredited analytical laboratory.



## 6. PERIOD 2 - MID-FEBRUARY UNTIL EAR EMERGENCE

Flow chart of measurements and records (weekly after Sample 5 see Section 3.3 for details)



## **6.1. Crop health check**

The health of the crop should be checked and recorded (on the 'Crop health record') weekly by examination in the field and by examination of a sample of ten shoots in the laboratory. A record should be made under each heading, even if there is no problem. Where a problem is identified this should be quantified by scoring, and the values of the scoring system should be defined. Any of the last 4 or 5 leaves, or the stem or ear, affected by disease should be identified, and appropriate keys (see Section 1.4.8.3) used to assess the percentage of the area affected. These assessments may be done in the lab on plants removed for quadrat sampling. Any pest attacks, (e.g. grazing by rabbits or aphid infestation), storm or mechanical damage (e.g. trampling), lodging (scored using Section 10.4/2) or symptoms of nutrient deficiency should also be recorded.

Records should be made of any remedial treatments carried out. If there are any major crop health problems the suitability of the crop as a Reference Crop should be re-considered.

## **6.2. Stage of development, leaf growth, plant height and date of ear emergence.**

### **6.2.1. Stage of development**

Record growth stage (GS) on the plants marked for growth stage recording on the 'Growth Stage Data' sheet (Section 10.4/4), using the growth stage key of Tottman (1987, see Section 1.4.8.1). The overall aim is to obtain at each date an accurate record of the stage which is exceeded by just half the plants (or shoots after GS 39). It is important to remember that arithmetic averaging is inappropriate for growth stages. Several growth stages can often be recorded on a single plant for example those referring to leaf number (GS 10-19), tiller production (GS 20-29) and stem elongation (GS 30-39). Only the greatest numerical stage will be required for summary purposes, other stages can be deduced from the separate records taken of leaf growth, shoot number, and node number.

### **6.2.2. Ear emergence**

As ear emergence commences objectively choose 10 'potentially fertile' shoots from quadrat position 28 in each sampling plot by selecting every other shoot in one row (30 shoots in all). Mark these shoots by placing coloured wire around the stem. Note: The marked stems in quadrat 28 cannot be used as they represent mainstems only. **Visit the field on Monday, Wednesday and Friday** (and more often if possible - this is a minimum), and as each plant reaches the stage when its inflorescence has fully emerged (ear collar above flag leaf ligule; GS 59), mark the plant by e.g. placing a second piece of wire around the stem. Record the number of plants tagged at each visit on the 'Ear emergence record' (Section 10.4/10a). When 15 shoots (i.e.  $15/30 = 50\%$  of the marked shoots) have reached GS 59 and have been tagged, record this as the date of ear emergence on part 1 of the 'Site report' (Section 10.4/1), interpolating between dates as necessary and proceed to PERIOD 3 (Section

7) for the new sampling protocol. If 50% ear emergence has occurred between visits, interpolate and enter the assumed date of 50% ear emergence. Leave the wire on these shoots, as the same ears should be used for assessment of date of anthesis (Section 7.2.2).

### 6.2.3. Plant height

Record plant height on the 'Plant height record' (Section 10.4/9) by measuring from ground level to the uppermost leaf ligule or the ear collar, whichever is the higher, of 10 plants chosen at random (it is not advisable to use marked plants for measurement of plant height as handling may affect growth).

### 6.2.4. Leaf Growth

Leaf extension growth is recorded on 10 of the 20 marked mainstems in each replicate, the other marked mainstems acting as spares in case of damage to plants or death of a recorded mainstem. Use the leaf data record sheet (Section 10.4/6) to record leaf lengths as shown in the example below. On each sample occasion on the mainstem of the marked plants, measure the lengths of all the expanding leaves in millimetres from the leaf tip to the ligule of the next older fully expanded leaf and record against the leaf number (counting from the base of the plant). Also in those leaves whose ligule has appeared, record the fully expanded lamina length (from its own ligule to its tip) against the leaf number and put a × in the next week's measurement column to indicate that the leaf is fully expanded. A label (see Sections 1.4.3 and 5.7) should then be placed around the mainstem below the leaf whose ligule has just emerged and its colour recorded on the record sheet.

#### 6.2.4.1. Example of leaf growth data sheet for leaf extension and growth stage.

Observation no.		1	2	3	4	5
Date		15/2	22/2	1/3	/	/
Recorded by		7P	7P	D7		
Leaf no.	Label colour					
2	purple	4	12	×		
3	yellow		8	19	×	
4				15		

On the first observation, leaf 2 has begun to expand, on the second, the ligule is visible so that after measurement a cross is put in the box to the right and the label colour noted in column 2; the length of leaf 3 is then recorded.

### **6.3. Other in situ measurements and sample removal**

#### **6.3.1. Sample dates 1, 2 & 3**

On these three sampling occasions, plants within the quadrats will be dug up or pulled up to allow accurate counting of whole plants. A plant is defined as all of the shoots, including the main shoot, arising from a single seed. The number of plants is recorded only on the first three sample dates. After digging up, the plants should be placed with their roots in the bottom of a bag or in a tray. In the laboratory plants should be counted then the roots (including any sub crown internodes) should be cut off and discarded, before recording weights for growth analysis. If any coleoptile tillers are present they should also be cut off at the level of the crown. On a sub-sample of 10 plants, count the number of detectable nodes see section 2.2.3 on the mainstem and record on the 'Node number sheet' (Section 10.4/8), and return these plants to the sample. Then process immediately as described in Section 6.5 or seal in polythene bags and store at 4-6°C before processing.

#### **6.3.2. Sample dates 4, 5 & 6**

On these sampling dates count the numbers of shoots *in situ* as well as in the laboratory in order to check for differences. These may arise when plants are removed from the field and small shoots become detached or when small shoots are lost during sample collection.

Within the centre of each quadrat to be harvested, mark out three lengths of row, each 0.33m long, and count the number of shoots in each of the three marked lengths. Measure the distance to the next row on each side of the counted row, recording counts and distances on the 'Field Shoot Numbers' sheet (Section 10.4/7). Note here that 'shoots' means all the stems, including the main stem, with a leaf greater than 1 cm long. Then calculate total shoot number and average row width from each plot on sheet 10.4/7 and enter these values on the 'Growth Analysis Data' sheet. Then sample the plants as described in Section 6.5

#### **6.3.3. Sample dates 4 onwards**

##### 6.3.3.1. Growth analysis sample

Using sharp scissors or secateurs, cut off all the shoots within the quadrat at the soil surface, then collect all of the plants and place as quickly as possible into a plastic bag, with the lower portion (most contaminated with soil) at the bottom of the bag to reduce the time spent washing. Then seal the bag to prevent the lamina drying out before projected areas are measured. If growth analysis is delayed, store samples in a cold room at about 4-6°C. If samples are contaminated with soil, wash AFTER storage in the cold room, as washing hastens deterioration of the lamina surface. Aim to do all growth analysis within two days of sampling.

### 6.3.3.2. Water soluble carbohydrate sample

From growth stage 32, take a random sample of 8 fertile shoots from just outside the quadrat area for determination of stem soluble carbohydrate concentration. These samples should be taken after all other field measurements have been made and if possible between 10.00 hours and 13.00 hours (to avoid differences due to time of day). Place the shoots in a plastic bag, seal and place in a chilled cool box. Return to the lab as quickly as possible and treat as described in Section 6.5.

### 6.3.3.3. Node number recording

As plants from the quadrat sample are cut off, not dug up as previously (Sample dates 1-3, Section 6.3.1) they are unsuitable for node number assessment which requires intact crowns and mainstems. Thus from sample 4 onwards dig up 10 plants from just outside the quadrat and count the number of detectable nodes (see Section 2.3.3 for definition) on the mainstem. The counts of nodes may take place in the field or laboratory and the data is entered on the 'Node number record' (Section 10.4/8) When a constant number of nodes has been recorded for three successive weeks, these recordings may stop.

## **6.4. Water -soluble stem carbohydrates (from GS 32)**

Record the fresh weight of the 8 shoots. Remove the laminae at the ligule (these can be discarded) and reweigh the 8 stems. Avoid cutting the stems which could allow sap loss; bend them if necessary. Immediately place the stems in a single layer in a gauze-based tray and "flash-dry" by placing in a forced-draught oven set at 102°C for 2 hours. The oven must be free of other material and be already at 102°C when the samples are added, to ensure that respiration is stopped as quickly as possible. After exactly 2 hours, record the dry weight. Store dried samples in sealed labelled polythene bags for water soluble carbohydrate analysis.

## **6.5. Division of quadrat sample**

Remove all plant material from the bag. Generally the samples should be almost free of soil. If plants are contaminated with soil, shake them so that the soil falls away or gently wash under a running tap. Using paper towels or by shaking, remove all surface water. Record the total number of plants on the first 3 dates and on all dates record the total number of stems including main shoots. Record the total fresh weight of the quadrat sample. Then sub-sample as described in section 2.3.1 to provide :

- 1) A subsample of 25% of the fresh weight for growth analysis (SS1).
- 2) A sub-sample of up to 500g Fwt (probably the whole of the remainder early in the season when the crop is small) for nitrogen analysis (SS2).

As quickly as possible record the weight of the sub-samples. Place the samples for green area determination (SS1) in a plastic bag to prevent dehydration, and store in a cold room at 4-6°C. If the crop was wet, dry the remainder at 80°C for 16 hours and weigh to allow a sub sample size check.

## **6.6. Measurements on the 25% sub-sample (SS1)**

Divide the sub-sample shoot by shoot into two categories (SS1(a) and SS1(b)) as described below and count the number of shoots in each.

- (a) Potentially fertile shoots (SS1(a)): A potentially fertile shoot is either clearly booting or its newest leaf is green and shows no signs of dying back from the tip.
- (b) Dead or dying shoots (SS1(b)): A shoot is dead or dying when it has no green material or its newest expanding leaf has begun to turn yellow at the tip, or its flag leaf is fully emerged but there is no evidence of "booting".

### **6.6.1. Measurements on the potentially fertile shoot group (a)**

Separate the material into :

- i. green lamina excluding flag leaf
- ii. green true stem plus sheath,
- iii. non-green stem plus sheath
- iv. dead material (dead lamina)

Yellowing parts of the leaves are classed as dead. A leaf is classed as dead if it is yellow across the whole width of the base of the lamina. Then, for green lamina and green stem, record projected areas using a suitable meter. Note that the leaf sheath is left on the stem, and is measured as stem. 'Leaf' means lamina only.

Dry all fractions separately for at least 16 hours at 80°C and record the dry weight of all fractions.

