Scotland's Rural College

Weed Competitiveness

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Handbook
Cereal variety testing for
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FOREWORD

This handbook is written and published within the SUSVAR network (COST Action 860); SUSVAR stands for 'Sustainable low-input cereal production: required varietal characteristics and crop diversity' and COST is an intergovernmental framework for European co-operation in the field of scientific and technical research. The SUSVAR network, initiated spring 2004, now includes researchers from more than 100 institutions in 28 European countries.

The main aims of the SUSVAR network are to ensure stable and acceptable yields of good quality for low-input, especially organic, cereal production in Europe. This will be achieved by developing ways to increase and make use of crop diversity, by establishing methods for selecting varieties, lines and populations taking into account genotype-environment interactions and by establishing common methodology for variety testing where appropriate.

Cereals are an important contribution to food production and the economy in Europe. As a consequence, reduced inputs of pesticides and chemical fertilisers are of general interest, and increasing the area grown under organic conditions receives much public support. The presently available crops and varieties may not be the best to ensure stable and acceptable yields under low-input conditions since most cereal varieties for the last 50 years have been developed to produce high yields under potentially unlimited use of pesticides and synthetic fertilisers. In many countries, national projects are in progress to investigate the sustainable low-input approach. These projects are coordinated in the SUSVAR network by means of exchange of materials, establishing common methods for assessment and statistical analyses, and by combining national experimental results. The network comprises scientists from many disciplines to investigate the complex interactions between the crop and its environment, in order to be able to exploit the natural regulatory mechanisms of different agricultural systems for stabilising and increasing yield and quality. The results of this cooperation will contribute to commercial plant breeding as well as official variety testing, when participants from these areas disperse the knowledge achieved.

This handbook is the result of the comparison of variety testing systems in the participating countries. It contains a description of different methodologies used in variety testing, their potential advantages and disadvantages and experimental conditions under which they may be applied. Special emphasis is on assessment of diverse crops, e.g., variety mixtures. and specific considerations needed for organic variety trials compared to conventional trials are highlighted and discussed. Each chapter has a list of literature references for more detailed information.

The handbook will be a useful tool for those involved with variety testing of cereals, including breeders, but also for researchers who are working on methodologies for studying genetic diversity and genotype-environment interactions in cereals.

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Chair of SUSVAR

INTRODUCTION

1. Why this handbook ?

Over the past ten years researchers in a number of European countries (e.g. Austria, Denmark, France, Germany, Netherlands, Switzerland, UK) have been engaged in research and discussions on the set-up of organic cereal variety trials. This has generated a wealth of knowledge and experience. COST-action 860 (SUSVAR, see also Foreword) has brought many of the researchers involved in testing wheat and barley varieties for organic and low input agriculture together. This concerted action has made it possible to compile all the available knowledge of professionals from different scientific disciplines like agronomy, weed science, phytopathology, food processing and biostatistitics.

The testing of varieties for organic and low input agriculture, compared to conventional variety testing not only deals with different growing environments, but also with different priorities for traits to be assessed. For example weed competitiveness and nutrient uptake efficiency have been reported as relevant characteristics to be evaluated in organic variety testing. As these traits are not incorporated in conventional variety testing protocols, evaluation methodologies have been developed recently in several of the countries mentioned above. These and other methodologies that may be important for organic and low input variety testing, are presented in this handbook. Apart from weed competitiveness and nutrient use efficiency, this includes lodging resistance, susceptibility to diseases and processing quality. Descriptions are limited to the crops barley and wheat, but most methodologies are applicable to other cereal crops as well.

Mixtures of varieties and heterogeneous populations are usually not included in regular variety trials. Increasing genetic diversity by e.g. mixing cultivars has proven to be an effective tool to manage diseases. This management option is especially important for 'low input' farmers, who want to use a minimum of synthetic fungicides and organic farmers, who do not apply any synthetic fungicides at all. Therefore the handbook also deals with the implication of including mixtures and heterogeneous populations in comparative trials.

The consequence of the integration of new traits to standard variety testing protocols and of changing priorities may be that variety tests for organic and low input agriculture lead to other variety recommendations than conventional tests. Moreover, conducting trials in a different growing environment may result in a change in ranking of the varieties. Although important, this topic will not be dealt with in this handbook.
At present statisticians of SUSVAR are conducting a meta-analysis of data from six different countries to deal with the variety ranking issue and results will be made available in a separate publication.

2. How to use this handbook

The first section deals with the statistical principles of variety testing, the aspects to be considered for setting up variety trials and for the interpretation and analysis of the results. Seed management is also included in this section. Unfortunately it is not included in the first edition of the handbook but will be added in spring 2007. It will be published on the Susvar website (www.cost860.dk) and can be downloaded from there. This website also offers the possibility to subscribe to an "e-mail alert service", which informs subscribers at the moment that updates are available for downloading.

The other chapters (on evaluation of weed competitiveness, diseases, lodging, nutrient use efficiency and processing quality) have all been written according to a more or less similar structure. After an introduction on the relevance of the characteristic involved, various methods to evaluate this characteristic are described and discussed. If relevant, special considerations for organic and low input agriculture and for variety mixtures and populations are mentioned. Some chapters include a survey of the methodologies applied by different institutions involved with variety testing in various European countries. This may be a helpful tool for optimizing existing variety testing systems but also for setting up new variety testing systems.

With regard to the comparison of methodologies used by different institutions, we have chosen to use the BBCH growth scale in all the chapters of this handbook. A description of this growth scale as well as a translation to the decimal growth scale of Zadoks et al can be found as an appendix.
All the chapters are completed with a literature list for additional reading and reference.
Each chapter has been written by a group of specialists in the specific topic. The names of the authors are mentioned with the title of the chapter. Names of institutions and (e mail) addresses of these authors are listed in the appendix.

SETTING UP VARIETY TRIALS for ORGANIC and LOW INPUT agriculture

Lilia Levy, Aart Osman, Irène Felix and Michael Oberforster

When setting up special variety trials for organic or low input agriculture several aspects - such as the choice of locations, design of the trials, traits to be assessed - need to be considered. Moreover, treatments and the management in certified organic fields should be in agreement with EU and national regulations on organic farming. This chapter is based on the experience of the authors with conducting organic and/or low input trials.

1.   Selection of the field location

Organic fields
The main objective of organic variety testing is to provide organic farmers, traders and producers with research results obtained from trials carried out in organic fields. Ideally, trial locations should be officially certified as organic and should be managed organically for at least five years (three years conversion period + two additional years) as experience shows that the crop performance still changes in the first years after conversion.

Soil type
Soil structure and soil type should be known in order to estimate the necessary amount of fertiliser. The balance should be corrected with regard to the expected yield, the amount of precipitation (soil nitrogen leaching) and the residues of the previous crop.

Field properties
The field should be as homogeneous as possible. An appreciation of its homogeneity can be obtained by growing a monoculture in the first year and testing the different parameters (especially the yield) that will be measured later in the real trial. Awareness of field gradients will enable a more accurate placement of the trial.
The ideal field should correspond to fields used by farmers for cereal production. As cereals are grown in different environments, one should try to include the most representative farming environments in the variety testing system.

Previous crop
Previous crops in the rotation influence the nitrogen level but also the preparation of the seedbed and the disease and pest pressure on the trial (Vullioud, 2005). One should try to select a field with a pre-crop that is typical for the farming system in the region. This may be more difficult for organic trials, as in a number of countries organic farmers usually grow a large number (six or more) of different crops in a crop rotation of at least six years. While fixing the pre-crop between locations may not be achievable, one should try to have the same pre-crop over the years at a given location
In cropping systems with spring-sown cereals, farmers sometimes grow a catch crop during the winter season. The species of the catch crop may influence the yield level and (baking) quality properties of the subsequent cereal crop (Mauscherning et al., 2006, Pedersen et al., 2006).

Distance to trial experts
The different locations of the trial system should be spread over the main growing areas of the crop. Therefore not all locations may be close to the professional who is in charge of the observations. Organic variety tests usually include a number of labour intensive observations, such as early ground cover and weed suppression. When resources are limited, it is advisable to concentrate the more labour intensive observations in trials that are as close as possible to the trial experts.

Human resources
Trials are often placed in farmers' fields. Special attention should be given to the selection of participating farmers. Their experience and motivation are fundamental for the successful outcome of the trial.

2.   Field Management

Field treatment
Treatments with synthetic products are not used in organic trials, and a reduced range is applied to low input trials. Plough and mechanical interventions (tine harrow, curry comb and hoe) are the main tools to prevent weed invasion. More information on the effectiveness of harrowing is given in the chapter on weed competitiveness in this Handbook.

Organic manure
The fertilisation of organic trials needs special attention, as manure usually is not homogenous and it is difficult to spread it uniformly over the field.
In some countries, organic farmers apply an additional gift of fertilizer after tillering and at flowering stage to enhance the yield level and baking quality of wheat. Machinery may cause considerable damage in the fields, especially when liquid manure is used for this purpose. Granulated organic fertilizer (commercial name e.g. Agro Biosol, Biofert) may be applied with a drill or by hand. The most appropriate way is to fertilize each plot separately with the help of a measuring jug.

3.   Trial layout and design

Variability
Random variability may be larger in organic and low input trials than in conventional trials, due to for example more heterogeneous soil conditions and the occurrence of weeds. Plot size and number of replicates may need to be increased to decrease experimental error (see Chapter Trial set up and Statistical Analysis in this Handbook).

Crop management and farm machinery
Weed management and the application of manure and other fertilizers are usually carried out with the farm machinery that is available at the location. To limit damage to the trial, plot size and trial lay-out should be adjusted to the dimensions of the farm machinery at the specific location and the direction of harrowing.

4.   Choice of varieties

Choice of standards
The standard varieties should include the most used varieties in organic or low input farming. Standards should be representative for the aimed level of quality, grain yield and disease resistance. It may be useful to include special standard varieties to evaluate specific characteristics, such as weed competitiveness.

Choice of varieties
Variety testing demands a high investment. If the number of applications is too large, a pre-screening in a simplified trial can help to identify varieties that are well adapted to organic farming (e.g. high protein content, high disease resistance, good weed competitiveness and a good yield potential).

5.   Seed material

5.1.   Choice of seeds in organic trials
Whereas the use of organically multiplied seed is compulsory for commercial organic farms, it may not always be available, especially for varieties/lines that have not yet officially been released. Conventional seed companies tend to delay the organic multiplication of their varieties until they have been released. The EU regulation on the use of organic seeds (EC) No 1452/2003 offers the possibility of derogation for research purposes (see paragraph 7). When organic seed is available for only part of the varieties to be tested, there are two options:
  − use organic seed for those varieties for which organic seed is available and conventional seed for the rest
− use conventional seed for all the varieties.

For variety testing it is important that seed quality is as similar as possible for the different varieties. As the seed quality may differ according to the provenance, the second option is preferable.

5.2   Seed health
Seed quality and health can influence the trial results and it has to be analysed more precisely than in conventional testing. The germination capacity of winter cereals should be analyzed at 10 °C instead of at 20 °C (which is commonly used).
As chemical treatment of seed is not possible in organic trials, it is likely that the trial results will be influenced by the presence of seed borne diseases. In this way varieties can be selected that produce healthy seeds, which is an interesting aspect for organic farmers. Wheat seeds should be treated if there are more than 5-10 spores of *Tilletia caries* on a kernel.
If one chooses to evaluate a set of varieties without the constraint of seed borne diseases, the following non-chemical methods, among others, may be used for this purpose:

Warm and hot water treatment
This old technique can be used for a range of diseases in several crops. Seeds are submerged in water of a fixed temperature for a fixed time, depending on crop and disease. In wheat it has been reported to be effective against root rot (*Microdochium nivale, Fusarium* spp.) and glume

blotch (*Septoria nodorum, Stagnospora nodorum*) (Winter et al., 1998; Schachermayr, et al., 2000; Osman et al., 2004).
In barley it can be used against leaf stripe (*Pyrenophora graminea*) and loose smut (*Ustilago nuda*) (Nielsen et al., 2000).

Hot humid air
Seeds are exposed to steam of a fixed temperature for a short period. Temperature and application period should be established for each separate seed batch, because the effectiveness of the treatment is influenced by the physiological condition of the seeds. The method has been commercially developed by the Swedish company Acanova. It has been reported to be effective against a wide range of diseases (Forsberg, 2005; www.acanova.se).

Electron treatment
This method is based on treating seeds with low-energy electrons. It is commercially applied in Germany (www.e-ventus.de). Whether this methodology is suitable for organic agriculture is a topic of debate within the organic sector. It shows effects against Common bunt (*Tilletia caries*) and to a lesser degree against Glume blotch (*Septoria nodorum*) in wheat (Tigges et al., 2002; Vogt-Kaute & Tilcher, 2004)

Mustard flour
Mustard flour (commercial name e.g. Tillecur) is mainly used against common bunt (*Tilletia caries*) in wheat (Borgen and Kristensen, 2000; Schachermayer, et al.; 2000; Vogt-Kaute & Tilcher, 2004).

Bacterial treatment
In Austria and other countries a bacterial treatment (commercial name e.g. Cerall, Cedomon) based on the soil bacterium *Pseudomonas chlororaphis* is applied. Cerall is used in common wheat, durum wheat, rye and triticale and effective against seed-borne *Tilletia sp., Fusarium sp., Microdochium nivale* and *Septoria nodorum*. Cedomon is suitable for barley and oats. (Widén & Annas, 2004).

5.3   Seed density
The same seed density should be used for all varieties. It is calculated on the basis of the thousand kernel weight and the germination rate of the grains. In organic trials, seed loss is usually higher than in conventional trials, due to the use of non-treated seeds, intensive harrowing and to a slower development, caused by lower nitrogen availability. Seed density in organic trials should be 15-30 % higher than in conventional trials to compensate for these losses.

6.   Additional traits to be observed

Some additional traits may be evaluated in organic variety trials, which are not commonly observed in conventional trials. An overview of extra traits that are proposed for wheat and barley in a selection of countries is given below:

TRAIT	Austria	France	Germany	Nether-lands	Switzerland
Tolerance to seed borne diseases	X	X	X		X
Early vigour	X	X	X	X	X
Weed suppression	X	X	X	X	X
Nutrient use efficiency	X				
Yield Stability	X				X
Product Quality					X
Bread quality under organic or low N input conditions	X	X	X	X	X
Baking test without additives			X	X	X
Wet gluten content			X		X
Stability of quality	X				X

Source: Oberforster (2004); Goyer et al (2005), Schnock (2003); Osman & Lammerts van Bueren (2003)

7. EU regulation on organic production and the implications for variety trials

When fields are organically certified this means that all practices have to comply with EU and national legislation on organic farming.

Field management.
The management aspects (e.g. use of inputs) of organic fields and products are dealt with in EU Council Regulation (EEC) No 2092/91. Annex 1 contains the principles of crop production, while Annex 2 lists the allowed crop protection products and fertilizers.

Seeds
A special regulation on the use of organic seeds came into force in 2004: Regulation (EC) No 1452/2003. According to this regulation organically multiplied seeds should be used. In certain cases derogations for the use of conventional, not chemically treated seeds, can be requested from the national certifying authorities. Article 5.1(d) specifically mentions that certifying bodies may grant authorization for the use of non-organic seeds in the case of research. More information on the procedure for requesting this derogation should be obtained from the national certifying body.
Full texts of both EU regulations can be downloaded from: http://eur-lex.europa.eu/

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TRIAL SETUP AND STATISTICAL ANALYSIS

Andreas Büchse, Pawel Krajewski, Kristian Kristensen and *Wiesław Pilarczyk*

1. Introduction

1.1. Background

The response from field trials is subject to random variation. This means that two neighbouring plots grown with the same variety and treated in the same way will always yield differently. This also applies to all other recordings made on a continuous scale. The size of the differences will depend on several circumstances such as the variability in the soil, variability in the applied fertilizer, historical events and uncertainty in the recording process. This means that a recorded difference between e.g. two varieties may be due to either a true difference in the response of the two varieties or may be due to random variations. In order to help decide whether the difference is caused by the different varieties or by random variation it is necessary to apply some statistical methods in order to estimate the actual size of the random variation in the field and compare the measured difference with the size of the random variation. In order to do that properly it is necessary to use properly designed trials and the correct way of analysing the recorded data.

This chapter gives some information on how to design the trials in such a way that the part of random variation that determines the uncertainty in the difference between varieties (treatments) is as small as possible. In designing the experiment it is essential to take into account the size of the difference that the researcher wants to become significant in order to design the trial with the number of replicates that is considered to be appropriate for the level of random variations expected in the trial.

This chapter also gives some information on how to analyse the most common types of measured variables in variety trials under organic and low input systems and the conclusions that can and cannot be drawn from the analyses.

The random variability may in some cases be much larger for organic grown trials than for similar conventional grown trials. In two series of comparable trials with spring barley in Denmark and Sweden, the random variability was largest in the organic grown trials in 19 out of 34 pairs of trials in Sweden and in 3 out of 4 trials in Denmark. On average the random variability was approximately: 5.0 $(hkg/ha)^2$ in the conventional grown trials and 7.2 $(hkg/ha)^2$ in the organic grown trials, but the maximum random variability was 2-3 times higher for the organic grown trials than for the conventional grown trial. This indicates that it may be necessary to have more replicates in organic grown trials than in conventional grown trials if one wishes to maintain the same precision.