### **6.6.2. Measurements on the infertile shoot group (SS1b)**

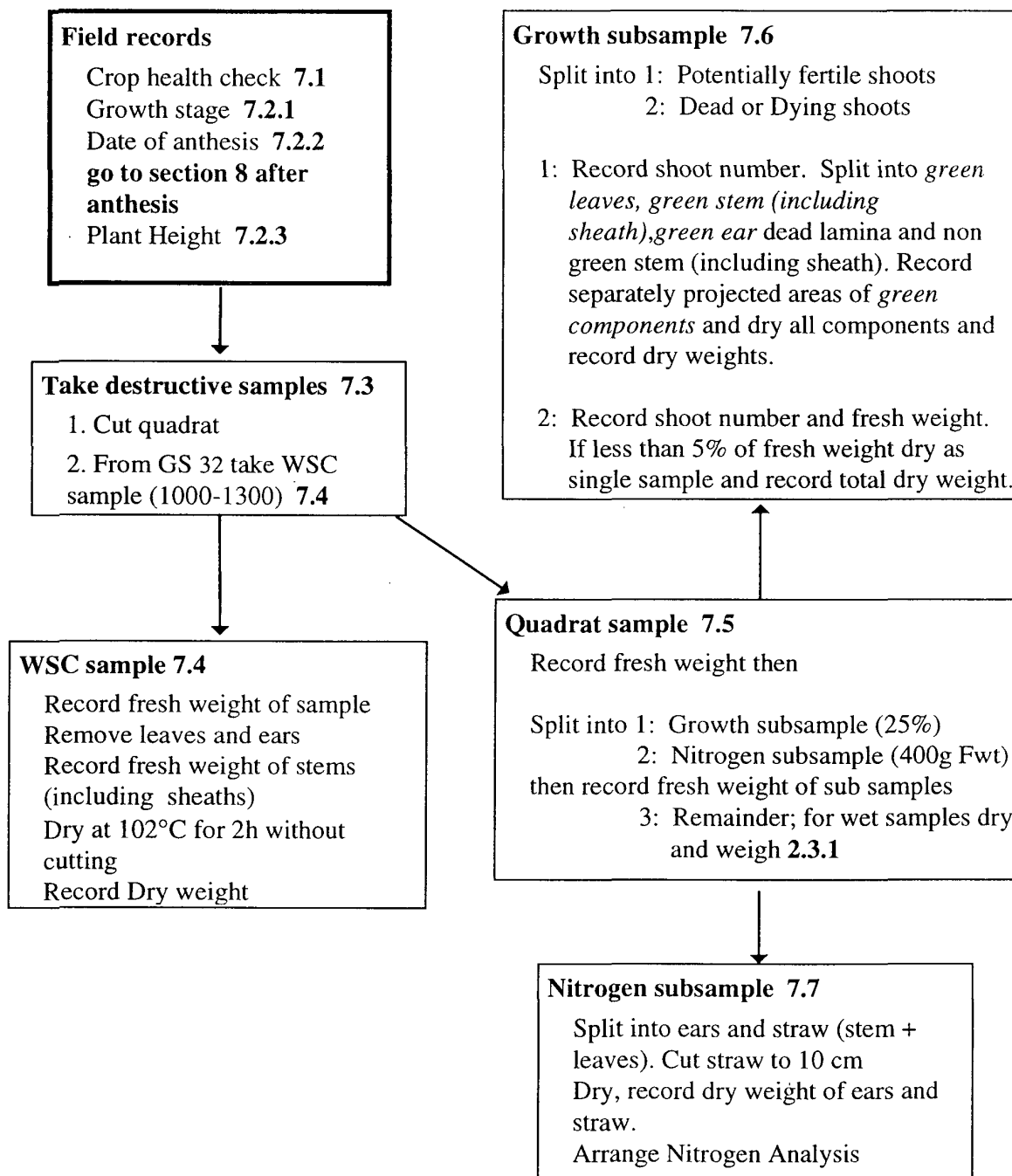
If the fresh weight of SS1(b) is less than 5% of the total fresh weight of SS1 then dry at 80°C and record the total dry weight. Otherwise separate into the same fractions and treat as SS1(a)

## **6.7. Samples for N determination (SS2)**

Record the fresh weight of the sample, chop into 10 cm lengths (if required by the analytical laboratory) before drying at 80°C for at least 16 hours. Check that the samples have reached constant weight (as described in Section 2.3.2), reweigh, then transfer into polythene bags, label and seal immediately, prior to submission for chemical analysis.

## 7. PERIOD 3 - EAR EMERGENCE UNTIL ANTHESIS

Flow chart of measurements and records (weekly after sample 5. see 3 for details)



## **7.1. Crop health check**

The health of the crop should be checked and recorded (on the 'Crop Health Record') weekly by examination in the field and by examination of a sample of ten shoots in the laboratory. A record should be made under each heading, even if there is no problem. Where a problem is identified this should be quantified by scoring, and the values of the scoring system should be defined. Any of the last 4 or 5 leaves, or the stem or ear, affected by disease should be identified, and appropriate keys (see Section 1.4.8.3) used to assess the percentage of the area affected. These assessments may be done in the lab on plants removed for quadrat sampling. Any pest attacks, (e.g. grazing by rabbits or aphid infestation), mechanical damage (e.g. due to hail or trampling), lodging (scored using Section 10.4/2) or symptoms of nutrient deficiency should also be recorded.

Records should be made of any remedial treatments carried out. If there are any major crop health problems the suitability of the crop as a Reference Crop should be re-considered.

## **7.2. Stage of development and height and date of anthesis**

### **7.2.1. Stage of development**

Record growth stage (GS) on the plants marked for growth stage recording on the 'Growth Stage Data' sheet (Section 10.4/4), using the growth stage key of Tottman (1987, see Section 1.4.8.1). The overall aim is to obtain at each date an accurate record of the stage which is exceeded by just half the plants (or shoots after GS 39) of the crop. It is important to remember that arithmetic averaging is inappropriate for growth stages. Several growth stages can often be recorded on a single plant for example those referring to leaf number (GS 10-19), tiller production (GS 20-29) and stem elongation (GS 30-39). Generally it is only the greatest numerical stage that will be required for summary purposes, but the other stages can be deduced from the separate records taken of leaf growth, shoot number, and node number.

### **7.2.2. Date of Anthesis**

It is important that an accurate record of date of anthesis is achieved. Date of anthesis is defined as the date on which 50% of the ears have some anthers dehisced (i.e. we have defined the start of flowering as 50% of shoots at GS 61). As anthesis approaches, return to the 10 'potentially fertile shoots' selected in quadrat 28 of each sampling plot for determination of ear emergence (total = 30 shoots marked with coloured wire around the stem). Visit the field on Monday, Wednesday and Friday and when anthers are visible on the marked shoots place another piece of wire (or a plastic drinking straw slit lengthways) around the stem below the ear. Record the number of shoots on the marked plants on which anthesis has occurred on the Anthesis record sheet (Section 10.4/10b). The date of anthesis is defined as the day 15 ears from the 3 plots have been tagged, and can be interpolated between the site visits if necessary. When reached, enter the date on part 1 of the Site report (Section



10.4/1) sheet, and as additional information in the summary file. Then proceed to PERIOD 4 for sampling protocol (Section 8).

### **7.2.3. Plant height**

Record plant height by measuring from ground level to the ligule of the uppermost leaf or the ear collar (whichever is higher) of 8 shoots chosen at random (it is not advisable to use marked plants for measurement of plant height as handling may affect plant growth) and enter on the crop height record sheet (Section 10.4/9).

### **7.3. Sample removal**

Using sharp scissors or secateurs, cut off all the shoots within the quadrat at the soil surface. Collect all above ground plant material and place as quickly as possible into a plastic bag, so that the lower portion of the plant (most contaminated with soil) is at the bottom of the bag. This will reduce any time spent washing the plants. After all the material has been collected, seal the bag to prevent the leaves drying out before their green areas are measured. If growth analysis is delayed, store the samples in a cold room at about 4-6°C. If the samples are contaminated with soil, wash AFTER storage in the cold room, as washing hastens deterioration of the leaves. Aim to do all growth analysis within two days of sampling.

Additionally take a random sample of 8 fertile shoots from just outside the quadrat area for determination of stem soluble carbohydrates. These samples should be taken after all other field measurements have been made and if possible between 10.00 hours and 13.00 hours (to avoid differences due to time of day). Place the shoots in a plastic bag, seal and place in a chilled cool box. Return to the lab as quickly as possible and treat as described in Section 7.4.

### **7.4. Water - soluble stem carbohydrates**

Record the fresh weight of the 8 shoots. Remove the laminae at the ligule and the ears at the collar (these can be discarded), and reweigh the 8 stems. Avoid cutting the stems which could allow sap loss; bend them if necessary. Immediately place the stems in a single layer in a gauze-based tray and "flash-dry" by placing in a forced-draught oven set at 102°C for 2 hours. The oven must be free of other material and be already at 102°C when the samples are added to ensure that respiration is stopped as quickly as possible. After exactly 2 hours, record the dry weight. Store dried samples in sealed polythene bags for analysis of water soluble carbohydrate.

### **7.5. Division of quadrat sample**

Remove all plant material from the bag. Generally the samples should be almost free of soil. If the shoots are contaminated with soil, shake them so that the soil falls away or gently wash under a running tap. Using paper towels or by shaking, remove all surface water. Record the total fresh weight of the quadrat sample.

Spread out the plant material and sub-sample (see Section 2.3.1) about 25% by Fwt (SS1) for growth analysis. Take a further sub-sample (SS2) of 15% Fwt (or approximately 400 g. Fwt, whichever is larger) for N% determination.

As quickly as possible, record the weight of the sub-samples. Place the samples for green area determination (SS1) in a plastic bag to prevent dehydration, and store in a cold room at 4-6°C. If the crop was wet, dry the remainder at 80°C for 16 hours and weigh to allow a sub sample size check.

## **7.6. Measurements on the 25% sub-sample**

Divide the sub-sample into two categories of shoot (SS1(a) and SS1(b)) and count the number of shoots in each. "Shoots" includes both tillers and mainstems. Categories are as follows:

(a) Fertile shoots (SS1(a)): A fertile shoot has an ear or is "booting" and has some green leaf.

(b) Infertile, dead or dying shoots (SS1(b)): A shoot is counted as infertile, dead or dying when it has no grain or green material, when its newest expanding leaf has begun to turn yellow at the tip or its flag leaf is fully emerged but there is no evidence of "booting".

For the fertile shoot group (SS1(a)), separate the material into :

- i. green lamina
- ii. green stem plus green sheath
- iii. green ear
- iv. non-green stem plus sheath
- v. dead lamina

'Leaf' means lamina only; leaves should be separated at the ligule, the junction with the sheath. Cut individual leaves into green and dead parts; yellowing parts of the leaves are classed as 'dead'. Leaves are also classed as dead if they are yellow across the whole of the base of the lamina.

The leaf sheath is left on the stem, and is measured as stem. Stems should be cut up into green and non-green material and the area of the green stems measured using a green area meter. Do not cut individual stems more than twice. (NOTE that measuring whole stems and estimating percentage green area is subject to major errors). Ears should be separated from the stem as soon as is practicably possible in order to detect the pattern of ear growth. As soon as most emerging ears can be easily pulled from the flag leaf sheath ( about GS 49) cut all unemerged, emerging and fully emerged ears at their collar, keeping ears which have more than half emerged separate from those which had less than half emerged.

Then record projected green areas for the laminae, stems, and the emerged ears. Recombine all ears and dry all fractions for at least 16 hours at 80°C (Section 2.3.2) and record dry weights.