It may be expected that the competition between neighbouring plots may increase when diseases are uncontrolled. This may be handled by increasing the guard areas between plots. However, increasing the guard areas too much will usually increase the random variation. Alternatively one could compensate for the increased competition by modelling the competitions (see the text on plot size and shape in section 2.2) or by increasing the number of replicates.

The validity of the statistical analyses depends on some basic assumptions.

Therefore, some information is given on how to check that the assumptions are fulfilled and how to proceed if they are not.

The text in this chapter tries to describe the principles and methods to be used together with the most important assumptions that are needed for the methods to work correctly. Details on how to do the calculations are not provided. More details on that subject can be found in the references and in the documentation of the statistical software that can be used to do the calculations such as Genstat (Payne, 2006), SAS (SAS Institute, 2006), R (http://www.r-project.org/) and others. Examples on the applications may be found in the references and in the documentation of the used software.

1.2. Definitions

In the following we will define a <u>plot</u> as the units to which the varieties are allocated. A plot may contain several plants from the same variety. In some cases a plot can be subdivided and each part of such a plot will be called a <u>sub-plot</u> and in such cases the plot that is subdivided is usually called <u>main-plot</u> (or <u>whole-plot</u>). Sub-plots may be used either for applying different treatments to each of these (as in a split-plot design) or for taking more samples in each plot (e.g. samples of plants for determination of dry matter). A <u>block</u> is a collection of plots within which the plots are randomised. If many varieties (treatments) are to be included in the design, a block with all varieties (treatments) may be so large that it will be difficult (impossible) to find blocks that are sufficiently homogeneous. In such cases the varieties are collected in <u>sub-blocks</u>, which are randomised within each block and subsequently the plots within each sub-block are also randomised. This is the case in the recommended types of incomplete blocks (see 2.1).

In order to describe the level and the variability of a given variable, e.g. yield, some measures are usually calculated. The most frequently used measure is the <u>mean</u>, which is given by:

$$\overline{y} = \frac{1}{n}(y_i + y_2 + ... + y_n).$$

The <u>median</u> is given by the value that separates the ordered observations in two groups of equal size. The median is more robust than the mean, but has a larger uncertainty than the mean if the data are normally distributed. The most frequently used measures to describe the variability are the <u>variance</u> and the <u>standard deviation</u> given by:

$$\text{Variance: } s^2 = \left(\frac{(y_1 - \overline{y})^2 + (y_2 - \overline{y})^2 + (y_3 - \overline{y})^2 + ... + (y_n - \overline{y})^2}{n-1} \right)$$

Standard deviation: $s = \sqrt{s^2}$

In the recommended statistical methods it is assumed that the recorded plot values for a variable are <u>independent</u>, which means that the observation made in one plot does not give any information on the observation in another plot. One feature of independent observations is that the variance of the mean is inversely proportional to the number of observations used for forming the mean. So if s^2 is the estimated variance on single observations then the <u>variance and standard error of the mean</u> based on n independent observations is given by:

Variance on the mean: $s_{\bar{y}}^2 = \dfrac{s^2}{n}$ and the standard error of the mean: $s_{\bar{y}} = \dfrac{s}{\sqrt{n}}$.

This can be used to calculate the <u>variance of a difference between two means</u>, e.g. for variety A and variety B:

$sed^2 = s_{\bar{y}_a}^2 + s_{\bar{y}_b}^2$ and equivalently the standard deviation of the difference: $sed = \sqrt{s_{\bar{y}_a}^2 + s_{\bar{y}_b}^2}$.

If the variances can be assumed identical for the varieties (which they most often can) this can be written as $sed = s\sqrt{\dfrac{1}{n_a} + \dfrac{1}{n_b}}$ where n_a and n_b represent the number of independent observations for the two varieties. This quantity can be used to calculate the minimum distance that must be found between two varieties in order to prove that the varieties are significantly different for the observed character. Assuming that the distribution of the variable in question is normal, this minimum distance can be calculated as $LSD^F = sed \times t_{f,1-\alpha/2}$, where $t_{f,1-\alpha/2}$ is the $1-\alpha/2$ fractile of a t-distribution with f degrees of freedom, where f is ($n_a + n_b - 2$).

The purpose of doing statistical analysis is usually both to <u>estimate</u> the parameters of interest, such as the mean yield of each variety and the mean difference between pairs of varieties as well as to <u>test</u> whether some <u>hypotheses</u> can be accepted or have to be rejected. In a simple situation such as a randomised complete block design without missing values the <u>estimates</u> of the mean yields of a variety are simply the averages over all observations on that variety. The <u>estimated</u> difference is simply the difference between the averages of the varieties. In more complicated designs or when some observations are missing the estimation is more complicated as it is necessary to use methods that take into account other factors such as the blocks in which a given variety is present.

Statistical <u>tests</u> are performed in order to know whether a <u>hypothesis</u> can be accepted or has to be rejected. Examples of such hypotheses could be the hypothesis that all varieties have the same yield, that the difference between variety A and B is zero or that all varieties react in the same way to nitrogen. The <u>tests</u> are made on some predefined significance levels, usually called α (alpha). There is a strong tradition to take α equal to either 5% or 1%. If α is 5%, it is said that the test is performed on the 5% level of significance. A <u>significant</u> result means that the hypothesis has to be rejected, i.e. the difference between variety A and B is different from zero at the 5% level of significance. A <u>non-significant</u> result means that the hypothesis can be accepted, i.e. the difference between variety A and B is not different from zero at the 5% level of significance. Note that this does not mean that the difference is zero; it only means that with the used number of replicates, the chosen design and the actual random variation there is no reason to conclude that the difference is not zero.

The application of statistical tests always implies some risks of making wrong decisions. These are usually separated into two types of risks. They are called <u>Type I</u> error and <u>Type II</u> error, respectively. The type I error is the error that arises when we decide the varieties to be distinct, when they are in reality identical. The type II error is the error that arises when we decide the varieties to be identical, when they are in reality different. The risk of type I error can be controlled easily as the risk here is α, whereas the risk of type II error, usually called β (beta), is more difficult to control as it depends on the size of the real difference between the varieties, the random variability, s, and the chosen design (number and replicates and lay-out in the field).

2. Experimental Designs

2.1. Type of designs

Randomised complete block design (CBD)
The experimental field is divided into blocks according to the number of replicates. Each Block is divided into a number of plots according to the number of treatments. The treatments are then assigned randomly to the plots. Each treatment occurs one time per block.
A benefit of block designs over completely randomised designs is, that differences between blocks (e.g. due to soil quality) do not influence the estimates of treatment differences and can be separated from the experimental error when performing analysis of variance.
One drawback of the CBD is that only soil differences in one direction can be modelled. Possible extensions of the block design for two directions are the Latin square, allowing for row and column effects.

A CBD is a good choice when there are no technical aspects that restrict the randomisation. Simple block designs are mostly used for one-factorial trials but two or more factors are also possible. The layout of blocks on the field has to be chosen in such a way, that soil differences between blocks are maximised and within blocks are minimised. Homogeneity of conditions within blocks requires that the treatment number and therefore the dimension of the blocks have an upper limit. Depending on plot size and soil conditions block designs are recommended for trials up to 20 treatments. In block designs the assumption is usually made that there are no interactions between treatments and blocks.

Fig. 1. A randomised complete block design
with 5 treatments in 4 complete blocks.

block 1	A	E	B	D	C
block 2	C	D	A	E	B
block 3	E	B	D	C	A
block 4	E	D	A	B	C

Incomplete block design (IBD)
In trials with high treatment numbers, e.g. variety trials, complete blocks are too large to give a good control of the experimental error due to soil heterogeneity. In these cases designs with incomplete blocks are useful. Every block only contains a fraction of the total number of treatments and is therefore *incomplete*. Several incomplete blocks form one complete replication. One type of such designs is the *lattice design*. The blocks of an incomplete block design can be arranged in any way that is useful for controlling soil heterogeneity.

With an IBD the arithmetic mean of a treatment is not the best estimator for the expected mean value. Treatment means have to be adjusted according to the linear model used for data analysis. One should use powerful software for the analysis (ALPHA+, GenStat and SAS).

Specialist software is also needed for the construction of the design (e.g. Alpha+ or CycDesigN, http://www.cycdesign.co.nz).

There are several types of lattice designs:

a.   <u>Square Lattices</u> need a quadratic or cubic number of treatments (9, 16 and 25). The number of plots per block (k) has to be the square root of the number of treatments (v). For example 36 treatments in 6 blocks of 6 plots per replicate.

b.   <u>Rectangular Lattices</u>: The number of treatments has to equal k(k+1) with k= number of treatments per block. This algorithm allows for treatment numbers like 12 or 20.

c.   <u>Alpha-designs</u>: More flexibility is reached with the new class of alpha designs or generalised lattices (Patterson & Williams 1976, Patterson et al. 1978). The following requirements have to be met: (1) The number of plots per Block (k) has to be smaller or equal to the square root of the number of treatments (v). (2) The number of replicates has to be smaller or equal to the ratio v/k. (3) The number of treatments has to be a multiple of k. Where the number of treatments does not meet these conditions, a design for the next possible number is developed and the redundant treatments are discarded.

Fig. 2. Example of an incomplete block design with 16 treatments in 3 complete replications. The replications are divided into 4 incomplete blocks with 4 plots each.

```
        Blocks of the design printed in rows

         rep  1 ----------------
         plot      1   2   3   4
         block +----------------
            1  |  15   3   1   8
            2  |  11   9   5  16
            3  |   7  14  12  13
            4  |  10   6   2   4

         rep  2 ----------------
         plot      1   2   3   4
         block +----------------
            1  |   3   8   6  16
            2  |   2  10   1  13
            3  |   7   4  12   5
            4  |  14  15  11   9

         rep  3 ----------------
         plot      1   2   3   4
         block +----------------
            1  |   4  13  11   3
            2  |   1  12   6   9
            3  |  10  14   8   5
            4  |  15   7  16   2
```

Split plot design
This type of design is often advantageous for factorial trials when one factor can not be allocated to small plots for technical reasons or when the factors should be tested with different precision.

Imagine a two factorial trial (tillage 1 and 2 and varieties A, B, C, D, E) with three replicates. First each block is divided into two main plots. The factor, tillage, is then allocated randomly to the plots. Each main plot is then divided into as many sub-plots as the second factor has levels, here 5. Then the levels of the second factor are allocated randomly to the sub-plots within the main plots.

In the analysis of variance the main plot factor has to be tested against the interaction main plot factor x block (the main plot error), whereas the sub plot factor is tested against the residual. Because the main plot factor is tested with less precision and with only a low number of degrees of freedom for the error term, usually only large differences become significant. A difference in sub plot factor means normally show much smaller standard errors. Since more than one error term occurs in split plot designs, the analysis should be performed in a mixed model framework. A description of the analysis of split plot trials is given in 3.3.

**Fig. 3   A split plot design with 2 treatments for the main plot factor (1 and 2),
             5 treatments for the sub plot factor (A-E) and 3 complete blocks.**

block 1	1-A	1-E	1-B	1-D	1-C	2-C	2-D	2-A	2-E	2-B
block 2	2-E	2-B	2-D	2-C	2-A	1-E	1-D	1-A	1-B	1-C
block 3	2-B	2-C	2-A	2-E	2-D	1-D	1-A	1-C	1-B	1-E

2.2. Trial set up and design

What type of design to choose?
Depending on the plot size and soil conditions complete block designs are recommended for trials up to 20 treatments. With higher treatment numbers incomplete block designs will normally give results with a lower standard error. Because of their great flexibility we recommend to use alpha-designs.
Complete blocks, incomplete blocks and split plot design can be combined in different ways to meet the technical and statistical requirements. The chosen structure may not be covered by examples in statistical textbooks. The only requirement is that the principles of replication and randomisation are kept in mind and that the model used for analysis is based on the randomisation structure of the trial (see Piepho et al. 2003 for details).

Number of replicates

For single trials four replicates are often recommended. But four replicates may not be enough to give results with a standard error of mean that is small enough to distinguish interesting treatment means significantly. Compared with randomised greenhouse or laboratory experiments, field trials utilise an extremely small numbers of replicates due to practical restrictions. Table 1 presents the detectable difference Δ as a k-fold of the standard deviation for the two-sided t-test for different numbers of replicates with a maximum false negative rate of 20% (Type II error) and the common false positive rate of 5% (Type I error). In a field trial with replicate or plot size of four, only effect differences larger than 2.02*SD will be detected with a maximum false negative rate (β) of 20% and a maximum false positive rate (α) of 5%.

Table 1. Detectable relative difference (Δ = Diff / SD) for various numbers of replicates with nominal values of α=0.05 and β=0.20 for type-I and Type-II experimental error respectively.

number of replicates	2	3	4	5	6	10	20
Δ (for α=0.05; β=0.20)	3.07	2.38	2.02	1.80	1.70	1.33	0.91

In trial series in different environments estimating the genotype x environment interaction is much more interesting than exact results in single trials. Therefore two or three replicates per location will be sufficient when the number of locations is high enough.

Block size and shape

The optimal block size and shape depends on the heterogeneity of the experimental field. If no additional information is available, a quadratic shape of the blocks is the best choice. The larger the blocks are, the higher the experimental error will be due to differences in the soil conditions. With more than 20 treatments, a lattice design (e.g. square lattice, generalised lattice) is recommended.

Plot size and shape (and guard areas)

A plot size larger than 20 square metres is seldom reasonable in variety trials. When the total experimental area is fixed, many small plots give a better control of the experimental error than a few large plots. Differences in the soil quality will be distributed more evenly on the different treatments. The minimum size of the plots also depends on the dimensions of the machinery to be used. A plot size between 5 and 20 square metres is commonly recommended. Variety trials are mostly performed in narrow plots. This has some technical advantages. For example if the harvester has a working width of 150 centimetres, it is practical to use plots of 150 centimetres wide.

Interplot interference can affect estimates of yield, quality and disease resistance due to differences in competitiveness of the tested genotypes (Talbot et al. 1995, Clarke et al. 1998). Interference may be caused by differences in plant height with consequent competition for light, and also by differences in disease resistance.

How can we reduce interference?

a.   Sow wider plots: harvest only the core of the plot, discard the guard rows.
b.   Grouping of cultivars: e.g. cultivars can be divided into a "short", "intermediate" and "tall" groups (if height is an issue) and tested in a split plot like design avoiding tall cultivars neighbouring short cultivars (see David & Kempton 1996).
c.   Use of covariates: correlated traits (plant height!) can be used as covariates, which could have an adjustment for competition (see Goldringer et al. 1994).
d.   Modelling of neighbour-effects: fit linear model with additive effects for cultivar and neighbour and calculate adjusted estimate for pure stand

An additional problem can occur at the front of plots. The plants located at the edge of plots have much better conditions to grow, because of the additional amount of light and nutrients available at the alleys between blocks. If genotypic differences are expected in using these better growing conditions, the front area of the plots should also be discarded (see Fig. 4).

Fig. 4.   Plots with core areas and different types of borders and guard areas.
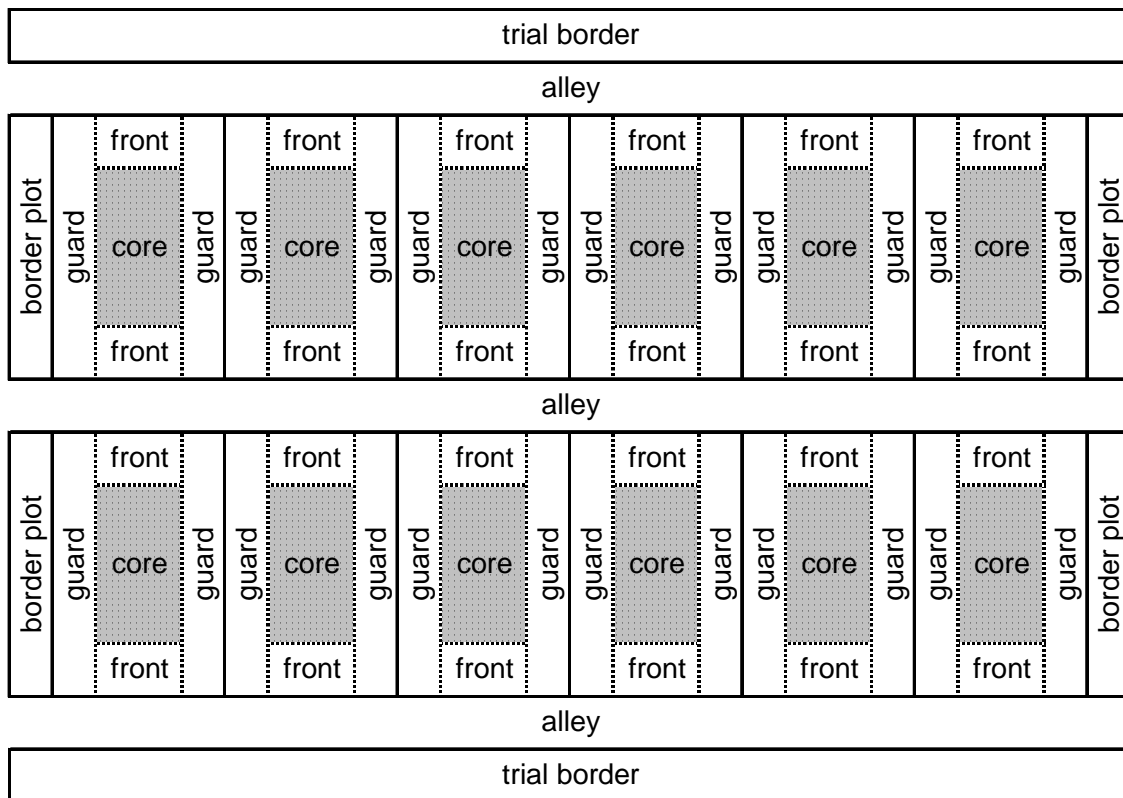
3.   Analysis of data

3.1.   Evaluation of data

3.1.1. Check for errors and assumptions

Every statistical analysis of trial data needs some assumptions to be fulfilled, otherwise the conclusions may be false. Among these assumptions the most common (for analysis of variance) are:

- independence of observations,
- normality of distribution,
- additivity of treatment and block effects,
- homogeneity of variances,
- lack of outliers.

Independence.

In the majority of statistical methods used for analysis of trial data, the independence of observations is a key assumption. On the other hand, it is commonly known that – for example in field trials – observations from adjacent plots are likely to be more similar than observations distant from each other. So, usually observations are correlated. Luckily a proper randomisation prevents statistical analysis from giving biased results. There are some statistical tools to detect correlations (lack of independence) between observations but for above-mentioned reasons there is no need to present them here.

Normality.

All the tests used in analyses of variance and analyses of regression are based on normality assumption. Normality means that the distribution of observations is "bell shaped" for all treatments under comparison. Mead et al. (1983) say "in most situations it is impossible to decide by examining the data whether the assumption of normality is reasonable and one has to rely on common sense in arguing whether the assumption is biologically likely". So this assumption is rather difficult to be verified unless the sample size is very large. There are some tests for checking this assumption but all of them are rather weak (in the sense that they very rarely reject the null hypothesis) when sample sizes are small and even moderately large. So they can be applied only for large sample sizes (sample size tending to infinity). As in routine experimentation the number of replicates is small (usually smaller than 6) and the sample size for a particular treatment is of the same order, the use of such a test is not possible. Graphical presentation of data can provide a visual inspection for lack of normality. Luckily the tests used in the analysis of variance (as well as regression), namely the F-test and t-test, are resistant against moderate deviations from normality. A method that is often used to check normality is the Shapiro-Wilk test, which is recommended for sample sizes not larger than 50 (Shapiro and Wilk, 1965).

Additivity.

In the analysis of variance of block trials (CBD or IBD, see section 2.1) it is assumed that there is no interference between blocks and treatments. In practice this means, that differences between any two treatments are the same in all blocks in which they appear together and that possible fluctuations are caused solely by experimental error. This assumption is usually fulfilled if the differences between blocks are not very large. When blocks differ considerably, e.g. an average yield of 20 kg/plot in one block and of 50 kg/plot in the other block, it is not reasonable to expect that the difference between two varieties of 4 kg in the first block will be of the same magnitude in the second block. A simple test for non-additivity in a CBD design was proposed

by Tukey (1949), known as "one degree of freedom for non-additivity". In this approach the sum of squares for error is subdivided into two parts. One is attributed to non-additivity, the other to the residual. Then, using the usual Fisher F-test with one degree of freedom for the numerator, the hypothesis that there is lack of additivity is tested. In the case of multiplicative effects, a logarithmic transformation can improve the situation.

Homogeneity.
The typical assumption in an analysis of variance is that the treatments do not influence the variance of experimental error, in other words that the variance is the same for all treatments. This assumption is likely to be fulfilled when levels of expression are similar for all treatments. When levels of expression (mean values) differ considerably between treatments, normality and additivity as well as homogeneity of variances can be violated. This assumption can be verified using Bartlett's or the Cochran test. In both tests, the estimates of variances are calculated for all treatments and next the hypothesis of equal variances is tested against the alternative that some of them (at least one) are different.
If the variances (standard deviations) are related to the level of expression (mean values) of the characteristic that is analysed, a logarithmic (or square root) transformation can improve the situation.

Outliers.
All the statistical analyses of trial data are carried out (possibly after checking all underlying assumptions) assuming that all collected data is correct. However, this is not always the case. Errors can occur when recording, copying or preparing data for computer processing. When such an error observation is out of the expected range of observations it is easily detected by a visual inspection of the data. Sometimes it can be detected after preliminary analysis, for example if such an observation "produces" an extremely high residual. In general, an observation is considered as an outlier if its value differs considerably from all other observations. If the value of one (or more) observation is far from the cloud of all other observations it is likely to be an outlier. The easiest statistical method to detect outliers is as follows:

a.   order all *n* observations in ascending or descending manner,
b.   temporarily remove the 'suspected' observation from your sample (it is either the smallest or the largest observation),
c.   calculate the (1-α) confidence limits for single observations by using the rest (*n*-1) of the observations (see footnote [1])
d.   if the 'suspected' observation is out of the calculated confidence limits, it is considered as an outlier and the reason for this should be checked. If the deviation is caused by a simple typing error, the error should be corrected. For other reasons such as damage to the plot caused by external factors independent of the treatment, the observation should be permanently rejected from the sample and be treated as missing data. If no reason can be found for the deviation the observation should be kept unless the deviation is so large that it will make the analysis unreliable (in such cases it may be wise to run the analysis twice – both with and without the outlying observation to see if the conclusion will change).

---

[1] For normal distribution, the lower X_* and upper X^* confidence limits are of the form:

$$X_* = \overline{X} - t_{\alpha/2}s \qquad\qquad X^* = \overline{X} + t_{1-\alpha/2}s,$$

where $\overline{X}$ is the mean value calculated over (n-1) observations, $t_{\alpha/2}$ and $t_{1-\alpha/2}$ are the Student t-distribution table values with (*n*-2) degrees of freedom and *s* is the standard deviation calculated over (*n*-1) observations. If there are more such "suspect" observations, the whole procedure can be repeated.

3.1.2.   Usefulness of the data for investigations

The choice of the most appropriate data to answer the question put in the investigation is not always simple and straightforward. In most cases, the investigator is forced to accept a compromise between the precision of the conclusions and the cost of the data. Clearly, cheap data is often sufficient to answer simple questions. However, this does not mean that expensive data will guarantee better reliability and accuracy of results and conclusions.

In general, the data will be useful for the investigations if the experiment in which they have been collected was properly designed. If the experimental design is faulty, no data cleaning, filtering, outlier detection or other processing techniques will be helpful. Also, no statistical method of data analysis is going to help to make proper conclusions. Statistical handbooks are full of recipes of how to properly plan experiments. It is noteworthy that rules as old as the ones given by Finney (1953, p. 173) are still valid.

Data used in statistical analyses are observations of random variables. The statistical procedures work only if there is a variability of the observations. The source of this variability must be known to the investigator if the conclusions are to be sensible. Thus, data obtained from carefully designed experiments are more valuable than data from observational studies or extracted from databases with an incomplete description of origin. A helpful discussion of this problem in the context of regression is given by Gomez and Gomez (1984, p. 417).

3.1.3.   Transformations.

When one or several of the mentioned assumptions is violated, the performed analysis is incorrect and decisions may be false. If, using some statistical tool or just after visual inspection of the data, deviation from the standard situation is detected, it is sometimes possible to 'improve' the situation by transforming data and then analysing the transformed data. Depending on which assumption is violated, several transformations may be applied. The most commonly used transformations are:

Logarithmic transformation.
This transformation is appropriate for data in which there is proportionality between mean values and standard deviations or when effects are multiplicative. Typical examples of such data (see Gomez and Gomez, 1984) are data concerning the number of insects per plot or the number of egg masses per plant (or per unit area). To transform original data (X), into a logarithmic scale (Y), simply take $Y = \log (X)$. When some observations are small (smaller than 10), the transformation $Y = \log (X+1)$ is suggested. The fulfilling of all assumptions must be again checked for the transformed data. If there are no serious deviations from assumptions, all the analyses and tests are applied to the transformed data. After performing treatment comparisons the mean values can be re-transformed into original scale.

Square root transformation.
This transformation is useful and effective for data for which variance tends to be proportional to the mean. This transformation can be applied for data consisting of small whole numbers. Such data appear when rare events are counted (in limited time or space). Typical examples are the numbers of weeds per plot (or per square metre) or the number of insects caught in traps. The square root transformation may also be appropriate for percentage data where all observations are in the range from 0% to 30% or in the range from 70% to 100%. For intermediate data (all observations between 30% and 70%) this transformation is usually not necessary. To apply this transformation simply calculate $Y = \sqrt{X}$ when all observations are in the range between 0 and 50 and calculate $Y = \sqrt{100 - X}$ when all observations are in the range between 50 and 100. Again

all the analyses are performed using transformed data. The final results (treatment means) can be presented on transformed scale or can be re-transformed to original scale.

The arcsine transformation.

To perform this transformation, use the formula $Y = \text{arcsine}\sqrt{X/100}$. This transformation is appropriate for data concerning fractions and expressed as percentages. Usually data obtained by dividing two counts (e.g. number of deceased plants and total number of plants) can be transformed using this transformation. The extreme values of 0% and 100% are to be substituted by (1/4n) and (100-1/4n) respectively before using *arcsine* transformation. This transformation can be easily performed using a computer or the tables of C.I. Bliss (1934) reproduced in many statistical textbooks. Because percentage data can also be transformed using other transformations, the practical advice is as follows (Gomez and Gomez, 1984):
-    for percentage data from the range between 30% and 70 % no transformation is needed,
-    for data sets where all data are in the range between 0% and 50% (or between 50% and 100%) the square root transformation is appropriate (see the text above),
-    for the data that do not belong to any of above-mentioned ranges the *arcsine* transformation is to be used.

The logit transformation.

This transformation is applicable for percentage data expressed as fractions. To perform this transformation simply apply formula $Y = \ln(X/(1-X))$, where X is the fraction to be transformed. Please note that this transformation is undefined for X=0 and X=1. This transformation is much simpler but almost indistinguishable from *probit* transformation described by Bliss [1934]. The logit transformation may be used to analyse the relative number of insects killed by different doses of an insecticide.

Additional remark.

If there is lack of homogeneity of variance in a data set and there is no relationship between means and variances (standard deviations), a possible solution is to split treatments into groups with similar (homogeneous) variances and perform independent analyses of variance for each of these groups or apply more advanced methods such as weighted analyses of variance or methods that allow the variance to be different (by using some approximations).

Instead of applying transformations to the recorded observations some characteristics e.g. percentages and counts may alternatively be analysed using generalised linear mixed models (see section 3.5).

3.2.   Methods for analysis

3.2.1.   Analysis of variance, F-tests, LSD-values

3.2.1.1. Randomised complete block design
Analysis of variance (ANOVA) is the main tool used for statistical interpretation of agricultural trial data. The analysis of variance is based on linear model of observation. For experiments performed in a randomised complete block design (CBD), the linear model is of the form

$$y_{ij} = \mu + \tau_i + \beta_j + e_{ij} \tag{1}$$

where y_{ij} denotes the value of observed trait for the i-th treatment ($i=1,2,…, t$), received in the j-th block ($j=1,2,…,r$) with a total number of observations $n = rt$; τ_i is the fixed effect of the i-th treatment, β_j is the effect of the j-th block and e_{ij} is an experimental error associated with observation of the i-th treatment in the j-th block.

Different assumptions can be made on the block effects β_j.
If the assumption is that β_j is fixed, meaning that the only random term in (1) is e_{ij}, the model is called fixed. In that case all conclusions are confined to treatments and blocks used in the analysed experiment.
More common is to consider β_j as the random component of model (1). In this case the model is called mixed. Such a model can be set up using the principle of randomisation, see Caliński and Kageyama (2000).
In the mixed model the blocks are treated as a random sample of an infinite set of all possible blocks and conclusions are not confined to the blocks actually used in experiments. The conclusions are "valid" in the population of blocks from which the blocks can be considered as a random sample.
Analysis of variance of trial data is based on a division of the sum of squares of total variability (SS_c) into a component attributed to blocks (SS_b) a component attributed to treatments (SS_t) and to the error (SS_e) according to the equality

$$SS_c = SS_b + SS_t + SS_e \tag{2}$$

Usually the main aim of the analysis of variance is to test the hypothesis, that there are no differences between treatments under comparison, namely the hypothesis

$$H_0: \tau_1 = \tau_2 = …. = \tau_t \quad \text{against} \quad H_1: \text{"}H_0 \text{ is not true"} \tag{3}$$

This hypothesis is always tested by application of a Fisher F-test of the form

$$F_0 = MS_t / MS_e,$$

where MS_t and MS_e are the mean squares for treatment and error respectively. Usually the results of ANOVA are presented in an analysis of variance table as in table 2.

Table 2.   Analysis of variance for a randomised complete block design (CBD)

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Blocks	r-1	SS_b	MS_b	
Treatments	t-1	SS_t	MS_t	F_0
Error	(r-1)(t-1)	SS_e	MS_e	
Total	n-1	SS_c	-	-

If $F_0 > F^{\alpha}_{(t-1);((r-1)(t-1))}$, where $F^{\alpha}_{(t-1);((r-1)(t-1))}$ is the critical value of the F distribution for (t-1) and (r-1)(t-1) degrees of freedom at α significance level, the hypothesis (3) is rejected, meaning that not all treatments are the same (some treatments differ from the others). If hypothesis (3) is rejected, the researcher is usually interested to identify which pairs of treatment are different. To answer this question usually the so-called least significant difference (LSD) is calculated. If the researcher is interested in one particular comparison (that was chosen before establishing the experiment), the best way is to calculate the Fisher LSD^F, using formula

$$LSD^F = sqrt(2*MS_e/r)*t^{\alpha}_{v},$$

where MS_e is taken from the analysis of variance table and t^{α}_{v} is the two-sided t-Student distribution critical value at α significance level for $v=(r-1)(t-1)$ degrees of freedom. If the absolute value of the difference between treatment-means calculated for e.g. treatment *1* and *2* is bigger than LSD^F, these two treatments are declared significantly different at α significance level. If more than one comparison with the use of LSD^F is made, the general significance level (for all comparisons) is larger than α.

If many comparisons between treatments are planned, it is recommended to use a method that minimises the risk of erroneously declaring pairs significant, such as the Tukey LSD^T which is of the form

$$LSD^T = sqrt(MS_e/r)*q^{\alpha}_{t,v},$$

where $q^{\alpha}_{t,v}$ is the critical value from studentised range distribution read at α significance level for t treatments involved in comparisons and v degrees of freedom (degrees of freedom for error in the ANOVA table).

The rules of using LSD^T are the same as for LSD^F, but now all treatment comparisons can be made and still ensure that the risk of erroneous declaring any of these significant will be less than α.

3.2.1.2. Incomplete block design (alpha design).
A slightly more complicated situation appears in the case of incomplete block design (which includes the alpha designs). Because blocks and treatments are not orthogonal to each other (which it was in CBD), the division of the total sum of squares into parts attributed to blocks and treatments is not unique. Usually the ANOVA table instead of single sum of squares for blocks (as in CBD), will mention two sums, the first attributed to complete replicates (superblocks), the second attributed to blocks (within superblocks) – ignoring treatments.

The linear model of observations in alpha design is of the form

$$y_{ijk} = \mu + \tau_i + \rho_j + \beta_{jk} + e_{ijk} \tag{4}$$

where y_{ijk} denotes the value of the observed trait for i-th treatment received in the k-th block within j-th replicate (superblock), t_i is the fixed effect of the i-th treatment ($i = 1,2,...,t$); ρ_j is the effect of the j-th replicate (superblock) ($j = 1,2,...,r$); β_{jk} is the effect of the k-th incomplete block within the j-th replicate ($k = 1,2,...s$) and e_{ijk} is an experimental error associated with the observation of the i-th treatment in the k-th incomplete block within the j-th complete replicate. There are $n = rt$ observations in total. The whole experiment consists of rs incomplete blocks forming r complete replicates. The whole discussion concerning randomness of blocks in randomised complete block design also applies to incomplete blocks and complete replicates in alpha design. In accordance with the linear model of observations (4), the analysis of variance is usually presented in the form given in table 3.