## *How to Run a Reference Crop*

### For the infertile, dead and dying shoot group (SS1(b)) :

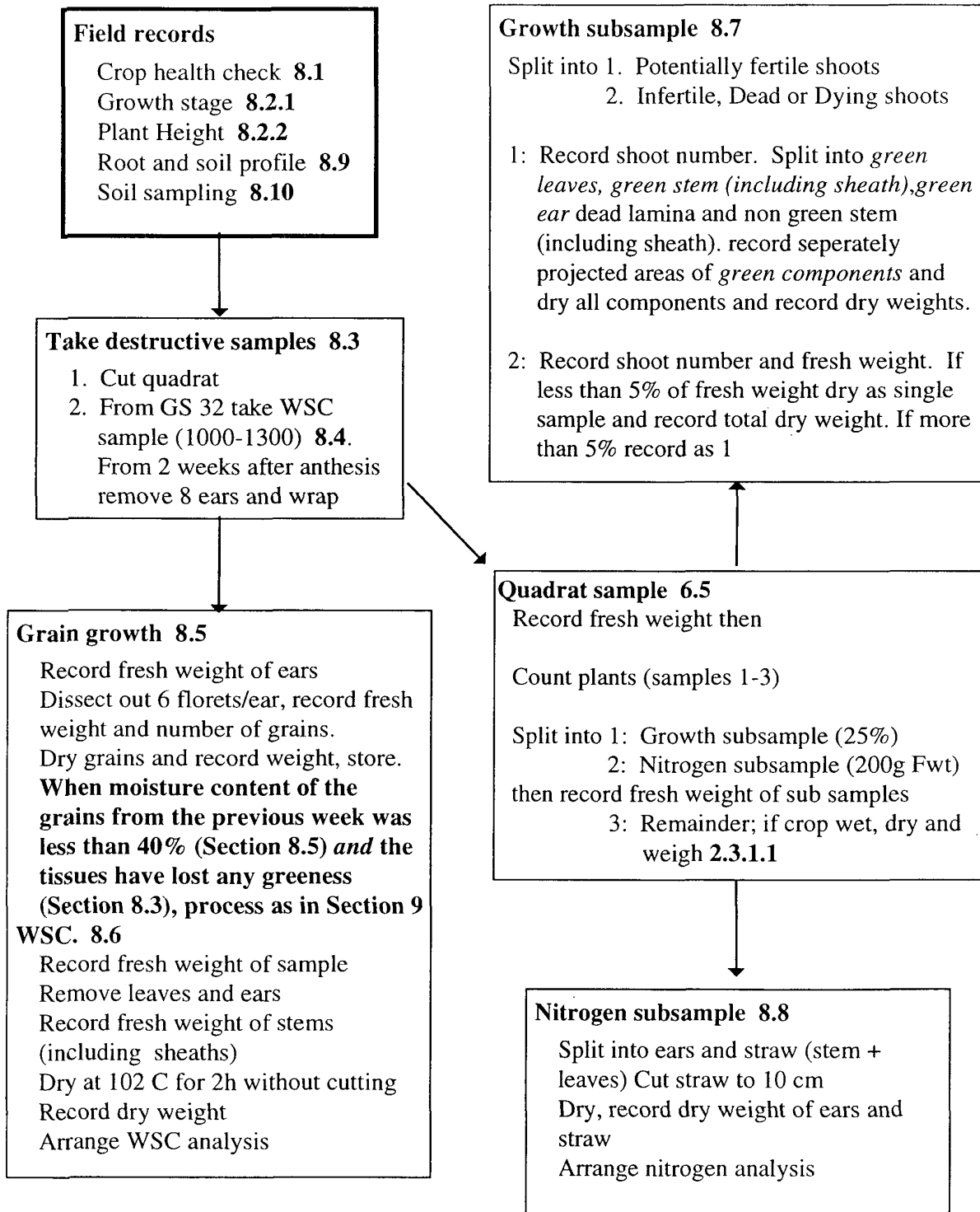
If the fresh weight of SS1(b) is less than 5% of the total fresh weight of SS1 then dry for at least 16 hours at 80°C and record the total dry weight of SS1 (b). Otherwise separate into the same fractions and treat as SS1(b)

### **7.7. Samples for N determination (SS2)**

Take a further sub-sample of plants from the remainder (about 15% of the total or approximately 400 g Fwt whichever is the larger) for N determination. Divide the sample into ears and straw (straw = stems + leaves). Remember that analytical laboratories may require the samples to be chopped into 10 cm lengths to speed processing. Record fresh weight of straw and ears and then dry at 80°C for at least 16 hours or until the samples reach constant weight. When the samples have reached constant weight (see Section 2.3.2) record dry weight, then place into separate sealed bags prior to submission for chemical analysis.

## 8. PERIOD 4 - ANTHESIS UNTIL 30% GRAIN MOISTURE

Flow chart of measurements and records (weekly after sample 5. see Section 3.3 for details)



## **8.1. Crop health check**

The health of the crop should be checked and recorded (on the 'Crop Health Record') weekly by examination in the field and by examination of a sample of ten shoots in the laboratory. A record should be made under each heading, even if there is no problem. Where a problem is identified this should be quantified by scoring, and the values of the scoring system should be defined. Any of the last 4 or 5 leaves, or the stem or ear, affected by disease should be identified, and appropriate keys (see Section 1.4.8.3) used to assess the percentage of the area affected. These assessments may be done in the lab on plants removed for quadrat sampling. Any pest attacks, (e.g. grazing by rabbits or aphid infestation), mechanical damage (e.g. due to hail or trampling), lodging (scored using Section 10.4/2) or symptoms of nutrient deficiency should also be recorded.

Records should be made of any remedial treatments carried out. If there are any major crop health problems at this stage the suitability of subsequent data as reference information should be reconsidered, but further monitoring should seldom be aborted.

## **8.2. Stage of development and plant height**

### **8.2.1. Stage of development**

Record growth stage (GS) on the plants marked for growth stage recording on the growth stage data sheet (Section 10.4/4), using the growth stage key of Tottman (1987, see Section 1.4.8.1). The overall aim is to obtain at each date an accurate record of the stage which is exceeded by just half the plants (or shoots after GS 39) of the crop. It is important to remember that arithmetic averaging is inappropriate for growth stages. Several growth stages can often be recorded on a single plant for example those referring to leaf number (GS 10-19), tiller production (GS 20-29) and stem elongation (GS 30-39). Generally it is only the greatest numerical stage that will be required for summary purposes, but the other stages can be deduced from the separate records taken of leaf growth, shoot number, and node number.

### **8.2.2. Plant height**

Record plant height by measuring from ground level to the collar of the ear of 8 shoots chosen at random (it is not advisable to use marked plants for measurement of plant height as handling may affect the plants). and enter on the 'Plant Height Record' (Section 10.4/9)

## **8.3. Sample removal**

Using sharp scissors or secateurs, cut off all the shoots within the quadrat at the soil surface. Collect all above ground plant material, and place as quickly as possible

into a plastic bag so that the lower portion of the plant (most contaminated with soil) is at the bottom of the bag. This will reduce any time spent washing the plants. After all the material has been collected, seal the bag to prevent the leaves drying out before their green areas are measured. If growth analysis is delayed, store the samples in a cold room at about 4-6°C. If the samples are contaminated with soil, wash AFTER storage in the cold room, as washing hastens deterioration of the leaves. Aim to do all growth analysis within two days of sampling.

Additionally take a random sample of 8 fertile shoots from just outside the quadrat area for determination of stem soluble carbohydrates (Section 8.4) and grain growth (Section 8.5). These samples should be taken after all other field measurements have been made and if possible between 10.00 hours and 13.00 hours (to avoid differences due to time of day). Then either :

- i. When grains are too small for removal by dissection (for about the 2 weeks after anthesis), place the shoots in a plastic bag, seal and place in a chilled cool box. Return to the lab as quickly as possible and treat as described in Section 8.4.

or

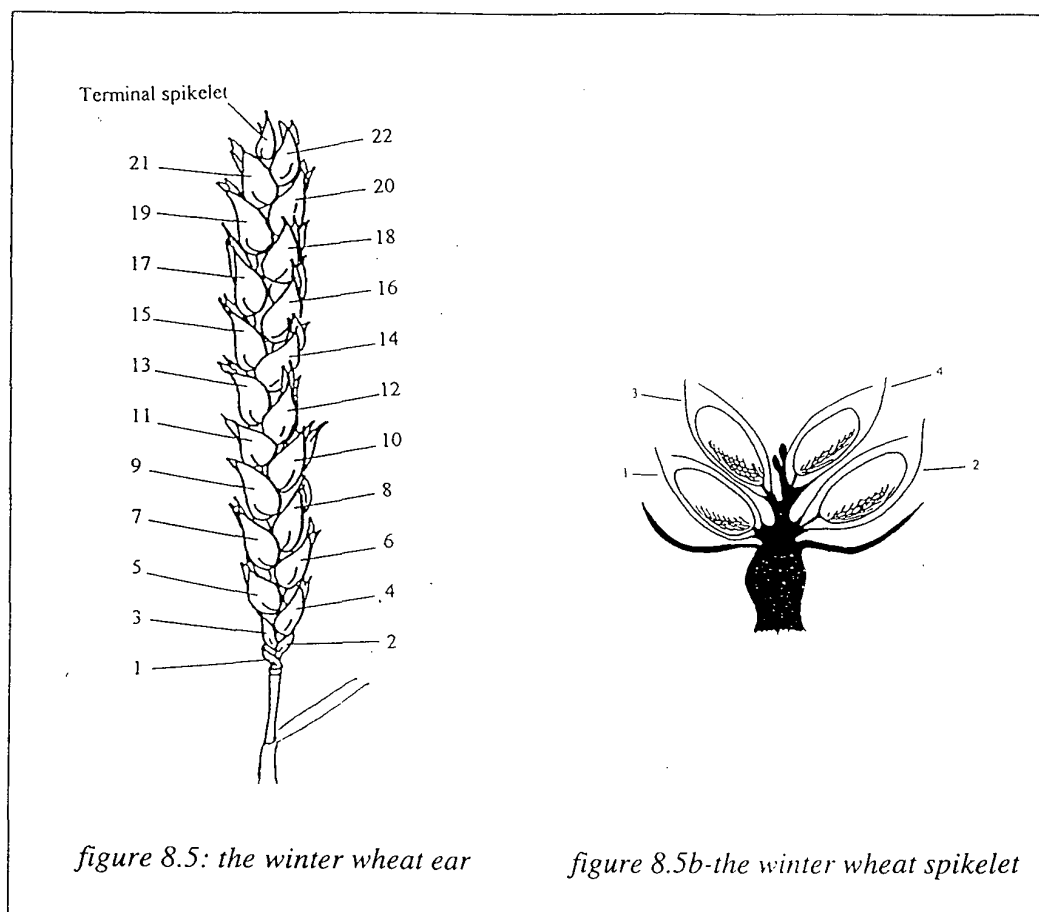
- ii. When grains are large enough to be removed from the ear by dissection (after about 2 weeks after anthesis), cut off the ears just below the collar. Tightly wrap the ears in clingfilm, or in a plastic bag, and seal these and the rest of the 8 shoots in a separate plastic bag. Place both ears and stems in a chilled cool-box. The 8 ears and stems must be processed immediately on returning to the lab. as described in Section 8.5.

#### **8.4. Water soluble stem carbohydrates**

Record the fresh weight of the 8 shoots. Remove the laminae at the ligule (these can be discarded) and reweigh the 8 stems. Avoid cutting the stems which could allow sap loss; bend them if necessary. Immediately place the stems in a single layer in a gauze-based tray and "flash-dry" for 2 hours by placing in a forced-draught oven set at 102°C. The oven must be free of other material and be already at 102°C when the samples are added, to ensure that respiration is stopped as quickly as possible. After exactly 2 hours, record the dry weight. Store dried samples in sealed labelled polythene bags for water soluble carbohydrate analysis.

## 8.5. Grain growth

Record the fresh weight of the 8 ears (collected as described above) and identify the central spikelet as in the figures below. Count upwards from the ear collar, half the total number of spikelets and round up to the next whole number. Identify this as the central spikelet ( i.e. spikelet 12 in the example shown in figure 8.5).



Remove the grains from florets 1 & 2 of the central spikelet, and from the two spikelets below it, giving a total of 6 excised grains per ear. The maximum will be 48 excised grains from the 8 ears but, if infertile florets or spikelets were present, do not collect additional grains to compensate. Immediately record the number of grains and their fresh weight (to 0.01g), and record the fresh weight of the ears remaining after removal of grains. Dry the grains and ears at 102°C for at least 40 hours and record their dry weight. Store for analysis by the laboratory.

When the moisture content of these grains becomes less than 40% (and all tissues have lost any greenness) the following weeks' samples should be processed as described under Section 9.

## **8.6. Division of quadrat sample**

Remove all plant material from the bag. Generally the samples should be almost free of soil. If the shoots are contaminated with soil, shake them so that the soil falls away or gently wash under a running tap. Using paper towels or by shaking, remove all surface water. Record the total fresh weight of the quadrat sample.

Spread out the plant material and sub-sample (see Section 2.3.1) about 25% by Fwt (SS1) for growth analysis. Take a further sub-sample (SS2) of 15% Fwt (or approximately 200 g. Fwt., whichever is larger) for N% determination.