Table 3.   Analysis of variance for alpha design

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replicates	r-1	SS_r	MS_r	
Blocks (within replicates, ignoring treatments)	rs-r	SS_b	MS_b	
Treatments(adjusted for blocks)	t-1	SS_t	MS_t	F_0
Error	rt-rs-t+1	SS_e	MS_e	
Total	n-1	SS_c	-	-

The term "ignoring treatments" means that the sum of squares for blocks is not free of treatment effects. Instead of the sum of squares for treatments (as for CBD), the sum of squares for treatments adjusted for block effects appear. It means that this sum of squares is free from block effects. The hypothesis tested is the same as in CBD (see (3)) and it is verified in exactly the same manner using a Fisher F-test. The value of $F_0 = MS_t / MS_e$ is now compared with the critical $F^{\alpha}_{t-1, \, rt-rs-t+1}$ value with $t-1$ and $rt-rs-t+1$ degrees of freedom. Treatment means are now not just simple averages over replicates as in CBD but are "adjusted". This adjustment is different for a fixed model of observation (in so-called intra-block analyses) and for a mixed model (in analyses with recovery of inter-block information). Additional difficulties arise when LSD is applied for treatment comparisons. Due to the lack of orthogonality, the variances of treatment comparisons (treatment contrasts) will often be different for different pairs of treatments. So in an extreme case for every pair of treatments a specific LSD (Fisher or Turkey) should be applied. However for moderate variations it may be acceptable to average the variance of treatment-comparisons and then use the average LSD value. But in this situation comparisons must be made with special caution. Usually the design is chosen so that the difference between the largest and the smallest variance of treatment comparisons is as small as possible. This means that balanced designs are preferable

3.2.1.3. Split-plot design

As described in 2.1, the split-plot design is applicable for two-factorial trials. The mathematical model of observations reflects the situation that experimental units (plots) of two different sizes appear. This implies that two different errors related to these plot sizes are present in a model of the form

$$y_{ijk} = \mu + r_i + a_j + \eta_{ij} + b_k + (ab)_{jk} + e_{ijk} , \qquad (5)$$

where y_{ijk} denotes the observations from experimental unit from i-th block (i=1,2,…r), concerning j-th level of main plot factor A (j = 1,2,…,a) and k-th level of sub-plot factor B (k = 1,2,…,b). r_i is the random effect of i-th block; a_j is the fixed effect of j-th level of factor A; b_k is the fixed effect of k-th level of factor B; $(ab)_{jk}$ is the fixed effect of interaction of j-th level of factor A with k-th level of factor B, and, finally, η_{ij} and e_{ijk} are the errors connected with main plots and sub-plots respectively.

There are n = rab observations in total. The analysis of variance of split-plot data is based on the division of sum of squares of total variability SS_t into the following components

$$SS_t = SS_b + SS_A + SS_\eta + SS_B + SS_{AB} + SS_e, \qquad (6)$$

where SS_b, SS_A, SS_η, SS_B, SS_{AB} and SS_e denote sums of squares attributed to blocks, factor A, main-plot error, factor B, interaction of factor A and B, and sub-plot error, respectively.

The traditional form of the related analysis of variance table is as follow:

Table 4.   Analysis of variance for split-plot

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Blocks	r-1	SS_b	MS_b	
Levels of factor A	a-1	SS_A	MS_A	F_A
Error η	(r-1)(a-1)	SS_η	MS_η	
Levels of factor B	b-1	SS_B	MS_B	F_B
Interaction A*B	(a-1)(b-1)	SS_{AB}	MS_{AB}	F_{AB}
Error e	a(r-1)(b-1)	SS_e	MS_e	
Total	n-1	SS_c	-	-

Usually, in a split-plot design three hypotheses are tested. First, the hypothesis of no differences among effects of factor A is tested, then the hypothesis of differences among effects of factor B is tested and finally the hypothesis of no interaction between levels of factor A and B is tested. Formally, the hypotheses tested are:

no effects for factor A: H_{0A}: "$a_1 = a_2 = \ldots = a_a$"   against H_{1A}: "H_A is not true"

(the appropriate F statistics $F_A = MS_A/MS_\eta$ is compared with the F-distribution critical value at chosen α significance level with (a-1) and (r-1)(a-1) degrees of freedom),

no effects for factor B: H_{0B}: "$b_1 = b_2 = \ldots = b_b$"   against H_{1B}: "H_B is not true"

(the F statistic to verify it is $F_B = MS_B/MS_e$, with (b-1) and a(r-1)(b-1) degrees of freedom), and finally the hypothesis that there is no interaction between Factor A and factor B, namely:

no interaction between factor A and factor B: H_{0AB}: "$(ab)_{11} = (ab)_{12} = \ldots = (ab)_{ab}$"
                                                                                        against H_{1AB}: "H_{AB} is not true"

(the F statistic to verify it is $F_{AB} = MS_{AB}/MS_e$, with (a-1)(b-1) and a(r-1)(b-1) degrees of freedom).

After rejecting these hypotheses the researcher is "entitled" to make comparisons between levels of appropriate factors. The researcher can use either LSD^F or LSD^T values as a threshold of significance between levels. Here only formulas for LSD^F are given but they can easily be modified to LSD^T. So, to compare two levels of factor A, the appropriate LSD^F value is calculated as

$$LSD^F = sqrt(2*MS_\eta/rb)t^\alpha_\nu,$$

where t^α_ν is the two sided t-Student distribution critical value read at α significance level for $\nu=(r-1)(a-1)$ degrees of freedom. The rules to use this LSD are exactly the same as in one-factorial designs (e.g. in CBD design).
To compare two levels of factor B, the LSD is calculated using the formula

$$LSD^F = sqrt(2*MS_e/ra)t^\alpha_\nu,$$

where ν are degrees of freedom associated with MS_e, i.e. $\nu = a(r-1)(b-1)$.
If the hypothesis H_{AB} is rejected some additional comparisons are possible. One can compare two levels of factor A within the particular level of B, or two levels of factor B within the particular level of factor A, or any combination of levels A and B. The appropriate LSD value for comparing two levels of factor B within a chosen level of factor A is calculated using the formula

$$LSD^F = sqrt(2*MS_e/r)t^\alpha_\nu,$$

where ν are degrees of freedom associated with MS_e. The formulas for other comparisons can be found in the literature (see e.g. Gomez and Gomez, 1984). To apply LSD^T instead of LSD^F, the presented formulas can be easily modified in a similar way to the description for CBD design.

Additional remarks on application of the split-plot design.

When there are only two levels of factor A (or B), there is no need to calculate LSD values to make a comparison of these two levels as rejecting the hypothesis H_{0A} (or H_{0B}) means that the two levels differ significantly. Another way of analysing the data from split plot trial is to split the overall (described here) analysis into independent analyses made within each level of factor A. It means that if there are "a" levels of factor A, then "a" separate CBD analyses are performed. But such an approach has two disadvantages: (1) the number of degrees of freedom for error for each partial analysis is smaller than the number of degrees of freedom for error "e" in a full split plot analysis and (2) the separate analyses do not allow to test the presence of interactions between levels of factor A and B, which are often the most interesting.

3.2.2. Analysis of variance including covariates (ANCOVA).

One of the aims of the researcher in the choice of an experimental design, the choice of plot size and shape, the choice of a mathematical model of observation etc. is to decrease the variance of the experimental error. The estimate of this variance is the mean square for error MS_e (appearing in the ANOVA table and in the denominator of the F-test) and the smaller the value is, the higher the probability of rejecting the null hypothesis of no difference between treatments and the higher the chance of declaring significant differences between chosen pairs of treatments.

Analysis of co-variance (ANCOVA) is one method that may be used to reduce the size of the error MS_e. Analysis of co-variance is a method of analysis that can be used to eliminate effects resulting from variables in which there is no interest. An example of such a variable in field experiments is the number of plants in a plot. Different numbers of plants for different treatments can influence the final results and decisions. Assuming that there are two variables observed in an experiment, the main variable Y and the additional variable X where X can influence Y but X is not influenced by the treatments (e.g. measured before the treatments are applied), then ANCOVA may be used to remove (at least partly) the influence of X on Y.

Analyses of co-variance consist of three parts: analysis of variance for main variable Y, analysis of variance for additional (also called concomitant) variable X and regression analysis of variable Y on X. The mathematical model of observations in ANCOVA is the same as for ANOVA but is extended by a term related to regression. So, for an experiment performed in CBD this model is of the form:

$$y_{ij} = \mu + \tau_i + \beta_j + \gamma x_{ij} + e_{ij} \tag{7}$$

where the meaning of used symbols is the same as in formula (1) with the additional symbol γ used for denoting the common (for all treatments) coefficient of regression of the main variable on the concomitant variable and x_{ij} denotes the value of the concomitant variable observed for the i-th treatment in the j-th block. The x_{ij} is assumed to be fixed and not to be influenced by the treatments. Usually in ANCOVA three hypotheses are tested in turn:

− the hypothesis of no differences between treatments for the concomitant variable. If such differences exist, it usually means that values of the concomitant variable are influenced by treatments and ANCOVA should not be applied;

− the hypothesis that there is a significant linear relationship between variable Y and X. If there is no such relationship (regression is not significant), ANCOVA can formally be applied but is ineffective in decreasing the experimental error;

− the hypothesis that there are no differences between treatment-means for the main variable adjusted for values of the concomitant variable.

In a similar way the model for analysis of variance for alpha designs can be extended to the ANCOVA model. It is possible to include more concomitant variables.

When comparing treatments after an analysis of covariance, the variance on treatment comparisons is additionally influenced by different values of the concomitant variable for each treatment. The average influence of the concomitant variable on the variance of comparisons can often be applied. One of the possibilities is to apply the approximation proposed by Finney (1946). More information on the interpretation of ANCOVA analysis can be found in Little and Hills (1978).

3.2.3.   Regression analysis

Regression is a statistical method to describe the association between two or more observed variables (traits) or between one observed variable and a design parameter (such as the amount of applied nitrogen or the year in which the observation is recorded). In the situation of two observed variables it can be used to estimate the effect of one of them (the assumed predictor variable) on the other (the assumed response variable), by expressing the response variable as a function of the predictor variable. Which variable is taken as predictor and which as response is a matter of biological knowledge; the basic regression methods do not check these assumptions. The simplest choice of the function linking the two variables is a linear function. It is equivalent to assuming a constant change of the value of the response variable for each unit change of the predictor variable in the whole range of observations. If we denote the observations of the predictor variable by X_i, $i = 1, 2 ... n$, and the observations of the response variable by Y_i, the linear regression means that

$$Y_i = a + bX_i + e_i,$$

where a and b are regression coefficients, and e_i is a random deviation of the i-th observation of Y from the exact linear relationship. The values of a and b are calculated using the principle of "least squares". The process of calculation is sometimes called "fitting". Statistical significance of the regression coefficients can be tested by a t test. The equation implies that expectation (mean value) of Y_i is equal to $(a + bX_i)$. In mathematical statistics expectation expressed in terms of a variable (in this case - X) is called conditional expectation. Thus, the fitted regression function informs us about the expected (mean) value of the response variable for a chosen value of the predictor variable. The values of a and b can only be interpreted when X_i is measured without error, as the values of a and b are biased if the variable X_i is influenced by random variation – although the formula can be used for prediction in both situations.

Although regression analysis is a computational estimation method, it has several important connections with less formal graphical exploratory procedures. This is not strange knowing that any consideration concerning two observed variables can be conveniently illustrated by simple two-dimensional Y-X scatter plots. The role of graphical data exploration is two-fold:
−   before computation, the scatter plot can indicate if a linear relationship between variables is plausible,
−   after computation, the plot supplemented by the fitted regression line can tell which of the data points (units) are very close to the line, and which deviate considerably.

Moreover, a scatter plot can show many data set properties that affect the quality of the estimated coefficients and consequently the quality of the conclusions. The analysis may, as an

example, strongly depend on some data points, which are particularly influential in the sense that the result will be quite different without these points.

Or, the data points can form clusters which, when considered separately, would show no significant linear relationship between variables. Thus, it is strongly advised to use the graphs as an aid and a presentation tool whenever a regression function is fitted.

The regression line fitted by the computational procedure should be used with caution. In addition to simple graphical procedures described above, there are several diagnostic methods which can be utilised to check if the assumptions of the regression model are met and whether the obtained regression equation can be used to describe a biological process. Weinsberg (1985) describes several techniques designed to find problems with the assumptions and influential data points.

The general rules for simple linear regression can also be applied with several extensions to more complex situations. The most important generalisations are:
−   nonlinear regression, used when the relation between X and Y cannot be assumed to be linear,
−   multiple (linear or nonlinear) regression, used when one wants to study the influence of several predictor variables on the response,
−   multivariate regression, used in case of more than one response variable.

The regression equations in each of these three cases are straightforward generalisations of the linear equation. The fitting method is in most cases the same, based on the least-squares algorithm, and the conclusions about parameters are very similar. However, the simple scatter-plots cannot be used for critical assessment for a multiple or multivariate regression because a (2-dimensional) plot of the response variable against one predictor variable may be masked by a second predictor variable. This makes the more advanced diagnostic tools like the analysis of deviations or partial leverage plots more relevant.

Due to its simplicity, regression analysis is broadly used in all types of experimental studies. Unfortunately, it is also misused in several manners (see, e.g., Gomez and Gomez 1984, p. 416). Let us mention just the most common cases.

Firstly, the user must realise, that a necessary condition for regression analysis is some variability of the observations, both in X and Y. This variability must be caused by well understood or controlled sources (factors) if the regression equation is to be interpreted in a sound way.

Secondly, the fitted equation can be considered as valid only within the range of observed values of the variables; generalisations outside of this range are not justified.

Thirdly, in designed (replicated) experiments, the regression equation should be fitted to treatment (variety) means instead of plot observations in order to remove experimental error from consideration and because the interpretation may be difficult/wrong if the regression is calculated across several levels of variations.

Finally it should be noted that a significant regression coefficient (b significantly different from zero) does not prove that the predictor variable causes the variation found in the response variable unless the predictor variable is controlled by the investigator.

3.2.4. Generalised linear mixed models (GLMM)

Analyses of linear regression and analyses of variance, as described above, rely on models that express the response variable (e.g., yield) as a sum of:
−   the so-called linear predictor, which is a linear function of parameters (that are fixed but unknown, such as regression coefficients) and random variables (such as block or sub-block effects in a mixed model of results of replicated experiments)
−   the residuals, which are assumed to have a normal distribution.

Such a formulation implies that the expectation of the observed variable itself is a linear function of the parameters and variables included in the predictor. Although linearity and normality are often acceptable approximations for many continuous variables, and many significance testing methods are quite robust against violation of these assumptions, there are situations in which it is better to do the analysis using a more general model. The formulation of the generalised linear mixed model (GLMM), as described e.g. by Engel and Keen (1994), allows for this, because it assumes that:
−   the expectation of the response variable is related to the linear predictor through the so-called link function (e.g., logarithm),
−   the residual variability follows one of the distributions belonging to the exponential family, e.g. a binomial, Poisson or gamma distribution.

Initially, the classical linear model of observations, involving only fixed effects, was extended to a generalised linear model (GLM) (see e.g. McCullagh and Nelder, 1989). After realising that inclusion of random effects in GLM can be equally helpful as in linear models, the generalised linear mixed model (GLMM) was described (see Breslow and Clayton, 1993; Engel and Keen, 1994). Appropriate statistical procedures of estimation and hypotheses testing were developed, and GLMMs can now be fitted and analysed using statistical systems such as SAS or Genstat.

Several examples of GLMM applications to biological problems can be found in literature. One of them is the analysis of disease incidence data described by Piepho (1999). The author considers a situation, in which an experiment is designed with three replications to compare the effect of six treatments against downy mildew of grape. In each plot, five randomly chosen shoots from each of three vines were scored for mildew by counting m, the number of leaves with at least one mildew lesion and the total number of leaves per shoot, n. Two ways of modelling the data are considered:
a) a linear mixed model for the observed disease incidence m/n,
b) a generalised linear mixed model, in which the logit function of unknown probability of disease incidence, $log[\pi/(1-\pi)]$, is assumed to depend linearly on the fixed treatment and block effects, random effects of plots, random effects of vines within plots, and random errors caused by sampling of shoots within vines.

Under b), two sub models are discussed, which differ in the definition of "shoots within vines" effect. One of them involves the so-called over dispersion parameter, which here describes the extent to which the variances on the recorded values exceed those expected in the binomial distribution. The other sub model assumes a random effect of each shoot.

According to the author's final remarks, the analysis using GLMM is not much different in interpretation from the one using a linear model. In order to set up the analysis, a basic knowledge of similar rules is necessary. The advantage is that the parameters of GLMM may have a better interpretation; a disadvantage is that some statistical tests are valid only asymptotically (for large samples).

A similar problem is considered by Madden (2002), who gives some general rules on superiority of different GLMMs in the situation of an experiment conducted over five years to study the effects of different fungicide treatments on the control of Phomosis leaf blight of strawberry. The recorded variable was the number of diseased leaves in a sample of 15 leaves, representing a given plot and treatment. In the formulation of GLMM the logit function was used.

Another example of an interesting application of GLMM is given by Candy (2000). The author describes a study of incidence of some insects in tree leaves. The experiment consists of a multi-level sampling of plots within compartments of the plantation (trees within plots, branches within trees and shoots within branches), to count the number of leaves on the shoot occupied by insects. As the total number of leaves per shoot is very large and counting them is impractical, the response variable here is assumed to have a Poisson distribution. The logarithm of the expected number of affected leaves per shoot is modelled by a linear function of fixed compartment effects and random plot effects. Apart from the estimation of model parameters, the analysis described in the paper is meant to give hints on a better design of the experiment, in particular about an optimal relation between the number of sampled plots and sampled trees within plots.

Finally, GLMM can be applied to predict weed intensity in the field based on soil properties and counts of weeds observed over years, in the context of development of site-specific farming techniques, described by Christensen and Waagepetersen (2002). Here, the model is a spatial one. A GLMM with the Poisson distribution and the log link function is used to account for a non-normal distribution of the response variable.

3.3.   Multi-environment trials (MET)

Multi-environment data originates from replicated experiments carried out in several years, at a number of sites, or in different environments defined by e.g. agricultural practice. Although in each case the observations are classified by environments, treatments and replications, the required analysis may be different for different meanings of the word "environment". Usually, full analysis of MET data with estimation and significance testing is completed for traits that are continuous and normally distributed, such as yield. Linear mixed models provide the most general analysis framework for such traits (Searle, Casella and McCulloch, 1992; Denis et al. 1997). Utilisation of linear models with only fixed effects may not be satisfactory due to the random nature of environmental sources of variation.

Most of the MET data are collected to study behaviour of plant genotypes (varieties, lines) in different environments. The analysis of such data can be done using two different approaches:
(a) a two-stage analysis, in which the data from all environments (experiments) are first analysed separately, and the estimated mean values are collected for the second stage devised to answer questions about the treatment-environment interaction,

(b) a one-stage analysis, in which plot-level data is modelled and analysed to give answers about the main effects and interaction.

Appropriate instructions for (a) are given e.g. by Patterson (1997). The methods for type (b) are described by Smith et al. (2001) and Caliński et al. (2005). The estimation method used extensively in mixed models for MET data is the REML algorithm (Patterson, Thompson 1971). The advantage of the recently developed approach (b) over the more traditional one (a) is that all observations are analysed within one model in order to estimate the parameters of interest and to test the corresponding hypotheses. A disadvantage of the one stage analysis (b) is that it is computationally more intensive and that it may require special algorithms.

Independently of the actual method of analysis, the MET data are collected to give answers about the variety x environment interaction. This interaction is defined as a differential response of genotypes to conditions in different environments. The presence of an interaction of a particular genotype with environment can also be understood as a situation, in which the genotype's reaction to the environments is different from the mean reaction of a set of reference (standard) genotypes or the mean reaction of all genotypes included in the trials. This definition implies a practical requirement for the trials: the set of genotypes used in different trials should be as uniform as possible. Although the REML algorithm can treat data even with a very incomplete (non-orthogonal) structure, caution should be taken when the variety x environment table contains many missing values.

The estimated genotype x environment interaction parameters, if statistically significant, can be submitted to some additional analyses aimed at explaining the nature of interaction. Very often the joint regression analysis (JRA, Eberhart and Russel 1966; Shukla 1972) is used for that purpose. JRA tries to explain genotype x environment interaction by an environmental index, usually calculated from the mean values for the environments. However, it should be noticed that a good determination of interaction variability by regression on such a simple index is seldom satisfactory. Therefore, more complicated indices are formed; for this task, the knowledge of weather and soil characteristics of the trial locations is extremely helpful.

Finally, one should acknowledge the importance of several explorative or analytic methods in the analysis of MET data. An initial component analysis of genotype x environment interaction deviations, and its graphical representation in the form of a bi-plot (Kempton 1984), can be very helpful in discovering advantage or disadvantage of genotypes for particular environments. Experience with using other geometrical methods is reported by Westcott (1986).

3.4.   Analysis of data recorded on a discrete scale

Several traits important for the behaviour and quality of genotypes are expressed on a discrete scale, usually from 1 to 5 or from 1 to 9. As an example we can take disease severity, which is visually assessed as percentage of the area (of plant or leaf) affected and recorded as a number from 1 to 9, according to a rating scale (see chapter Disease assessment page D 11). Another example is disease incidence measured as the percentage of the affected plants in the plot (see chapter Disease assessment page D 10). Statistical analysis of such data is not always straightforward, because the measurement scale may cause problems with the assumption of normality underlying several procedures. Therefore, some researchers do not carry out formal significance tests for disease or quality traits. This practice is acceptable, because in most of the

experiments the trait of primary interest is the yield, and ranking of the treatments for additional traits can provide sufficient basis for the breeder's decision. However, if the statistical analysis of the discreet scale traits is interesting, the following solutions are possible.

In an analysis of replicated experiments, assuming that the unit of measurement is plant or leaf as it is the case for disease severity, there are several measurements per plot, which can be averaged to provide the plot observation. Such means may be assumed to behave as a variable measured on a continuous scale, and can be subjected to analysis of variance, possibly after a transformation. If the unit of measurement is a plot, there is only one observation, and for such data, analysis of variance should not be used. A possible solution is to create replicated observations by sampling within experimental plots (Gomez and Gomez, 1984, p. 532).

In a regression analysis, estimation and testing of regression coefficients should not be done on plot data, but on treatment (variety) means estimated from the analysis of variance model. Distribution of such values can be approximately normal.

In multi-environment data analysis, mean values for treatments over replications within environments can also be considered as approximately normally distributed. Therefore, the analysis of main effects of treatments can be completed using the analysis of variance if the significance of these effects is tested by comparison with the treatment x environment interaction.

For some of the traits there is a possibility to keep the observations in the form of counts (of units affected out of total number of units investigated). Very often, it is found that such observations have a binomial or Poisson distribution and can be modelled by GLMM as described in Section 3.5.

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WEED COMPETITIVENESS

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1.   Introduction

In many European countries tests and trials are undertaken in order to know more about the genotypic effect on weed competitiveness and several studies have shown that varietal differences do exist. The selection of cereal varieties for competitiveness against weeds under organic conditions requires the identification of relevant crop characteristics (or traits) and the development of routine methodologies to measure them. An adequate evaluation and brselection for weed suppression will be an advantage to farmers practising integrated methods of farming, as well as benefit the breeding of suitable varieties.

Weed suppression cannot be attributed to one single characteristic but is the result of the interaction between a series of desirable characteristics. In the following paragraphs the theoretical basis and the practical methods to measure the different components of weed competitiveness will be discussed. Three main tools of weed control will be used as a continuous thread throughout these paragraphs:

- Plant physiology
- Allelopathy
- Harrowing

1.1   Plant physiology

The evaluation of new varieties in relation to weed suppression is mainly based on the above-ground characteristics of the crop. The competitive ability of the crop against weeds is clearly also determined by the growth and development of the root system (Wilson, 1988). However, root competition is relatively poorly understood, difficult to evaluate and very little is known about the genotypic variation between varieties. However, Bertholdsson and Jönsson (1994) found in a study of 25 barley and 25 oat cultivars that the relative growth rate of roots explained more than 50 % of the variance in weed biomass in barley, while the differences in shoot biomass did not explain any of the variance. In oat equal amounts of variance were explained by the root and the shoot growth rates.
Some varieties have higher weed suppression than others, though this is not attributed to one single characteristic. Rather, it is the interaction between a series of desirable characteristics that results in competitive ability against weeds, and varieties may compensate for weakness in one component with strength in others.

- *Crop ground cover*

The main above ground feature for competing against weeds is crop ground cover.

Crop ground cover is inversely related to weed ground cover and associated with the light interception of the crop. In other words: the more ground covered by the crop, the more shading and the higher the weed suppression.

Ground cover at early tillering is strongly correlated with weed suppression throughout the season. A high season-long crop ground cover is important for adequate weed competitiveness.

Ground cover measured from above the crop is a good indicator of shading characteristics and can be used as a parameter. However, total leaf area index (LAI) or green area index (GAI) are also good correlants with shading and weed suppression.

Crop ground cover is the result of a range of individual plant characteristics, such as growth habit, tillering capacity, rapid early growth and plant height. The balance between these components will determine the value of a variety for early, late and season-long weed control, and the suitability for specific climatic zones.

- *Growth habit*

An early prostrate habit (at the start of tillering) combined with a moderate to high leaf area index (either through rapid leaf development or good crop establishment) has been determined to be closely linked with reduced weed growth and seems to be a good indicator of competitive ability (e.g. in Scotland) (Davies et al, 2004 and Hoad et al, 2006). As the shading effect at this stage is low, it is likely that the root system plays an important role in this competition.

An erectophile habit at early tillering tends to require a higher crop establishment to be as equally competitive as an early planophile. [2]

The importance to combine an early prostrate habit (at the start of tillering) with a moderate to high leaf area index is not relevant under all growing conditions and also depends on the type of plant traits that are present in the available varieties.

In some countries (e.g. Austria) a minority of the varieties combine the requirements described above. Genotypes with a prostrate habit at BBCH 28 often show small leaf areas, begin to elongate the stem relatively late and are shorter. They permit more light penetration to the soil at a later date then varieties with a semi-erect or erect habit.

Selection for specific growth habits is based on understanding the role of different characteristics in weed competition. Different growth habits will be needed for different soil, nutritional and cropping conditions (this will be discussed in 5.1.1.).

- *Tillering capacity*

Shoot population density, measured as the number of shoots per m^2, is a function of plant population density and the ability of plants to produce and retain tillers. Some varieties have a relatively high shoot density because of good establishment, whilst others produce a higher than average number of shoots per plant; some varieties may have both characteristics.

High tillering ability is likely to be most important at low plant densities. As organic seed is not treated for disease and pest control, establishment in adverse conditions can be reduced significantly. The tillering phase must not be too long and if high tillering means late stem elongation, the competitive ability will be reduced. Thus, as a single measure, shoot density density it is not always a good indicator of competitive ability. It has to be considered in combination with other parameters.

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[2]*Prostrate is a description of the plant habit and means lying on the ground, planophile is a description of the leaf habit and means tending to be parallel with the ground. A very early planophile habit is the same as prostrate, but later planophile is not prostrate.*

- *Rapid early growth to stem extension*

Rapid early growth allows the crop to maintain a light interception and nutrient uptake lead over the rapidly growing weeds, and in combination with the right habit, shade newly emerging weeds. Rapid early growth of shoot is also related to the root development. An early root development is important for the competition of water and nutrients. During plant establishment this may be more important than the competition for light, especially in organic production with less availability of nutrients (Bertholdsson and Jönsson, 1994). Ground cover by the crop at the end of tillering is strongly correlated with weed suppression up to full canopy cover and up to harvest.

The date of end of tillering is related to local climatic conditions. In southern European regions, depending on sowing time, it ends between the beginning of February and mid March. As the weed emergence follows the same local pattern, it is likely that the correlation remains the same, albeit at an earlier stage.

Rapid early growth can be evaluated by recording the time (in days or weeks) it takes to reach a key growth stage or a certain stem length. The biomass production of varieties can be evaluated by a visual ranking or by using a scanning instrument such as Cropscan (*Cropscan Inc.*).

- *Plant height*

Although there is no clear evidence of plant height alone being able to increase crop competitive ability, tall varieties appear to be competitive at moderate to good plant densities. However, early stem elongation is more important than tall straw at maturity.

Height can compensate for an erectophile leaf habit, but a relatively short (and late season) planophile habit can give the same shading rate and weed suppression of shorter weeds. Tall varieties may have an advantage over some very tall grasses and scrambling weeds. On the other hand, tall varieties may cause problems, such as lodging, especially in winter sown crops at lower and medium latitudes.

The WECOF project ("Strategies of Weed Control in Organic Farming" www.wecof.uni-bonn.de) has indicated that crop ground cover (and shoot density) are good measures of crop competitive ability. The effect of crop height, however, is not consistent, most likely because changes in height are associated with changes in other characteristics such as plant growth habit. Plant height, does appear to compensate for low values in other crop characteristics such as low plant or shoot density.

1.2   Allelopathy

Allelopathy is an important mechanism of plant interference through the release of plant-produced phytotoxins to the plant environment. Chemicals with allelopathic potential are present in most plants and not the least in cereals. The allelopathic potential of rye is well known but also oat, barley and wheat show allelopathic properties to a varying extent. Even if the phenomenon of allelopathy has been known for a long time (Molish, 1937) very little has been done to exploit it in breeding.

Recent studies in wheat and barley show that allelopathy together with morphological traits can explain a large part of the genetic variation in weed competitive ability (Olofsdotter et al, 2002, Lemerle et al, 2001, Bertholdsson 2005). The explained variance from morphological and allelopathic traits varies between years. But if the two traits are combined in a multiple regression model the year to year variation is reduced (Bertholdsson 2005).

1.3   Harrowing

Harrowing can give good weed control in various agro-environmental conditions, on both autumn and spring-sown crops. The reliability of harrowing depends on factors that influence both its weed control efficacy and tolerance of the crop.

In variety trials the main purpose is to evaluate differences between varieties in their tolerance to harrowing. Comparative variety trials of durum wheat have shown that these differences do exist (Faustini and Paolini, 2005).

The crop tolerance to mechanical injury is strongly related to the management of harrowing, while the level of weed infestation and the traits of the variety can also be important.. The better the management, the lower the risk of crop damage. Therefore it is important to have sufficient background knowledge on the factors that influence the efficacy of weed control by harrowing. A number of these factors are described hereafter.

- *The level and type of weed infestation.*

As any other non-chemical mean, harrowing is the most effective under low to moderate levels of weed infestation. Moreover, the lower the proportion of grass weeds (mainly fast early growing, aggressive species like *Avena* spp.), the better the harrowing efficiency. Infestations resulting in no more than 2 t ha^{-1} of weed dry biomass at crop harvest and with no more than 30 percent of final density due to grass weeds, are likely to be well controlled in most cases, at least in autumn sown-crops. Indirectly, low to moderate infestations also minimize the risk of significant crop damage by harrowing, as make effective less severe passes (see below).

- *Time of application and number of passes.*

In wheat, harrowing is normally applied post-emergence, once or twice mainly depending on sowing time (autumn or spring) and agro-environmental conditions (Cirujeda et al, 2003; Hatcher & Melander, 2003).

In general, harrowing is the most effective when applied as early as possible during tillering. A timely pass should give the best compromise between three requirements: (1) to injure weed plants as young as possible, when they are most sensitive to mechanical injury, mainly due to uprooting (Kurstjens and Kropff, 2001); (2) to injure the highest number of emerged weeds and (3) to pass through the crop when the plants have developed sufficiently well not to be seriously damaged. An early, timely pass also leaves a longer interval suitable for a further pass when needed.

In autumn-sown crops, where infestation levels are not high, just one timely pass can often allow good control, particularly on moderate levels of grass weeds. It should be applied with a crop soil cover ranging from 30 to 40 percent, i.e. about two weeks earlier than the standard time for herbicide spraying, with a second pass applied two weeks later if needed. In spring-sown crops, two passes as above (eventually after one applied just prior to crop emergence) are often required (Rasmussen & Svenningsen, 1995), as weeds tend to be more developed and/or at higher densities at the beginning of the period for harrowing. The shortness of this period (3-4 weeks) makes further passes impracticable.

- *Soil texture and moisture.*

In light soils (sandy to sandy-loam) a moisture content suitable for working (around 50 percent of field capacity in the upper 10 cms) can more easily occur during the winter and the first half of spring, while compaction is also lower. As a consequence, there are more chances for timely passes, whilst the risk of tine jumping (too low moisture and high compaction) or kneading (too high moisture), making weed control uncertain and crop injury higher, can be minimized. Moreover, in light soils tines find less resistance and work better.

- *Tine setting and working modalities.*
Tine diameter, tine inclination and working speed must be
optimized in order to achieve the best harrowing results.
Tine diameters range from 7 to 12 mm. The tine inclination
influences the work severity with a decreasing work severity
at higher angles to the vertical (see figure, Johnson, 2002).
Inclinations to the vertical in the range 15-30° ensure good
work in most cases. Harrowing parallel to crop rows minimizes
crop damage without any significant decrease of weed control.
The working speed may vary indifferently between 5 and
8 km/h (Cirujeda et al, 2003).
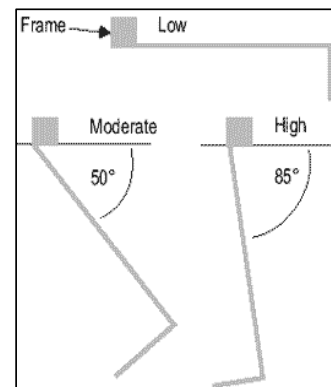
- *Integrating harrowing with other control means.*
Harrowing can be integrated with cultural means, i.e. any crop husbandry choice enchancing its
competitive ability As a higher competitive ability is always the result of higher early growth
rates (Lemerle et al, 1996, Paolini et al, 2002), more competitive crops generally have a higher
suppressive power on surviving weeds and higher tolerance to mechanical injury. The growing
of more competitive varieties represents the most promising cultural mean to be combined to
harrowing in integrated non-chemical control strategies. In fact, these varieties have no lower
yielding potential, nor undesirable agronomic traits (Lemerle et al, 1996), and there is direct
evidence that their growing improves the efficacy of harrowing and minimizes crop damage
(Faustini and Paolini, 2005). Other suitable cultural means are the use of higher plant densities
(Weiner et al, 2001) and, possibly, the growing of mixtures (Finckh et al, 2000).