As quickly as possible, record the weight of the sub-samples. Place the samples for green area determination (SS1) in a plastic bag to prevent dehydration, and store in a cold room at 4-6°C. If the crop was wet, dry the remainder at 80°C for 16 hours and weigh to allow a sub sample size check.

## **8.7. Measurements on 25% sub-sample**

Divide the sub-sample into two categories of shoot (SS1(a) and SS1(b)) and count the number of shoots in each. "Shoots" includes both tillers and mainstems. Categories are as follows:

(a) Fertile shoots (SS1(a)): A fertile shoot has an ear or is "booting" and has some green leaf.

(b) Infertile shoot group (SS1(b)): A shoot is infertile when it has no grain and there is no evidence of "booting".

For the fertile shoot group (SS1(a)), separate the material into :

- i. green lamina
- ii. green stem plus green sheath.
- iii. ears with any green present
- iv. ears without any green present
- v. non-green stem plus sheath
- vi. dead lamina

'Leaf' means lamina only; leaves should be separated at the ligule, the junction with the sheath. Cut individual leaves into green and dead parts; yellowing parts of the leaves are classed as 'dead'. Leaves are also classed as dead if they are yellow across the whole of the base of the lamina.

The leaf sheath is left on the stem, and is measured as stem. Stems should be cut up into green and non-green material and the green stems passed through the leaf area meter. Do not cut individual stems more than twice. (NOTE that passing through whole stems and estimating percentage green area is subject to major errors.)

For the ears, having recorded whole projected area, record percentage of non-green ear as assessed by visual estimation on a sample of 10-20 ears. Dry for at least 16 hours at 80°C and record dry weight of all fractions.



Then record projected green areas for the lamina, green stem, and the ear. Dry for at least 16 hours at 80°C and record dry weight of all fractions.

For the infertile shoot group (SS1(b)) :

If the fresh weight of SS1(b) is less than 5% of the total fresh weight of SS1 then dry for at least 16 hours. at 80°C and record the total dry weight of SS1(b). Otherwise separate into the same fractions and treat as SS1(a)

## **8.8. Samples for N determination.**

Divide the sample into ears and straw (straw = stems + leaves) for N determination. Remember that analytical laboratories may require the straw samples to be chopped into 10 cm lengths to speed processing. Record fresh weight of straw and ears and then dry at 80°C for at least 16 hours or until the samples reach constant weight. When the samples have reached constant weight (see Section 2.3.2) record dry weight, then place into separate sealed bags prior to submission for chemical analysis (Section 2.3.3.).

## **8.9. Soil sampling at anthesis**

On the weekly sampling date nearest to the date of anthesis, take soil samples for determination of mineral nitrogen in the profile to 90 cm depth as described in Section 5.8.2 and record the results on the 'Soil Analysis' sheet (Section 10.4/13) In order to assist with location of the inspection pit for the determination of rooting and soil structure (see Section 8.10 below), carefully observe the cores for any variation. Freeze the samples and submit to the analytical laboratory.

## **8.10. Assessment of rooting depth, soil type and root abundance at anthesis**

### **8.10.1. Digging of inspection pit**

Assessment of rooting and soil type is should be carried out at anthesis using an inspection pit. Dig the pit in a discarded area at the edge of the trial, at least 0.5 m from the edge of a plot and approximately half way along the length of the trial, unless some variability in soil profile is suspected (this should have been assessed by sampling with a soil auger before selection of the site). If the profile is at all variable across the site, pits should be dug at the extremities and their positions recorded accurately.

The pit should be rectangular in plan (1 m long by 0.75 m wide) and 1 m deep with one vertical face of 1 × 1 m oriented towards the sun for maximum light. Mechanical diggers can be used but it probably will be necessary to remove smearing of the face before root inspection (see below). When digging the pit, collect the topsoil and subsoil on separate polythene sheets to allow restoration of the profile after inspection.

It is important to record the profile and roots as soon as possible after digging the pit. When the sun is shining on the face of the pit, photograph the profile for future reference. Indicate the position and orientation of the pit on the back of the photograph.

### 8.10.2. Root abundance and depth

Root abundance is assessed by classing the root numbers emerging from the face of the soil pit in a series of 225 cm<sup>2</sup> (15 × 15 cm) squares arranged in a strip vertically down the profile. Divide the face into 7 adjacent squares. In each square, carefully pluck back the profile face with a trowel or knife to remove any smearing so that the emerging roots can be observed. Then estimate (according to class; see tables below) and record the abundance of very fine, fine, medium and coarse roots in each section, using the table in Section 10.4/11.

Note that dead roots (if these can be distinguished) are classed as plant remains and not included in measures of root abundance. Living plant material other than roots, such as bulbs, rhizomes, stolons etc., should be described in general terms, giving details of their average size, distribution and quantity.

This method of estimating the abundance of roots is difficult, especially at the deepest layers, but it is the quickest method available and the information obtained is vital for full interpretation of results from the site. Thus ample time should be allowed to undertake this operation.

Root size class	Root Diameter
Very Fine	<1 mm
Fine	1-2 mm
Medium	2-5 mm
Coarse	> 5 mm

Frequency	Root abundance per 225 cm <sup>2</sup>		Class
	very fine & fine	medium & coarse	
None	0	0	0
Few	1-25	1-2	1
Common	25-55	2-11	2
Many	55-450	>11	3
Abundant	>450	NA	5

### **8.10.3. Soil description and texture**

The aim of the soil description is to provide a dimensioned sketch of the soil profile and to identify any possible horizons of uniform appearance as far as possible. It is important to note any possible reasons for reduced root activity e.g. compacted layers and to record the structural units into which the soil is fragmented (such as crumby, blocky, angular, platy, prismatic). For each successive horizon from the surface, describe its depth, structure (shape and size of peds, and sizes of voids and channels; see the Soil Survey Field Handbook Section 1.4.8.2), colour including mottling (especially any changes between horizons), and stone content. For each horizon, take about a dessert spoonful of soil and use the key in Appendix 2 to determine the soil texture.

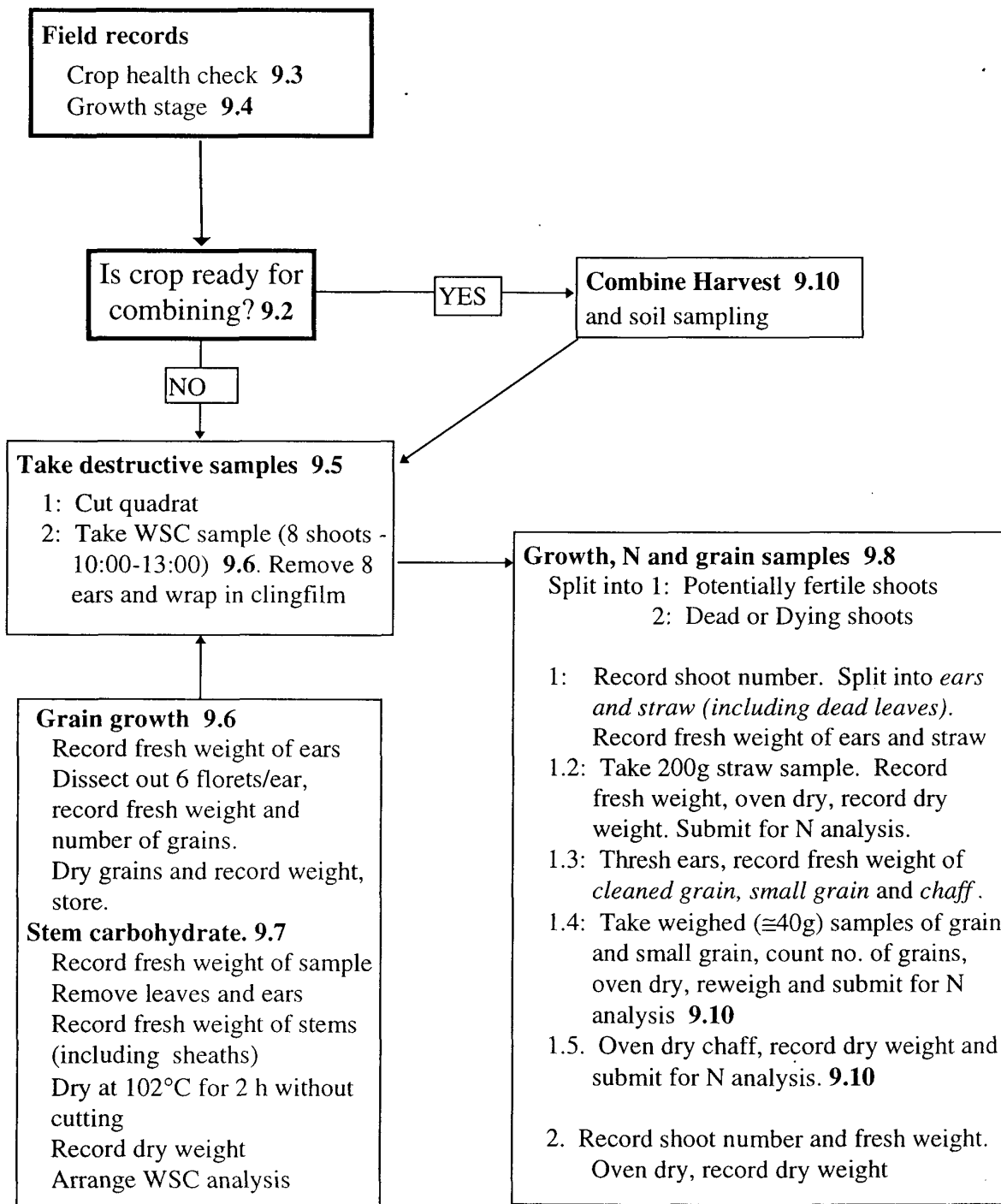
Further instructions and assistance can be found in the Soil Survey Field Handbook (see Section 1.4.8.2).

### **8.10.4. Renovation**

After completing the soil profile description, and taking any necessary samples, the pit should be restored as nearly as possible to its original state. The subsoil should be replaced first, a few spades at a time, stamping down frequently to restore a similar soil density. The topsoil should then be replaced using the same method. In heavy soils, not all of the removed soil can be replaced without leaving a raised surface. This is normal; the surface will eventually subside.

## 9. PERIOD 5 - ABOUT 30 % GRAIN MOISTURE UNTIL 2 WEEKS AFTER COMBINE HARVEST

Flow chart of measurements and records: see Sections 9.1 and 9.2 for scheduling



## **9.1. Introduction to harvesting and sampling in period 5**

Careful preparation, and therefore anticipation, will be needed to co-ordinate sampling and harvesting operations as the crop ripens. Quadrat sampling continues every week until two weeks after the combine harvesting so that the effects of weather on grain yield during this period can be established. Grain yield and its components are measured at each quadrat sampling, and one of these samples will be timed for the day of combine harvesting, so that the combine and quadrat yield can be compared. The usual weekly sampling interval is maintained but may be delayed if combining is anticipated within three days.

## **9.2. Timing of combine harvesting**

Combine harvesting should take place when the grain is mature and can easily be threshed, which can be assessed by rubbing ears out by hand. The aim should be to harvest at a moisture content of less than 16%, but in practice higher moisture contents may be used, for example later in the season, if the grain is rewetted by rain, or if in less favourable areas a higher moisture content is the best that is likely to be achieved under the prevailing conditions. The actual date will also be influenced by practical considerations such as availability of equipment and labour.

## **9.3. Crop health check**

The health of the crop should be checked and recorded (on the 'Crop Health Record') weekly by examination in the field and by examination of a sample of ten shoots in the laboratory. A record should be made under each heading, even if there is no problem. Where a problem is identified this should be quantified by scoring, and the values of the scoring system should be defined. Any of the last 4 or 5 leaves, or the stem or ear, affected by disease should be identified, and appropriate keys (see Section 1.4.8.3) used to assess the percentage of the area affected. These assessments may be done in the lab on plants removed for quadrat sampling. Any pest attacks, (e.g. grazing by rabbits or aphid infestation), mechanical damage (e.g. due to hail or trampling), lodging (scored using Section 10.4/2) or symptoms of nutrient deficiency should also be recorded.

Records should be made of any remedial treatments carried out. If there are any major crop health problems at this stage the suitability of subsequent data as reference information should be reconsidered, but further monitoring should seldom be aborted.

## **9.4. Stage of development**

At this stage of the crop it is only necessary to record the grain filling and ripening development stages. Record growth stage (GS) on the plants marked for growth stage recording on the growth stage data sheet (Section 10.4/4), using the growth stage key of Tottman (1987, see Section 1.4.8.1). The overall aim is to obtain at each

date an accurate record of the stage which is exceeded by just half the shoots. It is important to remember that arithmetic averaging is inappropriate for growth stages.

### **9.5. Quadrat sample removal**

Using sharp scissors or secateurs, cut off all the shoots within the quadrat at the soil surface. Collect all above ground plant material, and place as quickly as possible into a paper sack so that the lower portion of the plant (most contaminated with soil) is at the bottom of the bag. This will reduce any time spent cleaning the plants. Transport to the lab. and, if growth analysis is delayed, store the samples in a cold room at about 4-6°C. If the samples are contaminated with soil, shake or wash AFTER storage in the cold room, as washing hastens deterioration. Aim to do all growth analysis within two days of sampling.

Additionally take a random sample of 8 fertile shoots from just outside the quadrat area for determination of stem soluble carbohydrates and grain growth. These samples should be taken after all other field measurements have been made and if possible between 10.00 hours and 13.00 hours (to avoid differences due to time of day). Then cut off the ears just below the collar. Tightly wrap the ears in clingfilm, or in a plastic bag, and seal these and the rest of the 8 shoots in a separate plastic bag. Place both ears and stems in a chilled cool-box. The 8 ears and stems must be processed immediately on returning to the lab. as described in Sections 8.5 & 8.4 - .Water-soluble stem carbohydrates

### **9.6. Grain growth**

First weigh the 8 ears and stems (fresh) then process immediately as described in Sections 8.4 (Water soluble carbohydrate) & 8.5(Grain Growth ). Record the fresh weight of the 8 ears (collected as described above) and identify the central spikelet (see Figure 8.5). Remove the grains from florets 1 & 2 of the central spikelet, and from the two spikelets below it, giving a total of 6 excised grains per ear. The maximum will be 48 excised grains from the 8 ears but, if infertile florets or spikelets were present, do not collect additional grains to compensate. Immediately record the number of grains and their fresh weight (to 0.01g), and record the fresh weight of the ears remaining after removal of grains. Dry the grains and ears at 102°C for at least 40 hours and record their dry weight.

### **9.7. Water soluble stem carbohydrate**

Record the fresh weight of the 8 shoots. Remove the laminae at the ligule (these can be discarded) and reweigh the 8 stems. Avoid cutting the stems which could allow sap loss; bend them if necessary. Immediately place the stems in a single layer in a gauze-based tray and "flash-dry" by placing in a forced-draught oven set at 102°C for 2 hours. The oven must be free of other material and be already at 102°C when the samples are added, to ensure that respiration is stopped as quickly as possible. After exactly 2 hours, record the dry weight. Store dried samples in sealed labelled polythene bags for water soluble carbohydrate analysis.

## **9.8. Growth analysis, N and grain sample collection**

Remove all plant material from the bag. Generally the samples should be almost free of soil. If the shoots are contaminated with soil, shake them so that the soil falls away or gently wash under a running tap. Using paper towels or by shaking, remove all surface water. Record the total fresh weight of the quadrat sample.

Divide the whole sample into two categories of shoot (SS1(a) and SS1(b)) and count the number of shoots in each. "Shoots" includes both tillers and mainstems. Categories are as follows:

- (a) Fertile shoots (SS1(a)): A fertile shoot has an ear with at least one grain.
- (b) Infertile shoots (SS1(b)): A shoot is infertile when it has no grain.

### **9.8.1. For the fertile shoot group (a) :**

Count the number of shoots, cut off all their ears, count them, and as soon as possible, record separately the fresh weight of ears and straw (including dead leaves). Take a representative sub-sample of at least 200g Fwt of straw and record its fresh weight; chop into < 10 cm. lengths if required by the analytical laboratories, dry to constant weight (at least 16 hours at 80°C) and record the dry weight then seal in a polythene bag for later N analysis (Section 9.9). If the crop was wet also dry the remainder of the straw and record its dry weight ( see Section 2.3.1)

Immediately thresh and winnow the remaining ears (use a laboratory thresher which has been checked to avoid loss of grain) and collect all grain and as much chaff as possible. Sieve the grain over a 2 mm sieve, and add any remaining pieces of chaff or non-grain which passes through the sieve to the chaff fraction. Record the fresh weight of all 'cleaned grain', 'small grain' and the chaff separately. Then remove approximately 40 g of this 'cleaned grain' for mean grain weight determination (N.B. mean grain weight in mg = thousand grain weight in g); take the exact fresh weight, count and record the number of all these grain. Also count and record the number of all 'small grains'. Dry the 40g sample of 'cleaned grain', the 'small grain' (both for at least 40 hours at 102°C), and the chaff (at least 16 hours at 80°C) to constant weight, and record the dry weights. Keep sealed in polythene bags for later nitrogen analysis. (Section 9.9).

### **9.8.2. For the infertile shoot group (b) :**

Count the number of shoots and as soon as possible, record their total fresh weight. Dry to constant weight (at least 16 hours at 80°C) and record the dry weight.

## **9.9. Preparation for N determination and grain quality analysis**

Place the oven dried 'cleaned grain', straw and chaff into separate sealed bags prior to submission for N determination (Section 2.3.3.).

Save the remaining undried 'cleaned grain' for measurement of Hagberg Falling Number (HFN), and specific weight after air-drying the grain; either place in a forced-draught oven *without heating* or, if already close to 20% moisture content, spread the grain out in trays and leave to dry in a warm laboratory, turning each day. The grain should be dried gently and not heated, in order to avoid denaturing the proteins. Save the air-dried grain separately in clearly labelled paper or linen bags in a dry and vermin proof environment until analysis.

## **9.10. The day of combine harvesting**

Careful preparation, and therefore anticipation, will be needed for the day of harvest. In addition to harvesting with a combine harvester, a quadrat sample should be taken from the sampling plots on the day, or at least within one day of harvest (9.5) and the soil should be sampled for soil mineral N (Section 5.8.1). Do not forget to leave two quadrats in the sampling plots for 1 and 2 weeks after combine harvesting.

At combine harvesting, care is needed to ensure that accurate measures are taken of both area and weight, and that samples of grain are taken appropriately for moisture and grain quality determinations (Sections 9.6, 9.8 and 9.9).

The soil should be sampled for soil mineral nitrogen analysis as near as possible to the date of combine harvesting (Section 5.8.2).

### **9.10.1. Preparation**

Before commencing combine harvesting, ensure that everyone involved knows the particular requirements for accurate measurement of combine yield. Several checks and measurements are needed to identify different sources of possible bias between growth analysis and combine harvesting.

First, on a spare area of crop, set the combine to produce a relatively 'clean' sample of grain but avoiding undue loss of small grain. Check under the straw swath for grain losses. Check the zero and the calibration of the combine's weighing system using a known weight of appropriate size, e.g. 10 or 20 kg.

### **9.10.2. Harvesting and sampling**

Then, trim 0.75 m from the ends of the plots and cut out 0.75 m either side of the tramlines so that the crop edges do not form any part of the harvested area. This will leave two or three lengths of crop remaining in each plot. Using a tape measure, record each of these lengths on each plot accurately on the 'Combine Harvest Data' sheet

Then take one combine cut through the centre of each area designated for combine harvesting, leaving uncut areas either side to avoid edge effects. Whilst the combine is harvesting, take a 250 g sample from the flow of grain as it is being delivered to the weighing hopper and seal into a labelled polythene bag (for determination of grain moisture content). An additional sample of about 3 kg of grain may be taken in a labelled linen bag if required (for grain quality measurements including



breadmaking characteristics). Leave sample bags in the weighing hopper and record the weight of fresh grain from each plot separately. If particularly heavy bags or bottles are used for sampling they should be tared off or allowed for when recording the weight of the crop.

After combining, but before the guard areas are harvested, record the average width of cut of the combine harvester from at least three points along each plot. (Note that, since there is often play on the dividers, the distance between them should not be used to represent the average width of cut.)

Then assess the harvested plots for shed seed. It is important that differences between yields based on quadrat samples and combine-harvesting can be explained; seed losses at combining are an important possible source of difference. At three positions along the combine run but away from the beginning, end, or any halts in the combine's run (where there is no normal straw swath) remove the straw, shaking it so that grains or unthreshed ears fall to the ground, and place two canes across the plot 50 cm apart. In each position, count and record the number of seeds (rubbing out any unthreshed ears) in the whole plot width.

### **9.10.3. Sample processing**

Determine grain moisture content as soon as possible after harvest. Do not use a moisture meter; the sample for grain moisture should be treated in exactly the same way as samples from quadrat samples i.e. weighed, dried in a forced draught oven at 102°C for at least 40 hours and reweighed. Then take approximately 40 g of this grain (for mean grain weight determination; N.B. mean grain weight in mg = thousand grain weight in g), count all these grain, and record their number and exact dry weight.

Any samples taken for grain quality should be dried at room temperature to less than 15% moisture content and submitted for analysis (Section 9.9).

### **9.10.4. Soil sampling**

On a weekly sampling date near to the date of harvest, take soil samples for determination of mineral nitrogen in the profile to 90 cm depth as described in Section 5.8.2 and record on the 'Soil Analysis' sheet (Section 10.4/13) Freeze the samples and submit to the analytical laboratory.

## **10. DATA COLLECTION AND MANIPULATION**

### **10.1. Introduction**

The task of data organisation when running a reference crop is not trivial and the necessary calculations are sometimes more complex than first appears. Thus in this section some of the calculations are described and examples of appropriate data recording sheets are provided

### **10.2. Calculations of leaf development, appearance dates and phyllocron**

On each measurement occasion before full expansion of the flag leaf the total number of expanded leaves and the lengths of expanding and new fully expanded leaves (since the last date of measurement) are recorded. These data together with a knowledge of the thermal time above 0°C accumulated from the first measurement (see Appendix 3) allows accurate estimates of the phyllocron (the interval, expressed as thermal time), between the appearance of successive leaves and the date of full expansion of any leaf of interest. The method is as follows

1. Calculate accumulated thermal time for each measurement date.
2. Calculate the total number of fully expanded plus expanding leaves by adding the number of fully expanded leaves to the proportion of the length of the expanding leaf present (calculated retrospectively as the length of the expanding leaf divided by its length when fully expanded).
3. Fit a straight line to the relationship between accumulated thermal time (as  $x$  variable) and total leaf number, omitting records once the flag leaf is fully expanded.
4. The slope of the straight line estimates the phyllocron and the accumulated thermal time of appearance of any particular leaf can be estimated from the line, and converted back to the date by which the required thermal time was accumulated.

### **10.3. Calculations of growth and combine harvest parameters**

The collection of weekly growth data described here allows the calculation of many parameters of interest. However, because of the large amount of data, an organised approach to routine data processing is required and spread sheet software is recommended. To standardise calculations of the most common parameters, details of these calculations are given below in typical spread sheet format for growth analyses and harvest data. If, as is usual, the measurements are replicated on a number of plots, then parallel calculations are required for each plot measured, and appropriate summary statistics (e.g. means and standard errors) will need to be calculated.