2.   Description of methodologies

2.1.   Crop physiology

Depending on the crop (spring or winter) and the time available, one or more of the following
parameters can be used to describe the weed competitiveness of varieties:

- *Plant density after emergence*
Plant density after emergence should be evaluated if it appears too low in general or if there are
differences between plots. A method to evaluate a trial with considerable differences (> 20 %)
between plots, is to count the number of plants on a fixed surface (e.g. 0.25 m^2) of the plots with
the lowest and highest density (score 1 and 9) and assess all the plots with a score in this range.

- *Damage from harrowing*
Theoretically, crop damage results from a combination of various effects on the plant (uprooting,
burial, temporary growth reduction). Uprooting is the most important as, in general, plants
recover from burial 7-10 days after harrowing and non-uprooted plants recover quickly from
mechanical injury. The damage should be therefore evaluated at crop physiological maturity by
estimating the percentage of uprooted stems (see section 2.3 for more details).

- *Crop ground cover*

It can be estimated as a percentage or on a 1 to 9 scale at several intervals (e.g. every other week), starting from the moment that differences between varieties are visible (around BBCH 28) until the stage that the crop canopy is fully closed.

Crop ground cover is relatively quick to measure. Stand directly over the crop and visualise the crop as a 2-dimensional image of leaf and soil. Estimate the proportion that is leaf and express this as a percentage. Several measures should be taken per plot or section of crop. Although simple to measure, there can be considerable bias between assessors.

A comparison (carried out in the Netherlands) of three methods to measure groundcover (visual examination, a crop scan and a counting frame) showed that the visual assessment tends to over-estimate the highest score and under-estimate the lowest score.

Another possible but more laborious indicator to measure ground cover is biomass sub-sampling an area of 0.25 m^2 or single plant sampling. In Swedish trials the mean dry weight of 10 barley plants per plot showed a high correlation with the weed biomass ($r = 0.79$ with $p < 0.01$).

Instead of sampling, multispectral radiometry (e.g. *Cropscan*) can be used. In this case the plots must be weed free. Both the sampling and Cropscan-measurements should be carried out during stem elongation (BBCH 30-39) and a standard cultivar should be included.

At an early stage (between BBCH 28 and BBCH 49), it may be relevant to estimate the weed ground cover, for example when one part of the field has more and different species of weed than other parts. The data can be used as background information to explain yield differences.

- *Stem density*

Tillering capacity can be measured by counting stems on ten plants per plot at the beginning and end of stem elongation. It is best if plants are removed for inspection, though it may not always be practical and possible to separate individual plants, when many tillers have been formed.

Another option is to mark a part of the plot and count all plants at emergence and repeat the counting of all shoots at the same spot at the end of tillering. Number of shoots divided by number of plants gives an estimate for the number of tillers per plant.

Measures of stem density can be time consuming and plant counts are difficult to make once the crop has started to tiller. A single estimate of stem or plant number may require several minutes per plot.

- *Inclination of leaves:*

Can be measured on a 1 to 9 scale.

1   all flag leaves are rectilinear
3   about ¼ of the plants with recurved flag leaves
5   about ½ of the plants with recurved flag leaves
7   about ¾ of the plants with recurved flag leaves
9   all flag leaves are recurved

- *Leaf area index*

It can be estimated visually or measured (e.g. with a Sunscan or a Cropscan) at different growth stages. A Sunscan (*Canopy Analysis System of delta-T*) measures the fractional light inter-ception of the crop. The photosynthetically active radiation (PAR) below the crop is compared with incident light above the crop at the same moment.

The fraction of light intercepted by the crop and other leaf data information is used within the Sunscan to the estimate leaf area index. Generally, the Sunscan system gives a good estimate of leaf area index. However, differences between estimated and actual LAI will tend to increase when leaf canopies are patchy or when the plant growth habit is extremely erectophile or planophile.

Results obtained by Sunscan gave a good correlation with the indirect parameters of ground cover such as crop cover and crop height.

- *Plant height or canopy height*

Height can be measured at several times during stem elongation (e.g. BBCH 31-32) but should be measured at least once after full elongation. Measure height from the soil to the top of the leaf canopy (during stem elongation) or measure height from the soil to the base of the ear (after full elongation).

- *Growth habit*

Growth habit is relatively simple and rapid to measure, but one should consider a bias between different assessors.

The following 5-point scoring system (developed by the Scottish Agricultural College) for plant growth habit based on mean leaf angle can be used. This is a visual assessment of the whole crop or plot. The growth habits relate to mean leaf angle from the stem i.e. vertical.

Score	Leaf habit
1	erectophile habit with leaf angle $< 15^{o}$
2	tendancy towards erectophile habit with leaf angle $< 30^{o}$
3	intermediate, often with wide range of leaf angles 30-60°
4	tendancy towards planophile habit with wide range of leaf angles $> 45^{o}$
5	planophile habit with leaf angle $> 60^{o}$

- *Weed density*

Weed density can be estimated, counted or weighed.

An example from Sweden: all weeds are collected within two sample areas for each plot, with an area of 0.25 m^2 for each sample. Assess fresh and dry weights of the total amount of weeds (without roots).

- *Weed competitiveness index (WCI)*

In Denmark an index is calculated from three parameters:

WCI = Reflectance + LAI + Plant height

- *Special trials*

In Sweden special trials are carried out where part of the each plot in the organic variety trails is undersown with ryegrass to create a "simulated" weed population. The part sown with rye is 1.5 meter on a total plot length of 14 meter. At ripening stage a sample of 0.5 m^2 from each plot is harvested within the undersown area. This sample is sorted in three fractions, cereals, ryegrass and other weeds. The different fractions are weighed (Ericson and Nogren, 2005).

Undersown *Sinapis alba* (L) and a natural weed flora have been used in Sweden to study weed competitiveness in 6 barley cultivars. Weed competitiveness was measured by sub-sampling an area of 0.25 m^2 and analysing crop and weed biomass (Didon 2002).

Undersown weeds have been used in the Danish BAR-OF project. The crop (barley) was sown with a seed drill with a row distance of 12 cm. Seed rates were adjusted for weed weights and germination rate to give a target established population of 350 plants per m². As model weeds a mixture of 25% *Chenopodium album*, 25% *Phaselia tanacetifolia*, 25% *Brassica napus* ssp. *Napus* and 25% *Trifolium incarnatum* L, cv Poppelsdorfer was sown in plots without pesticide treatment. Photographic images were used, among several other techniques, to study the weed competitiveness to different weeds (Hansen et al. 2005).

2.2   Allelopathy

There are several bioassay methods available to measure the allelopathic activity. In some methods extracts are used, but in most donor and receiver plants are grown together (Wu et al, 2000; Belz 2004). Common to all methods is that only one receiver plant is used while it is known that the allelopathic activity has a certain degree of specificity. However, there are reports of cultivars that are allelopathic to several weeds, both mono-and dicotyledonous.
A simple and rapid bioassay used both in Australia (Wu et al, 2000) and Sweden (Bertholdsson 2005) is an agar-based method where cereal plantlets are grown together with rye grass and the potential allelopathic effect is measured as the rye grass root inhibition after 10 days. The method is very simple and has a high output.

2.3   Harrowing

- *Ranking varietal tolerance to harrowing*

When evaluating varieties for their tolerance to harrowing, one must first be sure that the observed differences are not due to factors other than genotype.
The best parameter to evaluate crop damage is the counting of uprooted stems after harrowing as compared to the non harrowed crop. It should be evaluated at crop physiological maturity by estimating the percentage of uprooted stems as $(A - B) / 100$, where A is the stem density on a sample area kept weed-free by hand weeding in a non-harrowed strip of the plot (6-10 rows wide), while B is the stem density on the remaining harrowed area of the plot.
This way of evaluating allows saving of space and time compared to a trial with a factorial or split plot design with two levels (harrowed/non harrowed crop) allocated to plots or sub-plots of the same size. The only way to get punctual information on crop damage is to compare the final crop biomass in harrowed and non harrowed plots, that have been kept weed-free throughout the period of competition (from the beginning of tillering until heading). This allows to separate the effects of mechanical injury and weed competition. However, for practical reasons, comparing the percentage of uprooted stems as described above gives a reasonable estimation of the varietal differences in tolerance to harrowing.

- *Special trials*

In Central Italy detailed trials are conducted where the response of various durum wheat (winter sown) varieties is evaluated in terms of both crop tolerance and weed control efficacy. Each variety is split into two harrowing levels (none and standard harrowing with tine inclination of 30°), two times of pass (early and late) and two types of sown infestation (different proportions of grass weeds on total weed density). Three types of samples are taken: (1) kept weed-free in the non-harrowed sub-plot; (2) non weed-free from crop emergence onwards in the harrowed sub-plot and (3) kept weed-free from harrowing onwards in the harrowed sub-plot. This allows an evaluation of crop plant mortality, actual crop damage (as the results of plant mortality + growth reduction) and a response in terms of yield reduction compared to the weed-free crop (comparable to a sprayed crop showing negligible toxicity). The first results (Faustini and Paolini, 2005) show important varietal differences in weed control efficacy (from 60 to 90 %) and crop tolerance (with a crop plant mortality ranging from 5 up to 18 %), mainly depending on the type of weed infestation and crop competitive ability, which appears to be closely correlated to high early growth (within 60 days from sowing).The experiments give valuable information on the suitability of durum wheat for organic farming.

2.4   The Austrian way as an example

As an example of a possible time schedule for measurements on weed competitiveness, the schedule used by AGES (Austria) is described hereafter.

Growth stage	Measurement	scale
BBCH 28	Rate of coverage	%
BBCH 31-32	Rate of coverage	%
	Canopy height	cm
	Photosynthetically active radiation	%
	Leaf area index	LAI
BBCH 34-47	Rate of coverage	%
	Canopy height	cm
	Photosynthetically active radiation	%
	Leaf area index	LAI
	Frequency of plants with recurved flag leaves	1-9

In addition to these parameters variety rank orders are established.

In the Austrian descriptive list of varieties a separate chapter on farming under ecological conditions is published. The varieties are classified for the most important parameters of weed suppression, as in the table below.

Table 1: Classification of Austrian winterwheat varieties for weed suppressing parameters.

Variety	Crop cover tillering	Crop cover stem elongation	Plant height stem elongation	Leaf inclination stem elongation	PAR stem elongation
Erla Kolben	+	++	++	+++	+++
Emerino	++	++	++	0	+++
Josef	+	+	+	+	+++
Capo	++	++	++	0	++
Stefanus	+	+	++	+	++
Exklusiv	+	+	+	++	++
Edison	+	+	0	++	+
Pireneo	+	+	+	++	+
Saturnus	+	+	+	+++	+
Renan	+	0	-	+++	0
Pegassos	0	0	-	0	-
Ludwig	+	0	+	- - -	- -
Romanus	-	- -	-	- - -	- -
Granat	- -	- -	- -	- -	- - -
Dekan	- - -	- - -	- -	- -	- - -

+++ = very good; 0 = middle; --- = very poor

3. Special considerations for organic and low input variety testing compared to conventional variety testing

3.1 Crop physiology

Although plant communities behave differently with different nutrient availability, as occurs under organic and conventional farming, the traits that make a crop more competitive should be the same in both situations. That is why the described parameters should also be measured and estimated under both conditions. Depending on type of weed infestation, the outcome of crop/weed competition for a given variety can be different in the two farming systems, as lower nutrient availability (mainly nitrogen) can increase the aggressiveness of some species, mainly, but not only, grasses.
The time available for the assessment of ground cover in organic trails is likely to be longer than in conventional trials, due to a wider range of varieties.

3.2 Harrowing

The idea that harrowing is only relevant for organically managed crops is misleading, although experimental evidence to support this statement is not easy to be found. A satisfactory level of weed control (higher than 80 %) can be obtained in conventional wheat with a moderate to medium infestation level, provided that mineral fertilization does not give considerable competitive advantage to the weeds relative to the crop. It should be possible to keep the weed infestation at an acceptable level by applying a proper rotation and a good ploughing practice. Mineral fertilization does not necessarily imply a competitive advantage for weeds. If properly timed at top dressing, it can result in an advantage for the crop, as observed for bread wheat (Angonin et al, 1996). In general, the growing of competitive varieties should give more opportunities for satisfactory weed control.

4. Populations, varietal mixtures and single varieties

4.1 Allelopathy

The allelopathic trait is probably easier to be exploited in populations and mixtures than in single varieties. If specificity is high for different weeds it may be possible to find cultivars highly allelopathic to certain weeds and in mixtures get combined effects. Some cultivars show allelopathic interaction with other cultivars within the same species. Some cultivars are donors and others are receivers; the donors may influence growth or insect resistance of the receiver plants. It could therefore be of interest to find donor and receiver cultivars and use them in mixtures.

4.2 Harrowing

Variety intercropping is likely to give resource use complementarities, with crop biomass advantages and thus weed niche and growth reduction. So far, however, there is no clear evidence of these effects, nor that they can favour early crop growth or improve harrowing efficacy. In organic systems, wheat is sometimes intercropped with cut legumes such as clover

(Caporali et al, 1993), which can improve mineral nutrition of the crop and the residual fertility to the benefit of the whole system. On these mixed crops harrowing is not applicable, and weed control is exerted by both preventive, indirect means (soil tillage, crop rotation, stale seed bed) and the weed suppressive ability of the two intercrops.

5.   Discussion and recommendation

The reaction of the genotypes is often similar in different environments.
However, one should always be on the look-out for the possible occurrence of interaction between variety, location and year. These interactions are inevitable and can even be used to the advantage of a farmer who may adapt his production technique accordingly.

5.1   Crop physiology

5.1.1. The ideal plant growth habits to suppress weeds
For plant breeders, targets for plant and crop characteristics should be considered in relation to generalised growth habits that are exploitable in different agro-environmental and management scenarios, e.g. organic farming.
Seasonal variations in plant establishment and individual genotype responses in tiller production and/or tiller retention can make difficult to group varieties in field trials into consistently good or poor ground cover and light interception. However, it is possible to grade or measure early vigour and growth and describe general growth habits of current varieties in such a way that this can be of benefit for breeding programmes specifically aimed at the selection of more competitive varieties.
During plant establishment, competition for water and nutrients are far more important than competition for light especially in organic systems. A rapid early growth of both shoot and roots is of essential importance to compete for water and nutrients and to improve the use efficiency of the crop. The early root development will also increase exudation of allelochemicals and hence affect the allelopathic activity. Later on competition for light becomes more important and the growth habit starts to play a central roll. But still the shoot growth is important and the relative growth rate of the shoot should be higher than the growth rates of the weeds (Dock Gustavsson, 1989).
A continuous planophile habit has a clear advantage over the erectophile habit at a given plant or shoot density. It appears to be particularly beneficial in shorter varieties and under conditions where a crop requires to be strongly weed suppressive, from a few weeks after emergence until stem elongation. An early planophile to later erectophile habit can compensate better for lower crop establishment than early erectophiles, though rapid leaf development or large leaves would enable varieties of this type to take full advantage of their leaf habit. A variety that changes from planophile to erectophile over the season will give continuous good shading so long as it is tall later on. The early erectophile to later planophile habit is a good model when crop establishment is high and if crops are sown in narrow rows. This structure, can provide high fractional light interception throughout the season. The later planophile habit is the most beneficial habit where there is late weed growth i.e. from stem extension onwards. In drier summer climates, later shading is less important as weed growth stops earlier in the season.
The erectophile habit has been the long established ideotype for high yields in cereals for high-input agriculture and unlimited nutrient supply- particularly in drier climates. This habit can be an advantage when weed levels are low, but it is a risky strategy when competition from weeds is high, especially early in the growing season. If an erectophile habit is desired, then increased

height may be of value. Shorter varieties would benefit from an ability to produce and retain a high number of shoots per plant.

5.1.2. Considerations for different climatic zones

The balance between the above mentioned characteristics will determine the suitability of a variety for early, late and season-long weed suppression under various climatic conditions. The following priorities are suggested for three broad climatic zones.

- *Cool, moist, temperate*

In cool, moist, temperate regions a planophile habit is useful throughout the season as weed growth may continue through to canopy closure and beyond in some cooler, wetter summers. If the variety is or becomes erectophile, it must be tall with large leaves. The risk of poor crop establishment can be high, and a crop with large leaves and high tillering ability should reduce the risk of poor competitiveness. Some compensation is also possible if varieties are taller around flowering time. For late sown crops in cool temperate regions a rapid early spring growth is particularly required to shade a largely spring emerging weed flora. In early sown crops rapid autumn tillering is required, as well as rapid early spring growth.

- *Continental*

In continental regions a planophile habit in the spring is useful; dry and warm conditions in summer reduce weed growth, so later growth may be erectophile. A tall early erectophile variety with large leaves may be suitable. In continental regions or in late sown crops in cool temperate regions a rapid early spring growth is particularly required to shade a largely spring emerging weed flora. In early sown crops rapid autumn tillering is required, as well as rapid early spring growth.