## *How to Run a Reference Crop*

In the examples below, raw data (shaded background) is differentiated from calculated items (clear background). The first column contains the data row references. The second column (A) provides the name of the raw data item entered, and the name of the calculated value; it also provides the units in which data must be entered, and the units for the calculated values. The third column (B) provides either an 'example' value for the raw data item or the equation used to provide a calculated value. The fourth column (C) provides the results of the calculations, as well as repeating the data entered. Some of the calculations have been included to provide a check that sampling operations have been conducted properly. (N.B. The example shows all calculations that may be made through a season and includes data that could not all be collected at the same time.)

The equations are shown in typical spread sheet format. For example the equation in column B row 28 [=B27/B26\*100], gives the number of shoots in Sub Sample 1 expressed as a percentage of total number of shoots in the quadrat, by dividing the number of shoots in Sub Sample 1 (found in column B row 27) by the total number of shoots in the quadrat (found in column B row 26) and multiplying by 100 (to change values to percentages). Thus all descriptors with no prefix are constants and descriptors prefixed with capital letters refer to other measured or calculated values.

In the growth analysis data sheet Cell B75 is a special case. If the sub sample is not divided enter the total infertile, dead and dying DM in grams to replace the formula. If the sub sample is divided the spread sheet the formula will calculate the total dry weight.

How to Run a Reference Crop

Growth analysis calculations

	A	B	C
1	Growth analysis data and calculations	Data values entered or method of calculation	
2	Site		
3	Date		
4	Harvest no.		
5	Recorder(s)		
6	Replicate number		
7	Quadrat size (m <sup>2</sup> )	0.72	0.72
8	INITIAL SAMPLES		
9			
10	DATES 4 TO 6: (from field counts)		
11	No. shoots in 3 x 0.33 m rows	46	46
12	Row width (cm)	12.5	12.5
13	Shoots/m <sup>2</sup>	=B11/(B12*0.99/100)	371.7
14			
15			
16	FRESH WEIGHTS (g)		
17	Sub sample 1 (SS1) for growth analysis	326.4	326.4
18	Sub sample 2 (SS2) for nitrogen analysis	219.9	219.9
19			
20	Total for whole quadrat	1283.7	1283.7
21			
22			
23	SS1 FOR GROWTH ANALYSIS		
24			
25	Samples 1 to 3:		
26	Number of plants in quadrat sample	169	169
27	Number of plants in subsample	42	42
28	Number of shoots in SS1 as % of total	=B27/B26*100	24.9
29	Number of fertile tillers/plant	=B40*B20/B17/B26	2
30			
31	Samples 4 to 6 (lab counts):		
32	Total number of shoots in quadrat sample	338	338
33	Number of shoots in subsample SS1	=B40	86
34	Number of shoots in SS1 as % of total	=B33/B32*100	25.44
35	ratio field shoots/lab shoots	=B13/B125	0.99
36			
37	SS1 as % of total by fresh weight	=(B17/B20)*100	25.42
38			
39	FERTILE SHOOTS IN SS1		
40	No. of fertile shoots in sub sample	86	86
41	Projected areas (cm <sup>2</sup> )		
42	Total green lamina area (cm <sup>2</sup> )	2948.28	2948.28
43	Green true stem + sheath (cm <sup>2</sup> )	1616.66	1616.66
44	Whole ear area (cm <sup>2</sup> )	654.41	654.41
45	% green ear (visual estimate)	100	100
46	Green ear area (cm <sup>2</sup> )	=B44*B45/100	654.41
47			
48	Dry weights (Fertile shoots)		
49	Total green lamina (g)	17.5	17.5
50	Green true stem + sheath (g)	98.55	98.55
51	Non-green true stem + sheath (g)	42.93	42.93
52	Whole ear (g)	91.83	91.83
53	Dead lamina (g)	17.16	17.16
54			
55	INFERTILE DEAD & DYING SHOOTS IN SS1		
56	(Only measure projected areas if dead/dying shoots form >5% by FwI of subsample)		
57	No. of dead/dying shoots	12	12
58	Dead + dying shoots FwI (g)	2.4	2.4
59	% by FwI of (dead + dying) / total	=B59/B17*100	0.735
60	Total green lamina area (cm <sup>2</sup> )		
61	Green true stem & sheath (cm <sup>2</sup> )		
62	Whole ear area (cm <sup>2</sup> )		
63	% green ear (visual estimate)		
64	Green ear area (cm <sup>2</sup> )	= B63 * B64 / 100	
65			
66			
67	Dry Weights (Infertile, dead & dying shoots)		
68			
69	Total green lamina (g)		
70	Green true stem + sheath (g)		
71	Non-green true stem + sheath (g)		
72	Whole ear (g)		
73	Infertile, dead & dying lamina (g)		
74			
75	Total infertile, dead & dying DM (g)	2.15 or = sum(B69:73)	2.15
76			

## How to Run a Reference Crop

	A	B	C
77	SS2 FOR N ANALYSIS		
78	(include live + dead material)		
79	Straw Fwt (=whole plant before ear em.)	=B18-B80	101.8
80	Ear Fwt	118.1	118.1
81	Straw or whole plant DM (g)	91.5	91.5
82	N (%) of straw or whole plant DM	0.8	0.8
83	Ear DM (g)	106.68	106.68
84	N (%) of ears	2.02	2.02
85			
86	SS3 FOR INDIVIDUAL GRAINS		
87	Number of ears (should be 8)	8	8
88	Fwt. ears (g)	17.313	17.313
89	DM ears (g) [ A + B below]	=B92+B93	15.88
90	Total number grains	46	46
91	Fwt. grains (g)	2.141	2.141
92	A DM grains (g)	1.91	1.91
93	B DM ears - grains (g)	13.97	13.97
94	SS4 FOR GRAIN QUALITY		
95	NOTE: Air dry grain to <20% MC		
96	No. of ears (should be >250)	250	250
97	Fwt of ears (g)	516.4	516.4
98	Total Fwt of threshed grain (g)	388	388
99	Fwt of SS for TGW (c.40g)	40	40
100	No of seed in TGW SS	994	994
101	DM of TGW SS (g)	37.06	37.06
102	Grain MC %	=(B99-B101)/B99*100	7.35
103	TGW (g DM basis)	=(1000/B100)*B101	37.28
104	Grain HFN		
105	Grain (N%)		
106	Fwt of chaff SS for drying (g) (whole sample)	119.8	119.8
107	DM of chaff SS (g)	110.44	110.44
108	Chaff MC%	=(B106-B107)/B106*100	7.81
109			
110			
111	SAMPLE FOR W.SOL CHO's		
112	No of shoots (6-8 fertile shoots)	8	8
113	Shoot Fwt (stems&leaves&ears) (g)	31.1	31.1
114	Stems Fwt [lamina and ears removed] (g)	10.6	10.6
115	Stems DM [lamina and ears removed] (g)	9.7	9.7
116	W. Soluble CHO (% of DM of stems only)	3.33	3.33
117			
118			
119	CROP GROWTH DATA SUMMARY		
120	Total crop GAI	=B124+B123	2.85
121	Total crop DM (t/ha)	=(B83+B81)*(B20/B18)/B7/100	16.07
122	Dead and dying shoots DM (t/ha)	=B75*(B20/B17)/B7/100	0.117
123	Fertile crop GAI	=(B42+B43+B46)*(B20/B17)/B7/10000	2.851
124	Dying crop GAI	=(B61+B62+B65)*(B20/B17)/B7/10000	0
125	Potentially fertile shoots/m <sup>2</sup>	=B40*(B20/B17)/B7	469
126	Dead + dying shoots/m <sup>2</sup>	=B58*(B20/B17)/B7	65.5
127	GA / fertile shoot (cm <sup>2</sup> )	=B123/B125*10000	60.7
128	GA / dying shoot (cm <sup>2</sup> )	=B124/(B68/B7)*10000	0
129	Total crop N (kg/ha)	=((B82/100*B81)+(B84/100*B83))*(B20/B18)/B7*10	234
130	N/GA ratio excluding ears (kg/ha)	=(B82/100*B81)*(B20/B18)/B7*10/B123	20.82
131	N/GA ratio including ears (kg/ha)	=((B82/100*B81)+(B84/100*B83))*(B20/B18)/B7*10/B123	82.1
132	Total crop green lamina area index	=(B42+B61)*(B20/B17)/B7/10000	1.61
133			
134		Partitioning summary	
135	Stems (t/ha) fertile shoots	=(B50+B51)*B20/B17/B7/100	7.72
136	Ears (t/ha)	=B52*B20/B17/B7/100	5.01
137	Lamina (t/ha)	=(B49+B53)*B20/B17/B7/100	1.89
138	D+Dshoots (t/ha)	=B75*B20/B17/B7/100	0.117
139	Total (t/ha)	=SUM(B135:B138)	14.75
140	Total from SS2 (t/ha)	=B121	16.06
141	TDM check SS1/SS2%	=B139/B140*100	91.8
142	WSC (t/ha)	=B116/100*B135	0.257
143	DM of ears (t/ha)	=B20/B18*B83/B7/100	8.65
144	DM of straw (t/ha)	=B20/B18*B81/B7/100	7.42
145	N uptake of ears (kg/ha)	=B143*10*B84	174.7
146	Nuptake of straw (kg/ha)	=B144*10*B82	59.34
147	Nuptake check	=(B145+B146)/B129	1
148	DM check	=(B143+B144)/B121	1
149	Sink size		
150	Ears/m <sup>2</sup>	=B40*B20/B17/B7	469.8
151	Ear Dwt (g/ear)	=B136*100/B150	1.067
152	Ear Fwt (g/ear)	=B80/B83*B151	1.182

Combine harvest data analysis spreadsheet

	A	B	C
1	<b>Combine harvest data and calculations</b>		
2		<b>Data value or method of calculation</b>	
3	Site		
4	Date		
5	Recorder		
6			
7	Combine Area		
8	Average combine width (m)	2.48	2.48
9	Total combine length (m)	21.8	21.8
10	Total combine area (m <sup>2</sup> )	=B8*B9	54.064
11			
12	Grain Yield / Moisture		
13	Total grain fwt (g)	39100	39100
14	Sub-sample fwt (g)	100	100
15	Sub-sample dwt (g)	87.9	87.9
16	% moisture content	=(1-B15/B14)*100	12.1
17	Grain yield @ 0% moisture (t/ha)	=(B13/B10)*(1-(B16/100))*1/100	6.357
18	Grain yield @ 15% moisture (t/ha)	=B17/0.85	7.479
19			
20	Grain Quality		
21			
22	HFN		
23	% N		
24	Specific weight (kg/l)	82.08	82.08
25			
26			
27	THOUSAND grain weight		
28	No of seeds in subsample	1069	1069
29	Dwt of subsample (g)	46.52	46.52
30	Thousand seed wt (gDM)	=B29*1000/B28	43.517
31			
32	Assessment of shed grain		
33	Width of plot where assesed (m)	0.5	0.5
34	Length of plot assessed (m)	0.5	0.5
35	Area assessed (m <sup>2</sup> )	=B33*B34	0.25
36	No of seeds counted	116	116
37	Seed shed /m <sup>2</sup> (estimate)	=B36/B35	464
38			
39	Grain loss (t/ha; estimate)	=B37/1000*B30/100	0.202
40	Loss as % of combine yield	=B39/B17*100	3.176
41	grain dry wt allowing for loss	=B39+B17	6.559
42			

## 10.4. Data Sheets

An organised approach to the collection and handling of data is essential if data is to be easily referred to. For example details of the site, location, year, soil type, cultivars, previous cropping, critical dates and other infrequent measurements and crop husbandry should be collated as a site report. Thus a collection of suggested data recording sheets follows. It is strongly recommended that sufficient copies of each sheet are made for every crop studied, and that they are bound in a book to form a comprehensive source of data .

	Data Sheet Title	Frequency of use	No. of pages	No. of copies
1	Site Report	1 per season	2	1
2	Crop Health Record	fortnightly then weekly	1	30*
3	Crop Emergence record	1 per season	1	1
4	Assessment of Growth stage	every sample date	1	2
5	Growth Analysis Raw Data	every sample date	2	25*
6	Leaf Growth Data	every sample date until flag emerges	1/plant /season	30
7	Field Shoot Numbers	1 per season	1	1
8	Node Number Record	1 per season per rep	1	3
9	Plant Height Record	1 per season	1	1
10	Ear Emergence and Anthesis Record	1 per season	1	1
11	Soil Texture and Root Abundance	1 per season	1	1
12	Combine Harvest Data	1 per season	1	1
13	Soil Analysis	1 per season	1	1

\* note further copies may be required in a long season

# 1: Site Report Part 1- Location, Soil, Seed and Dates

<i>Location attributes</i>		<i>Summarised by</i>	
Location Name		Year	
Field Name			
OS map no.:		Grid Ref.	
Altitude (m)			

Drainage -natural	good		moderate		inadequate		poor	
Drainage- artificial	present		absent		not known			
Soil Series Name								
Parent Material								

<i>Site History</i>		<i>Summarised by</i>			
Years	-1	-2	-3	-4	-5
Crop name					
Yield					
N applied (kg/ha)					
Animal manures -type					
Animal manures (t/ha DM)					
Fate of residues					

<i>Soil Analysis Summary</i>		<i>Summarised by</i>					
<b>Top Soil</b>	pH	P mg/l	K mg/l	Mg mg/l	CaCO <sub>3</sub> %	OM %	N mg/kg
Mean over reps							

<b>Deep Soil</b>	Date	Average 0-90 % Dry matter	Total N 0-90 cm kg/ha
February			
Anthesis			
Harvest			

*Crop summary*

Present Cultivar		<i>Summarised by</i>	
Sowing date		Anthesis date	
Seed rate		Ear emergence date	
Seedling Emergence date		Harvest date	



## Site Report Part 2 Cultivations and Treatments

<i>Cultivations</i>	<i>Date</i>	<i>Recorder</i>
Before sowing		
Others- after sowing e.g. harrowing, rolling		

<i>Fertiliser</i>	<i>Product</i>	<i>Rate (kg/ha)</i>	<i>Active Ingredient</i>

<i>Summary of pests, weeds and diseases, from Crop Health Checks</i>	
--	--

<i>Treatment</i>	<i>Product</i>	<i>Rate (l/ha)</i>	<i>Active Ingredient</i>
Herbicide			
Insecticide			
Molluscide			
Plant Growth Regulators			
Fungicides			

## 2: Crop Health Record

Site  Date  Recorded by

Weeds	Y	N	Details:
	<input type="text"/>	<input type="text"/>	

**Disease** If Yes, assess 10 tillers/rep in the lab -enter % area affected for each leaf for each replicate

Take all

Eye spot

Mildew

Septoria

Yellow Rust

Brown rust

Other (specify)

Y	N	Rep 1	Rep 2	Rep 3
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

**Pests**

Slugs

Aphids

Other (specify)

Y	N	Details of problem
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>

**Crop damage**

Rabbits

Other (specify)

**Frost damage**

see key in Section (5.4)

**Deficiencies**

<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>

**Lodging**

If yes, then record for each rep % in each index and comment on the cause of lodging

	Y	N	1	2	3	4	5	total	R	S
<b>Rep 1</b>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	100%	<input type="text"/>	<input type="text"/>
<b>Rep 2</b>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	100%	<input type="text"/>	<input type="text"/>
<b>Rep 3</b>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	100%	<input type="text"/>	<input type="text"/>

Key (Index)

- 1 upright
- 2 leaning
- 3 lodged
- 4 flat (ears less than 10 cm off ground)
- 5 Stem brackled

Type of lodging

- R= root failure/uprooting
- S = stem weakness
- State %R and % S

Cause of lodging

Crop not healthy

Action taken

Action recorded on site report



Crop completely healthy

### 3: Crop Emergence Record

Site

Year

Count the number of plants emerged in 5 m of row every 2-3 days in each rep

Date	Recorded by	Number of plants emerged		
		Rep 1	Rep 2	Rep 3

	Rep 1	Rep 2	Rep 3
Row width (m)			
Number of plants in 5m *			

\* sum all the plants within 10 x 0.5 m rows

#### 4: Assessment of Growth Stage

Sheet number  of  Site  Year

**Growth Stage** Record the growth stage just exceeded by half the plants in each rep, then half the plants overall.

Date	Recorded by	Rep 1		Rep 2		Rep 3		Overall growth stage
		Growth stage		Growth stage		Growth stage		
		Tillering	Elongation/ flowering	Tillering	Elongation / flowering	Tillering	Elongation/ flowering	

Note: leaf growth stage will be assessed from data entered on the leaf growth data sheet.

## 5: Growth Analysis Raw Data page 1

Site		
Date		
Harvest no		
Recorder(s)		
Replicate number		
Quadrat size (m <sup>2</sup> )		

**Field Records from initial samples**

Samples 4 to 6 (field counts)

No. shoots in 3 x 0.33 m rows

Row width (cm)


**Laboratory records**

**FRESH WEIGHTS**

Sub sample 1 (SS1) for growth analysis (g)

Sub sample 2 (SS2) for nitrogen analysis (g)

Total for whole quadrat (g)


**SS1 FOR GROWTH ANALYSIS**

Samples 1 to 3:

Number of plants in quadrat sample

Number of plants in subsample


Samples 4 to 6 (lab counts):

Total number of shoots in quadrat sample

--	--	--

**FERTILE SHOOTS IN SS1**

No. of fertile shoots in sub sample

--	--	--

**Projected areas**

Total green lamina area (cm<sup>2</sup>)

Green true stem + sheath (cm<sup>2</sup>)

Whole ear area (cm<sup>2</sup>)

% ear green ( visual estimate)


**Dry weights (Fertile shoots)**

Total green lamina (g)

Green true stem + sheath (g)

Non-green true stem + sheath (g)

Whole ear (g)

Dead lamina (g)


**INFERTILE, DEAD & DYING SHOOTS IN SS1**

No. of infertile, dead & dying shoots

Infertile, dead & dying shoots Fwt(g)

Total green lamina area (cm<sup>2</sup>)\*

Green true stem & sheath (cm<sup>2</sup>)\*

Whole ear area (cm<sup>2</sup>)\*

% ear green (visual estimate)\*


\*Only measure projected areas if dead/dying shoots form >5% by Fwt of subsample

**5: Growth Analysis Raw Data**

Dry weights (infertile, dead and dying shoots)

- Green lamina (g)\*
- Green true stem + sheath (g)\*
- Non-green true stem + sheath (g)\*
- Whole ear (g)\*
- Dead lamina (g)\*
- Total dead + dying (g)


\*Only record separately weights of components if dead/dying shoots form >5% by Fwt of subsample

**SS2 FOR N ANALYSIS**

(include live + dead material)

- Straw Fwt (= whole plant before ear em.,(g))
- Ear Fwt (g)
- Straw or whole plant DM (g)
- Straw or whole plant N (% in DM)
- Ear DM (g)
- Ear N (%)


**SS3 FOR INDIVIDUAL GRAINS**

- Number of ears (should be 8)
- Fwt ears (g)
- Total number grains
- Fwt grains (g)
- 1. DM grains (g)
- 2. DM ears less grains (g)


**SS4 FOR GRAIN QUALITY**

NOTE: Air dry grain to <20% MC

- No. of ears
- Fwt of ears (g)
- Total Fwt of threshed grain (g)
- Fwt of SS for TGW (c.40g)
- No of seed in SS for TGW
- DM of TGW SS (g)
- Grain HFN
- Grain N (%)
- Fwt of chaff SS for drying (g)
- DM of chaff SS (g)
- Air DM remainder of grain (g)


**SAMPLE FOR WATER SOLUBLE (WSC)**

- No of shoots (6-8 fertile shoots)
- Shoot Fwt (stems & leaves & ears)(g)
- Stems Fwt [lamina and ears removed] (g)
- Stems DM [lamina and ears removed] (g)
- WSC (% of DM in stems )


**REMAINDER DRY WEIGHT**

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## 6: Leaf Growth Data

Site  Replicate No.  Plant number  Year

**Records:** Record lengths of mainstem leaves in millimetres, count leaves from base of plant

For expanding leaves (i.e. until the ligule is visible), record length of lamina exposed.

For expanded leaves (i.e. when ligule appears), record length from lamina to tip and put a cross in the next box to the right, then put a label under the leaf and record its colour.

Observation no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Date	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Recorded by														
Leaf no.														
Label colour														

## 7: Field Shoot Numbers (samples 4, 5, and 6)

Site  Year

**Number of shoots:** Record the total number of shoots in  $3 \times 0.33$  m rows chosen at random from within the quadrat.

**Distance between rows:** Measure the distance in centimetres to the next row from either side of each of the chosen rows.

Number of shoots in row				Distance between rows (cm)						Mean				
				Row 1		Row 2		Row 3						
<b>Sample 4</b>				Row 1	Row 2	Row 3	Total	side 1	side 2	side 1	side 2	side 1	side 2	
Date		Rep 1												
Recorded		Rep 2												
by		Rep 3												

<b>Sample 5</b>				Row 1	Row 2	Row 3	Total	side 1	side 2	side 1	side 2	side 1	side 2	Mean
Date		Rep 1												
Recorded		Rep 2												
by		Rep 3												

<b>Sample 6</b>				Row 1	Row 2	Row 3	Total	side 1	side 2	side 1	side 2	side 1	side 2	Mean
Date		Rep 1												
Recorded		Rep 2												
by		Rep 3												



## 8: Node Number

Site  Plot  Rep  Year

**Measurements:** Select ten plants at random from outside and adjacent to the quadrat and count the number of nodes on each plant. Enter the maximum node number for each plant

**Calculation:** In the lab, calculate and enter the mean.

Date	Recorded by	Number of nodes										mean	

### 9: Plant Height

Site

Plot

Year

**Measurements:** Select ten plants at random from the plot and measure their heights in millimetres from ground to uppermost leaf ligule or the ear collar whichever is higher.

**Calculation:** In the lab, calculate and enter the mean.

Date	Recorded by	Plant height										mean

### 10a: Ear Emergence Record

Site  Year

Label 10 plants in each rep; every 2 days record number of plants with ear collar above flag leaf ligule)

Date	Recorded by	Number of ears emerged		
		Rep 1	Rep 2	Rep 3

### 10b: Anthesis Record

Label 10 plants in each rep; every 2 days record number of plants with anthers exerted

Date	Recorded by	Number of plants at anthesis		
		Rep 1	Rep 2	Rep 3

## 11: Soil Texture and Root Abundance

Depth	Roots		texture class	stones		pore size		*other information	sketch of profile
	Root class	abundance		size (mm)	content %	size (mm)	content %		
Top of profile ↓ 15 cm	Very fine								
	Fine								
	Medium								
15 cm	Coarse								
	Very fine								
	Fine								
30 cm	Medium								
	Very fine								
45 cms	Fine								
	Medium								
60 cms	Very fine								
	Fine								
75 cm	Very fine								
	Fine								
bottom profile 90 cm	Very fine								
	Fine								

Guide to Root Size Classes	
	diameter of live roots
Very Fine	< 1 mm
Fine	1-2 mm
Medium	2-5 mm
Coarse	> 5 mm

Guide to Root Abundance			
Frequency	class	Very Fine and Fine Roots	Medium and Coarse Roots
None	0	0	0
Few	1	1-25	1 or 2
Common	2	25-55	2-11
Many	3	55-450	>11
Abundant	4	>450	

Other Information  
 Make notes on:-  
 Depth of horizons  
 Soil colour and mottling  
 Cracking  
 Composition etc.  
 Organic matter

## 12: Combine Harvest Data

Site						Year	
<b>Date</b>							
Recorder(s)							
Plot number							

Combine Area

Combine width (m)

W1						
W2						
W3						
Mean						

Combine length (m)