- *Mediterranean and Dry areas*

In Mediterranean regions, rapid autumn and winter growth is required, mainly to contain early emerging and growing weeds. In Mediterranean regions a planophile habit in autumn and early spring would be useful. However, early growth rates over the whole tillering period (generally lasting from the beginning of December to the beginning of March) are particularly important, given also the long emergence pattern of grass weeds (*Lolium* and *Avena* spp.), particularly competitive in these areas. However, highly erectophile habits (especially after stem extension) may be preferred for later moisture conservation. Later weed growth is also reduced by the hot, dry conditions. Most varieties in this region are erectophile at present, so large leaves and height become more important characters in maintaining shading. In such situations early ground cover, and yield may be of greater consequence for weed suppression, along with leaf size, and possibly tillering ability. In such zones, early weed suppression is generally the requirement anyway and the characteristics indicated would provide that benefit.

5.2 Allelopathy

There is insufficient information on allelopathy to make recommendations for a variety testing protocol. Until further results are available the best practice is to undertake variety field tests in the presence of weeds, or possibly of a model weed. In this way both morphological and allelopathic traits can be accounted for.

5.3 Harrowing - Factors influencing crop tolerance to harrowing

Factors influencing the control efficacy of harrowing also influence crop tolerance to mechanical injury, which mostly consists in plant uprooting. The better these factors are managed, the lower the risk of crop damage. Particular care should be given to the time of application to avoid injury to small plants, and in adjusting tine setting to avoid too severe work, mainly when moisture in the upper soil layer tends to be high. Higher crop tolerance can also be achieved by the integration of harrowing with husbandry choices, such as increased seeding rates and the growing of competitive varieties.

In well established crops, the need for a good weed control exceeds by far the need for a good crop tolerance. In fact, in most cases wheat plant mortality can easily be contained below 15 percent. Table 2 summarizes the most relevant traits of different harrowing techniques for a number of typical agro-environmental scenarios. Variants are possible, mainly depending on level and type of weed infestation and on the thermo-pluviometric trend. In general, spring wheat needs more attention, given a context which tends to favour weed growth in combination with a shorter period suitable for harrowing.

Table 2 – Advised harrowing technique (pre-emergence passes unless differently specified) on organic wheat in different scenarios

Crop type	Soil type	Infestation level	Number of passes	Harrowing intensity[2]	Integration with other means	Others
autumn sown crop southern to middle European latitudes	sandy to sandy loam	moderate	1 early	moderate (20-40 °)	---	1 more pass in HGWP
		medium to high	1 early (+1)[1]		MC or HD	MC + HD in HGWP
	clay-loam to clay	moderate	1	medium (15-20 °)	MC	1 more pass in HGWP
		medium to high	1 (+1)[1]		MC + HD	---
spring sown crop northern to middle European latitudes	sandy to sandy loam	moderate	1 early + 1	moderate (20-30 °)	MC or HD	eventually 1 more pass just prior to crop emergence
		medium to high			MC + HD	
	clay-loam to clay	moderate	2	medium-high (10-20 °)	MC or HD	
		medium to high			MC + HD	

[1] especially in case of HGWP (see below) but often also in no HGWP   [2] tine inclination to the vertical
MC = more competitive variety   HD = crop density near to the upper extreme of local optimum range for yield   HGWP = high grass weeds proportion (generally ≥ 30 %) on total weed density

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DISEASE ASSESSMENT

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1. Introduction

Disease assessment is the basis for describing disease resistance characteristics of commercial varieties and is often the translation of a complex field situation into one single score.

Disease assessment will become especially important in sustainable systems of crop protection, where critical evaluation of disease levels is required in order to assess the effectiveness of proposed low-input, environmentally friendly strategies, such as the use of cereal cultivar mixtures. Low-input systems often result in lower nitrogen levels and higher weed populations in crops, and this has been shown to affect the fitness and movement of pathogens and hence the rate of development of disease lesions in cereal crops; thus the timing and frequency of assessments could be different in such crops compared to conventional cropping systems.

Disease assessment in relation to growth stage
Disease assessment data must be qualified by the growth stage of the crop or plant at the time of assessment. This is because the effects of a given level of disease on plant growth and yield and the importance of that disease level in relation to the progress of an epidemic will vary at different plant growth stages.

Evaluations in the early growth stages might serve the purpose of more detailed and specific genotype x pathotype interactions. In later growth stages, detailed studies of specific interactions could be hampered by the presence of several diseases on a high level. On the other hand, a higher infection pressure in later growth stages can provide knowledge on partial resistance of a variety. Therefore, the decision of the best time(s) of disease evaluation, will depend on the dynamics between genotypes and pathotypes and present level of specific diseases. In some cases, for the sake of creating balanced datasets, evaluations must be carried out at specific growth stages, not considering the level of the diseases.

Consequently it is important to be familiar with the keys currently available and other methods for determining stages of plant growth, and to correctly determine the frequency with which assessments should be carried out. Assessments should be related to a stage of plant development that determines an important physiological function – for example grain filling in cereals. For many years growth stages in cereals were scored on the Feekes scale, illustrated by Large (1954). This was superseded by the decimalized key of Zadoks *et al*. (1974) illustrated by Tottman *et al*. (1979) and Tottman and Broad (1987). A uniform decimal code for growth stages of crops and weeds (which could be important for sustainable systems) was produced by Lancashire et al (1991) and is known as the BBCH scale. The scale and codes are based on those of the Zadoks scale for cereals but in addition deal with rice, maize, oilseed rape, field beans, peas, sunflower and weeds, the aim being to establish a universal scale using a consistent set of numeric codes which can readily be adapted to all crops.

Other methods of determining crop growth stages include dissection of the shoot apex under a microscope; such an approach is considered to be more precise than examination of growth stages by the naked eye using the decimal code, and therefore permits more exact timing of plant protection measures such as disease assessment. The Cereal Development Guide by Kirby and Appleyard (1981) clearly illustrates these important stages of early apical development in wheat and barley. The use of remote sensing with hand-held radiometers also offers possibilities for indirectly measuring crop growth stages based on spectral reflectance changes from a healthy crop during plant growth. However, accurate calibration of radiometer readings with existing decimalized codes for crop growth stages in order to aid and standardize disease assessment would be desirable.

2. Methods of assessment

Methods should satisfy three criteria: consistency between observers, simplicity for speed of operation and high reproducibility across locations and years. Thus methods should be well defined and standardized. There is usually little disagreement between observers at either end of a descriptive disease severity scoring scale but wide variation can occur in the central (often critical) part of the scale. Direct methods of assessment are likely to be better correlated with yield losses in the crop than indirect methods (such as spore traps and remote sensing) and can be quantitative or qualitative in nature.

Direct quantitative methods are largely concerned with disease incidence (number of infected plant units / total number of plant units assessed) x 100, and disease severity (area of diseased tissue / total tissue area) x 100. Although incidence is traditionally based on visual disease symptoms, the definition can easily accommodate other more modern methods such as ELISA and PCR. Incidence is suitable for assessing systemic infections (such as viruses and smuts) as well as many root diseases, or where a single lesion causes death (such as axil lesions in barley caused by *Rhynchosporium secalis).* In general, incidence is easier and quicker to assess than severity and is therefore more convenient to use in disease surveys where many observations are needed. The relationship between incidence (*I*) and severity (*S*), (the so called *I-S* relationship) has been studied by many authors and is especially important in *Fusarium* ear blight of wheat where the two parameters are highly correlated, since severity (number of infected spikelets) can be predicted by scoring infected ear incidence. The relationship is important for predicting the risk of mycotoxin contamination in grain, important in sustainable systems. Most assessment keys measure % severity using standard area diagrams; it is important to avoid the use of arbitrary categories such as slight, moderate or severe. Since the ultimate aim is to relate disease to yield loss, the plant units assessed should ideally be important contributors to yield, for example the top two leaves of a cereal plant. Despite the use of standardized keys, assessment suffers from fundamental errors. Variegated patterns of disease and leaf size can affect the accuracy of assessment. Thus computer training programmes have been developed such as AREAGRAM, DISTRAIN, Disease.Pro. and Severity.Pro. Other direct quantitative methods of assessment involve computing coefficients and indices (such as for eyespot infection) and measuring components of partial disease resistance (PDR) (such as incubation and latent periods for *Microdochium nivale* that might be an early indicator for *Fusarium* head blight resistance). Direct qualitative assessments of disease are used to differentiate host responses or interactions, such as in race surveys and breeding programmes.

Finally it should be noted that data from visual assessments of disease often do not correlate with the amount of fungal biomass in the diseased tissue, leading to inaccurate disease-yield loss relationships. Precise techniques can now measure fungal biomass using chitin or ergosterol content, or quantitative PCR. In addition, in *Fusarium* ear blight of wheat, kernels in asymptomatic spikelets may be infected and mycotoxin content may not be correlated with visible symptoms in this case. Other confounding factors are often earliness and plant height, illustrating the complex task of relating the yield of crops to assessment data.

Terms and concepts for plant disease assessment are reviewed by Nutter et al (1991, 1993), and an overview of disease assessment and yield loss is given by Cooke (2006).

2.1. Methods of assessment and inoculation of seed-borne diseases

A number of illustrations referred to in the following paragraphs can be found on the full colour pages at the end of the chapter on disease assessment.

Disease: Loose smut of wheat
Pathogen: *Ustilago tritici*

Inoculation method:

Injection method
- 1 g spores per 1 l water
- Injection of one drop of spore suspension into the florets at early to mid-anthesis growth stage. Inject the inoculum with a 5- or 10-ml syringe with a needle that is 10 to 20 mm long.

Assessment method:

Visual assessment of disease incidence (= % infected ears) in the field.

Literature:
Wilcoxson RD and EE Saari (eds.) (1996). Bunt and Smut Diseases of Wheat. Concepts and Methods of Disease Management. Mexico, D.F., CIMMYT.

Disease: **Common bunt of wheat**
Pathogen: *Tilletia tritici*

Inoculation method:

Dry inoculation
- 1-2 g spores / kg seed, 1 minute shaking or 5 minutes in "Turbulamischer"
  (used in Switzerland)

Assessment methods:

Visual detection of early symptoms in the glasshouse
- Pot test with naturally infected or artificially inoculated seed in a mixture
  (1:3, vol:vol) of sand and commercial potting substrate. Sow 2 cm deep and water carefully.
  For pots with 18 cm diameter take 15 wheat seeds.
- Incubate at 4-5°C in the dark until appearance of the coleoptiles (approximately 3 weeks)
  and under fluorescent lamps in a growth chamber (15-20°C, 16/8 h day/night) until 3-4
  leaves have developed (GS 13-14).
- Examine the seedlings for chlorotic flecking indicative of *T. tritici* infection
  (Fig. 1-colour). Presence of early symptoms of *T. tritici* is a clear indication of a successful
  infection of the plant, but they are only of limited value in predicting the development of
  bunted ears.

Visual assessment of disease incidence in the field
Count the percentage of bunted ears (Fig. 2-colour).

Literature:
Johnston CO and Lefebvre CL (1939). A chlorotic mottling of wheat leaves caused by infection
    of bunt *Tilletia laevis*. Phytopathology 29: 456-458.
Kendrick EL and Purdy LH (1959). A seedling reaction of wheat indicative of bunt infection.
    Phytopathology 49: 130-132.
Koch E and Spieß H (2002). Characterisation of leave symptoms of common bunt (*Tilletia
    caries*) and relationship to ear attack in nine wheat varieties. Journal of Plant Diseases and
    Protection 109: 159-165.
Borgen A and Kristensen L (2003). Macroscopic leaf symptoms in wheat infected by *Tilletia
    tritici*. Journal of Plant Diseases and Protection 110: 432-436.

Disease: **Loose smut of barley**
Pathogen: *Ustilago nuda*

Inoculation method:

Injection method
- 1 g spores per 1 l water
- Injection of one drop of spore suspension into the florets at BBCH 61-65.
  Inject the inoculum with a 5- or 10-ml syringe with a needle that is 10 to 20 mm long.

Assessment method:

Visual assessment of disease incidence (= % infected ears) in the field.

Literature
Poehlman JM (1945). A simple method of inoculating barley with loose smut. Phytopathology 35, 640-644.

Eckstein PE, Voth D, Krasichynsky N, Rossnagel BG, Scoles GJ (2000). Identification and Development of Markers for the Un8 Gene for True Loose Smut (*Ustilago nuda*) Resistance in Barley. LOGUE,S.(editor): Barley Genetics VIII, Proceedings of the 8th International Barley Genetics Symposium, 22.-27.Oct. 2000, Adelaide, ISBN 0-086396-680-2, Vol.2, p 98-100.

Disease: **Leaf stripe of barley**
Pathogen: *Drechslera* (*Pyrenophora*) *graminea*

Inoculation method:

Sandwich method
- Sterilize seeds in 70% ethanol for 30 s and 5% NaClO for 5 min, rinse well in several changes of deionized water.
- Incubate in petridishes between two layers of potato dextrose agar (PDA) colonized by the actively growing mycelium of an isolate of *D. graminea* (30-35 seeds per dish).
- After 20 days of incubation in the dark at 6°C, the emerged seedlings can be transplanted into pots and grown in the greenhouse. For pots with 12 cm diameter take 5 seeds.
- At heading, harvest the plants and examine for leaf stripe symptoms. This method can be used for the screening of cultivars for resistance and the development of markers. It is a very successful inoculation method and causes heavy infection. It is not suitable for the inoculation of seeds for field experiments.

Assessment method:

Visual assessment of disease incidence (= % infected plants) in the field (Fig. 3-colour).

Literature:
Pecchioni N, Vale G, Toubia-Rahme H, Faccioli P, Terzi V, Delogu G (1999). Barley - *Pyrenophora graminea* interaction: QTL analysis and gene mapping. Plant Breeding 118: 29-35.
Skou JP and Haahr V (1987). Screening for and inheritance of resistance to barley leaf stripe (*Drechslera graminea*). Risø Report 554, Risø National Laboratory, Roskilde, Denmark.

Disease: **Covered smut of barley**   D 8
Pathogen: *Ustilago hordei*

Inoculation method:

Dry inoculation
- 1 g spores / kg seed, 1 minute shaking

Assessment method:

Visual assessment of disease incidence (= % infected ears) in the field.

Literature
Grewal TS, Rossnagel BG and Scoles GJ (2003). Effect of storage of inoculated seed and
     dehulling on the development of covered smut in barley. Barley newsletter 46
     (http://wheatr.pw.usda.gov/ggpages/BarleyNewsletter

Disease: **Loose smut of oats**
Pathogen: *Ustilago avenae*

Inoculation methods:

Vacuum method (spelt oats)
- Suspend 5 mg spores of *U. avenae* in 100 ml of water, add 25 g oat seed to the suspension and stir. Place the container in an exsiccator (Fig. 4-colour).
- Keep stirring and evacuate for 20 minutes, in between times let the vacuum build up and collapse several times.
- Pour inoculated seeds into a sieve and und let it dry over night at room temperature on a filter paper.

Dry inoculation (naked oats)
- 1 g spores / kg seed, 1 minute shaking.

Assessment method:

Visual assessment of disease incidence (= % infected ears) in the field (Fig. 5-colour).

Literature
Thiede H (1963). Untersuchungen zur Biologie und Bekämpfung von *Ustilago avenae* (Pers.) Rost. sowie der Infektionsmethodik. Phytopathologie. Z. 48: 29-72.

2.2   Methods of assessment and inoculation of leaf and ear diseases

Disease: **Fusarium head blight of wheat**
Pathogen: *Fusarium culmorum, F. graminearum*

Inoculation methods (field):

Maize-stubble method
- Plant the wheat directly in late-harvested maize (no tillage)
or:
- Spread maize stubbles (optimal from kernel maize) of about 20-25 cm length with 6 stubbles per m^2 in December - January

Oat kernel method
- Cultivation of *F. graminearum* isolates and incubation of sterilized oat kernels
- Incubate until mycelium growth and eventually perithecia on grain surface are visible
- Spread colonized oat kernels with 10g/m² at BBCH 31/32 (mid to end of April)

Spray inoculation
- Spray conidial suspension ($3x10^5 – 2x10^6$ conidia/ml) on a set of genotypes three to five times at intervals of three days during flowering in the evening with a water volume of 600-1000 l/ha. Exact timing depends on temperature and length of flowering period. Each genotype should be inoculated at least once at its respective mid-flowering date
or:
- Spray each genotype once according to its exact mid-flowering date with the conidial suspension

Assessment methods:

- Visual assessment (Fig. 6-colour) of disease incidence (= % infected heads) and severity (= % infected spikelets/head); calculation of Fusarium head blight index for each variety as:
FHB index = (Incidence [%] x severity [%])/ 100
or combined rating as % infected spikelets/plot
- Rating scale 1-9 (see description and figure 7-colour)
Two to three assessment dates during pathogenesis in 5-7 days interval starting with first genotypic differentiation. Calculate the arithmetic mean of all differentiating ratings or area under disease progress curve (AUDPC) in spray inoculated experiments or number of infected heads per m^2 in maize-stubble method, when disease severity is low.
- In spray inoculation, the rating dates must be adjusted to flowering date by selecting similar periods between flowering and rating date or by covariance analysis (SAS) with flowering date as covariate.