L1						
L2						
L3						
Mean						

Grain Yield / Moisture

Total grain Fwt (kg)

Sub-sample Fwt (g)

Sub-sample DM (g)


Grain Quality

HFN

N(%)

Specific weight (kg/hl)


Thousand Grain Weight

No of seeds in subsample

DM of subsample (g)


Assessment of shed grain

Width of plot where assessed (m)

Length assessed (m)


No of seeds counted

--	--	--	--	--	--

## 13 Soil Analysis

Site  Year

<i>Pre-Season top soil</i>	Plot	Rep 1	Rep 2	Rep 3
<b>Date</b>	pH			
<input style="width: 100px;" type="text"/>	P m./l			
	K mg/l			
	Mg mg/l			
	CaCO <sub>3</sub> %			
	Organic Matter % DM			
	NO <sub>3</sub> -N mg/kg			
	NH <sub>4</sub> -N mg/kg			

<i>February N</i>		Depth	Rep 1	Rep 2	Rep 3
<b>Date</b>	NO <sub>3</sub> -N mg/kg	0-30 cm			
<input style="width: 100px;" type="text"/>		30-60 cm			
		60-90 cm			
	NH <sub>4</sub> -N mg/kg	0-30 cm			
		30-60 cm			
		60-90 cm			
	Total-N in profile mg/kg				
	× 4 (kg/ha)*				

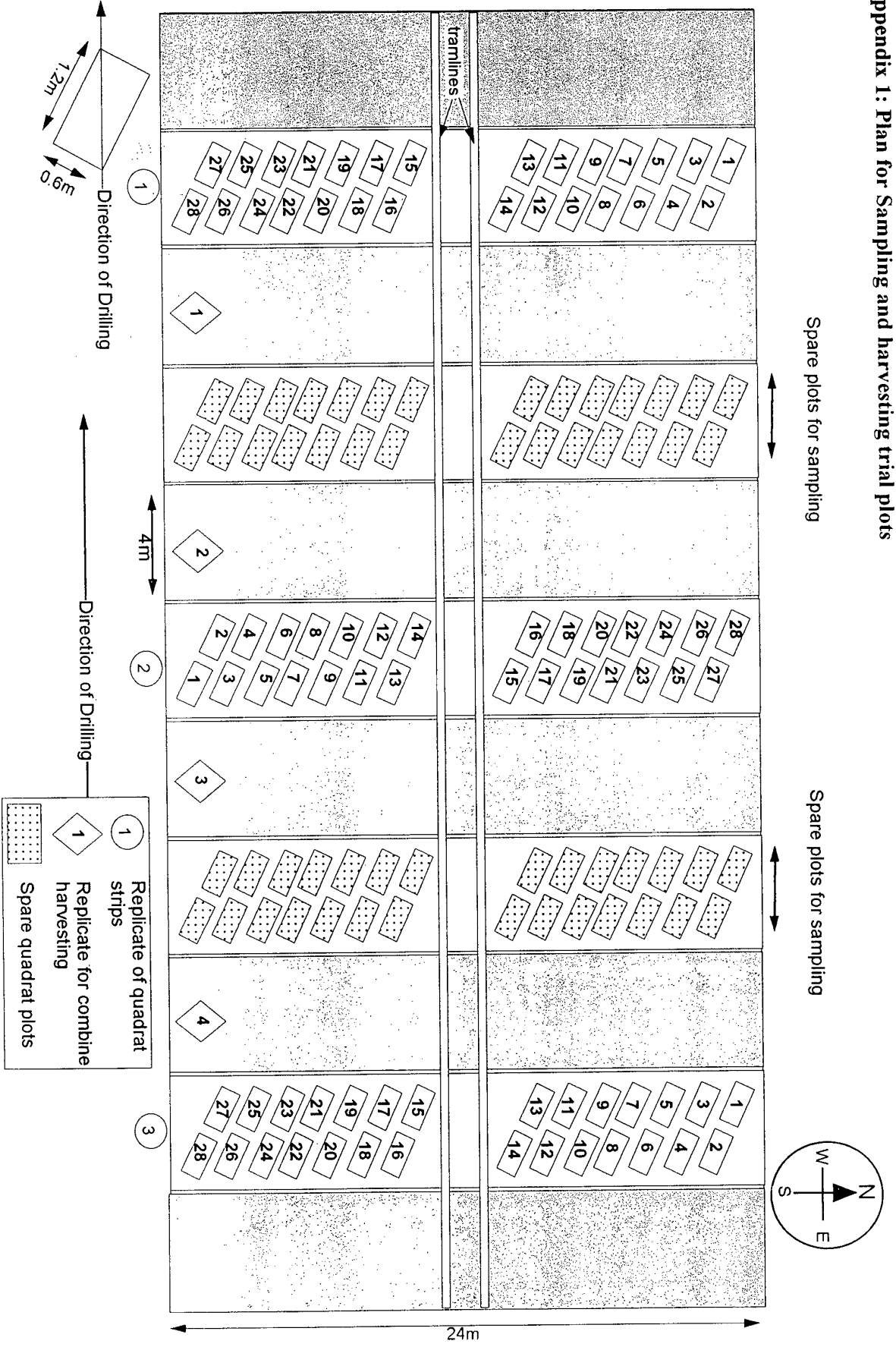
<i>Anthesis N</i>		Depth	Rep 1	Rep 2	Rep 3
<b>Date</b>	NO <sub>3</sub> -N mg/kg	0-30 cm			
<input style="width: 100px;" type="text"/>		30-60 cm			
		60-90 cm			
	NH <sub>4</sub> -N mg/kg	0-30 cm			
		30-60 cm			
		60-90 cm			
	Total N in profile mg/kg				
	× 4 (kg/ha)*				

<i>Harvests N</i>		Depth	Rep 1	Rep 2	Rep 3
<b>Date</b>	NO <sub>3</sub> -N mg/kg	0-30 cm			
<input style="width: 100px;" type="text"/>		30-60 cm			
		60-90 cm			
	NH <sub>4</sub> -N mg/kg	0-30 cm			
		30-60 cm			
		60-90 cm			
	Total N in profile mg/kg				
	× 4 (kg/ha)*				

\*this factor assumes soil has a bulk density of 1.33 kg/l and 30 cm sample depths

## **11. APPENDICES**

# Appendix 1: Plan for Sampling and harvesting trial plots

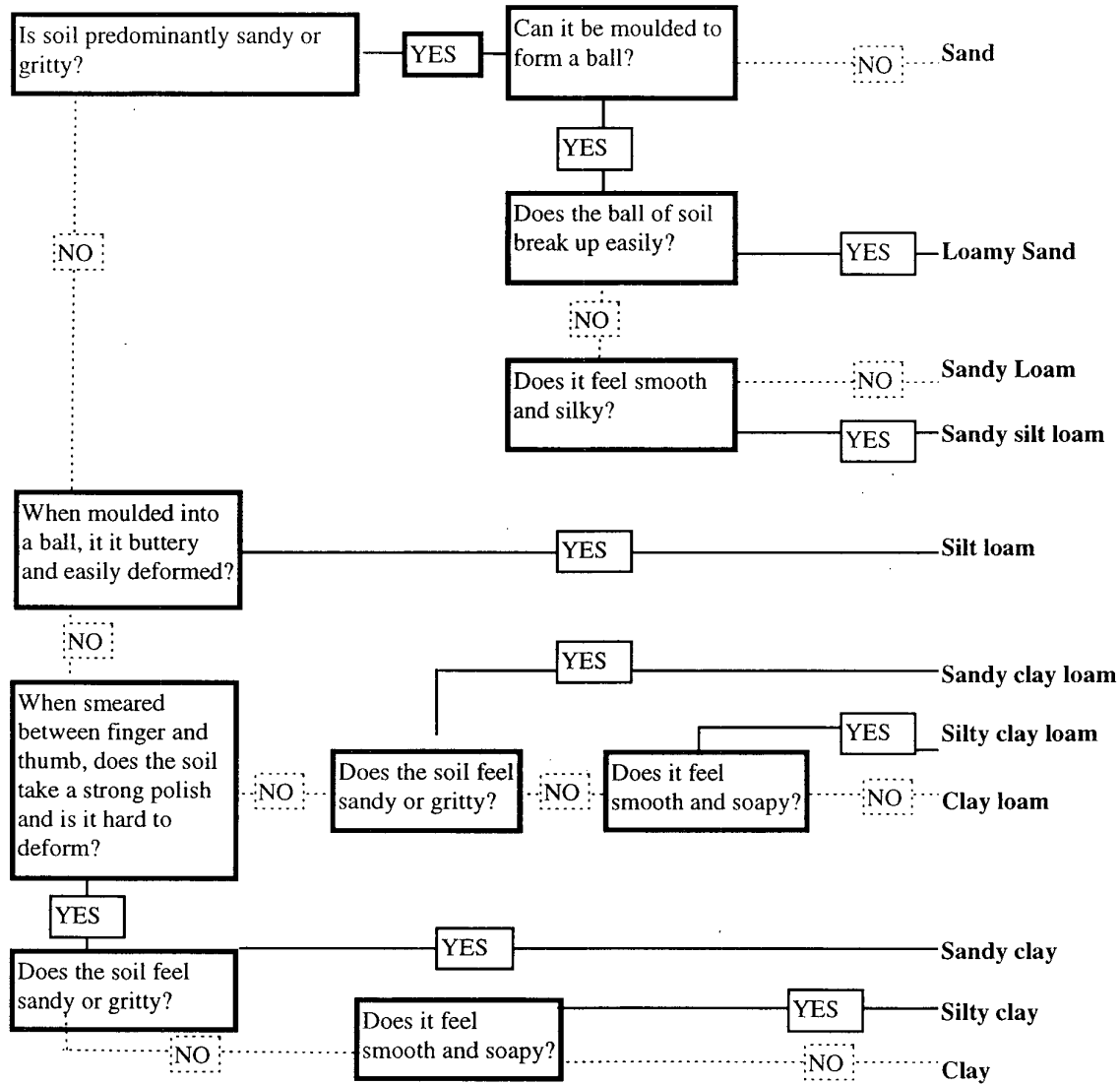


How to Run a Reference Crop



**APPENDIX 2: Identification Of Soil Type By Hand Texture**

Method: take about a dessert spoonful of soil. If dry, wet up gradually while kneading between fingers and thumb until there is enough moisture to hold the soil together and for the soil to exhibit its maximum cohesion. Follow the key.



**classes and abbreviations**

Sands	sand	S
	Loamy Sand	LS
Light Loams	Sandy loam	SL
	Sandy silt loam	SZL
Light Silts	Silt loam	ZL
Medium Loams	Sandy clay loam	SCL
	Clay Loam	CL
Medium Silts	Silty clay loam	ZCL
Clays	Sandy Clay	SC
	Clay	C
	Silty Clay	ZC

### Appendix 3      Calculation of accumulated thermal time

The influences of time and temperature on crop development can be summarised through the concept of 'thermal time'. This is calculated by accumulating daily temperatures, in relation to a base temperature below which the process of interest stops. Different base temperatures are used according to the process being studied. For example, 0°C is used for leaf development but 9°C is often used grain development. Ideally, the accumulation of temperature should be continuous through the diurnal cycle. However, a simplified calculation, which provides a good estimate, only requires measurements of daily maximum (max.) and minimum (min) temperatures. This method is as follows :

Each day start from condition 1 and, if this condition is not met, go on to next condition, until you have a successful match. Then calculate the daily thermal time as shown on the right of the table. Accumulated thermal time is the sum of the daily values over the period of interest.

Condition	Daily thermal time
1. base less than min	mean - base
2. base less than mean	$(\text{max.} - \text{base}) / 2 - (\text{base} - \text{min}) / 4$
3. base less than max.	$(\text{max.} - \text{base}) / 4$
4. max. and min less than base	0

*Note: mean is calculated as  $(\text{max.} + \text{min})/2$*