Description of rating scale 1-9:

Rating	% of diseased spikelets
1	No symptoms visible
2	< 5%
3	6-15%
4	16-25%
5	26-45%
6	46-65%
7	66-85%
8	86-95%
9	>95%

Note: this rating scale is roughly linear and
can easily be transformed into percentages

Literature

Fernando WGD, Paulitz TC, Seaman WL, Dutilleul P, Miller JD (1997). Head blight gradients
    caused by *Gibberella zeae* from area sources of inoculum in wheat field plots.
    Phytopathology 87: 414-421.

Mesterházy A (1978). Comparative analysis of artifical inoculation methods with *Fusarium* spp.
    on winter wheat varieties. Phytopath. Z. 93:12-25.

Miedaner T, Moldovan A, Ittu M (2003). Comparison of spray and point inoculation to assess
    resistance to Fusarium head blight in a multienvironment wheat trial. Phytopathology 93:
    1068-1072.

Xu XM, Parry DW, Edwards SG, Cooke BM, Doohan FM, Van Maanen A, Brennan JM,
    Monaghan S, Moretti A, Tocco G, Mule G, Hornok L, Giczey G, Tatnell J, Nicholson P and
    Ritieni A (2004). Relationship between the incidences of ear and spikelet infection of
    Fusarium ear blight in wheat. European Journal of Plant Pathology 110: 959-971.

Disease: Septoria leaf spot of wheat
Pathogen: *Septoria tritici* (*Mycosphaerella graminicola*)

Inoculation method (field):

Spray inoculation
- Spray conidial suspension ($4x10^5$ pycnospores/ml) on a set of genotypes at two times at intervals of 5-7 days with a water volume of 600-1000 l/ha, beginning at BBCH 37/39 (appearance of the flag leaf).

Assessment methods:
- Visual assessment (Fig. 8-colour) of disease severity on flag, first and second leaf
- Rating scale (Fig. 9): percentage infected leaf area on flag, first and second leaf
- Two to three assessments during pathogenesis in 5-7 days interval starting with first genotypic differentiation (mostly 25-30 dpi)
- Calculate arithmetric mean of all ratings or AUDPC beginning at BBCH 69-71.

Fig. 9 Rating scale for *Septoria tritici*
(Source: Syngenta Agro)

Literature

Obst A and Gehring K (2002). Getreide – Krankheiten, Schädlinge, Unkräuter, Verlag Th. Mann Gelsenkirchen: 57-64.

Bartels G and Backhaus GF (2000). Die Prüfung von Pflanzen auf ihre Widerstandsfähigkeit gegen Schadorganismen in der Biologischen Bundesanstalt, Mitt. Biol. Bundesanst. Land- u. Forstwirtsch. 373: 30-31.

Disease: **Tan spot of wheat**
Pathogen: *Drechslera tritici-repentis* (*Pyrenophora tritici-repentis*)

Inoculation methods (field):

Straw inoculation
- Plant directly in early harvest winter wheat (no or minimum tillage) OR:
- Cultivation of DTR isolates and incubation of sterilized straw stubbles
- Incubate until mycelial growth and pseudothecia on stubble surface are visible
- Spread inoculated wheat stubbles of about 5-10 cm length with 100 g/m² between December and January
- Dense crop will keep a humid microclimate in plots

Oat kernel method
- Cultivation of DTR isolates and incubation of sterilized oat kernels
- Incubate till mycelium growth and pseudothecia on grain surface are visible
- Spread colonized oat kernels with 25-30g/m² at BBCH 15-21 (December to January)

Assessment methods:

- Visual assessment (Fig. 10-colour) of disease severity on flag, first and second leaf
- Rating scale (Fig. 11): percentage infected leaf area on flag, first and second leaf
- Two to three assessments during pathogenesis in 5-7 days interval starting with first genotypic differentiation.
- Calculate arithmetic mean of all ratings or AUDPC beginning at BBCH 69-71.
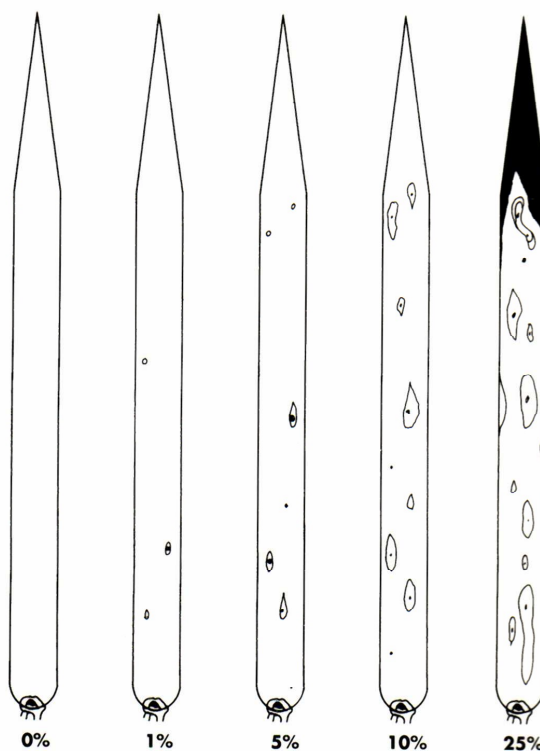

Fig. 11   Rating scale for *Drechslera tritici-repentis* (Source: Syngenta Agro)

Literature:

Mielke H and Reichelt A (1999). Studien zur Biologie des Erregers Drechslera tritici-repentis, zur Anfälligkeit des Weizens und verschiedener Artverwandten sowie zur Bekämpfung der DTR- Weizenblattdürre. Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem: 366.
Bartels G and Backhaus GF (2000). Die Prüfung von Pflanzen auf ihre Widerstandsfähigkeit gegen Schadorganismen in der Biologischen Bundesanstalt, Mitt. Biol. Bundesanst. Land- u. Forstwirtsch. 373: 27-29.

Disease: Leaf rust of wheat and barley
Pathogen: *Puccinia triticina, Puccinia recondita*

Inoculation methods:

Field
- Sow susceptible genotypes (e.g. Little Club) through the field that will act as spreaders. The natural infection will increase and will be more uniform.
- Artificial infection can be achieved by inoculating the spreaders or by placing sporulating plants from the greenhouse in the field.

Greenhouse
- Blow a mixture of rust spores and talcum powder (1:10, vol:vol) over the plants.
- Place inoculated plants with humidity at saturation, darkness and about 20º C for 12 hours.
- Symptoms (pustules or uredosori) will show up about five days after incubation.

Assessment methods:

Field method
- Visual assessment (Fig. 12-colour) of infected leaf surface, using modified Cobb scale (0-100 %) at BBCH 69 (Fig. 13).

Greenhouse method
- Infection type (to record the presence of a hypersensitive reaction):
  McNeal scale (0-9) (McNeal et al 1971).
- Low infection type (incompatible or resistant reaction): from 0 to 6
- High infection type (compatible or susceptible reaction): from 7 to 9

- Latency period (to assess partial resistance that is believed to be durable). It is measured by counting the numbers of visible pustules on marked areas of the leaves every day until no more pustules appear. From these data the time between incubation the appearance of the 50 % of the total number of pustules is the latency period (Parlevliet 1975). These tests can be carried out in seedling or in any other mature leaf (including flag leaf), being the highest correlations with field tests with the more mature leaves. To measure the latency period you need a very virulent isolate to overcome the hypersensitive resistance genes.

Description of infection types (McNeal scale):

Rating	Description
0	No symptoms
1	Small necrotic or chlorotic flecks
2	Flecks somewhat larger
3, 4	Minute uredosori surrounded by necrotic or chlorotic tissue
5, 6	Small uredosori surrounded by some necrotic or chlorotic tissue
7	Larger uredosori surrounded by some chlorotic tissue
8	Uredosori surrounded by very faint chlorosis
9	Well developed uredosori, no chlorosis or necrosis. Often there is a pale halo around the uredosori.
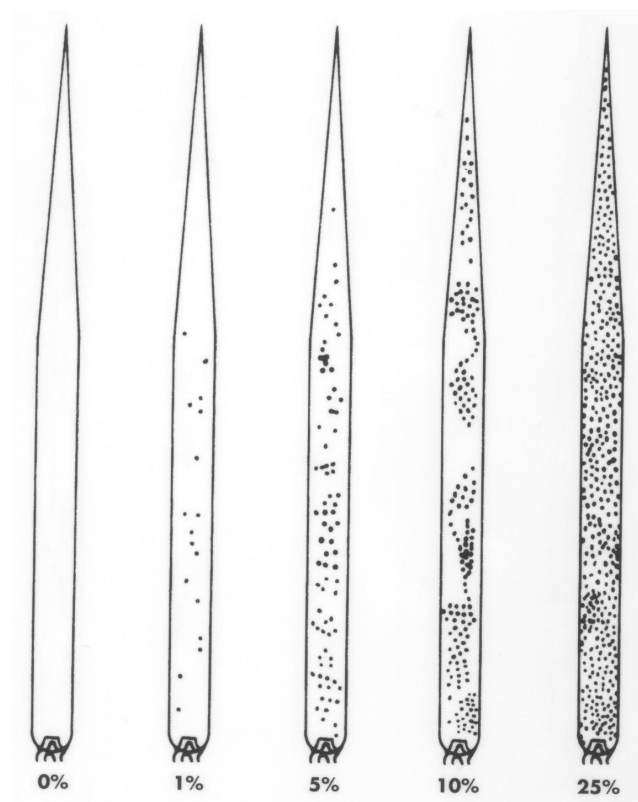
Fig. 13 Rating scale for *Puccinia triticina (P. recondita)*
(Source: Syngenta Agro)

Literature

McNeal FH, CF Konzak, EP Smith, WS Tate and TS Russell (1971). A uniform system for recording and processing cereal research data. USDA, Agricultural Research Service, Washington, D.C. ARS: 34-121.

Parlevliet JE (1975). Partial resistance of barley to leaf rust, *Puccinia hordei*. I. Effect of cultivar and development stage on latent period. Euphytica 24: 21-27.

Disease: Scald or Rhynchosporium leaf blotch (RLB) of barley
Pathogen: *Rhynchosporium secalis*

Inoculation methods

Field nursery method
- Incorporate chopped Rhynchosporium infected straw by surface cultivation. This is not necessary if the same site is used repeatedly.
- Sow the outer rows with a very susceptible winter barley genotype expressing no specific resistance to Rhynchosporium but preferably mlo resistance to mildew.
- Sow test varieties as short (0.5-1.0m) single rows between spreader rows.
- Apply regular field sprinkler irrigation, e.g. one hour on alternate days.

Detached leaf method
- Seedlings are best grown under sporeproof, air-conditioned glasshouse conditions. (The relatively uniform environment and low light levels of many growth cabinet / controlled environment rooms often produces atypical Rhynchosporium reactions).
- Prepare barley seedlings and detached leaf boxes by standard methods (Newton, 1989).
- Harvest spores by scraping conidia from CzV8CM agar plates (Newton & Caten, 1988) and macerating for one minute at high speed using a 'MSE' macerator.
- filter material through a glass-fibre plug in a funnel, and adjust the spore concentration using a haemocytometer.
- Prior to inoculation, gently abrade the centre of the leaves by brushing with a sable hair paintbrush with the bristles cut to 3 mm long.
- Wash the spores to remove any germination inhibitors, by diluting in sterile distilled water, centrifuged at 3,000 g for 2 minutes and resuspended again in sterile distilled water up to three times.
- Dispense aliquots of 10µl spore suspension containing a range of concentrations from 1×10^3 to 1×10^7 spores per ml onto the centre of each leaf. The optimum is around 1×10^5 spores per ml.
- Randomize all boxes in blocks in the incubator at 15°C under continuous light.

Assessment methods

Field nursery method
- Carry out visual assessment (Fig. 14) of disease severity every 2 weeks from the moment that the first symptoms can be observed using the whole plant scale below including half points.
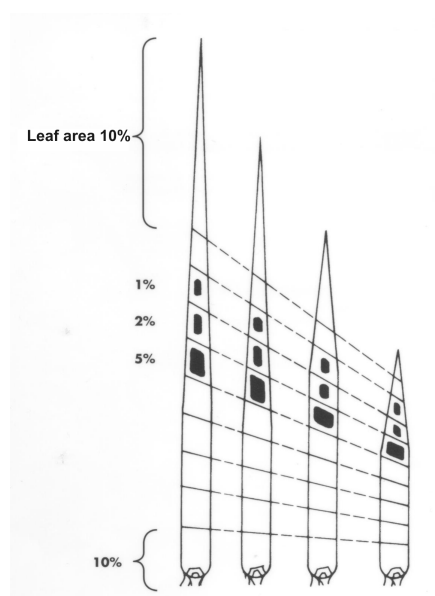

Fig. 14 Rating scale for *Rhynchosporium secalis*
         (Source: Syngenta Agro)

- Analysis of variance of the AUDPC calculated on the converted to percentage equivalent scores.

Detached leaf method
- After eight days, observe the inoculated leaves daily.
- The time of maximum lesion size, when a typical brown margin develops, should be recorded as well as the length of the lesion .
- Use the number of lesions produced by the isolates to determine whether an isolate is virulent or avirulent on a particular cultivar.
- Use the binary/octal system (Gilmour, 1973) for race designations to classify each isolate (Jones and Clifford, 1985).

Description of rating scale 1-9 (using half points as well)

Rating	Specific description	General description	Percentage
1	No symptoms visible	No infection	0%
2	1 lesion per 10 tillers	1% infection on lower leaves	0.2%
3	1 lesion per tiller	5% infection on lower leaves	1%
4	2 lesions per leaf but discrete lesions on most leaves	25% infection on lower leaves	5%
5	Leaves coalescing but overall appear green	50% infection on lower leaves	10%
6	Leaves appear ½ infected ½ green		25%
7	Leaves appear more infected than green		50%
8	Very little green leaf tissue left		75%
9	Leaves dead - no green leaf left		100%

Literature
Gilmour J (1973). Octal nomenclature for designating physiologic races of plant pathogens. Nature, London 242: 620.

Jones ERL and Clifford BC (1985). Rhynchosporium of barley. United Kingdom Cereal Pathogen Virulence Survey Annual Report for 1984: 63-69.

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Disease: **Net blotch of barley**
Pathogen: *Pyrenophora teres*

Inoculum production:

Rapid and abundant conidial production can be obtained in *Drechslera teres* on detached barley leaves incubated with 80 ppm benzimidazole under continuous near-ultraviolet (NUV) light or in the dark, with no aerial hyphae, leaf chlorosis or necrosis developing in the dark. Conidia produced on detached leaves are highly pathogenic.
Place 7 cm clean barley leaf segments (ideally produced in a semi-sterile environment) on filter paper soaked in 2.5 ml 80 ppm benzimidazole (to maintain leaf greenness) in the base of a sterile plastic Petri dish. Leaf segments can be held in place by segments of water agar containing the same concentration of benzimidazole. Inoculate each leaf segment with three 5 ul drops of conidial suspension containing 5×10^3 spores ml^{-1} with Tween 20 surfactant added at the rate of 2 drops per 100 ml. Incubate the leaf segments for 9 days at 17°C under continuous NUV light or in the dark. At the end of this period, harvest the conidia by removing the leaf segments and agitate in sterile distilled water with surfactant to facilitate spore removal.

Inoculation methods:

Detached leaf method
Inoculum can be generated using the above method or by plating out surface-sterilized naturally infected barley leaves on PDA and incubating under diurnal (12 h light/12 h dark) NUV at 17°C. After sporulation, make single-spore cultures on PDA and grow for 7 days at 17°C under diurnal NUV. Conidia are removed with a fine paint brush and Tween 20 surfactant added to the spore suspension in sterile distilled water at the rate of 2 drops per 100 ml.
The method described above for inoculating detached leaf segments is used with the following amendments. The optimum temperatures to be used are 15, 20 and 30°C. This allows the development of chlorosis at the inoculation sites and gives satisfactory correlations with field performance (% leaf area affected, 1000 grain weight, sieving grain index) of the same cultivars used under field conditions. Score the mean number of days required for each cultivar to develop chlorosis on detached leaf segments as a measure of cultivar susceptibility.

Field nursery method (straw)
- Both straw (= natural mixture) and infected leaf material (= selected isolates) are workable.
- When irrigation is not available the use of straw or dried, infected leaf material is better than the use of a suspension.
- Cultivation of *P. teres* inoculum in greenhouse: select isolates, inoculate a susceptible cultivar at the 2 leaf stage, cut down the plants two weeks after inoculation, gentle drying.
- Spread straw or dried leaf material on plots at tillering stage.
- Irrigate (if available) one hour per day before sunset.
- Dense crop will keep humid microclimate in plots.

Field nursery method (suspension)
- Cultivation of *P. teres* inoculum during winter. Inoculum maintains its aggressiveness at -20ºC for one year.
- Selection of isolates is based on the aim of the trial. Differences in virulence and in aggressiveness might be significant.
- Inoculation with a suspension after tillering stage (2×10^4 spores/ml) late in the evening (400 l/ha or 3 ml/hill plot).
- Keeping the leaves moist with help of an irrigation system for two days.
- If possible repeat the inoculation at flag leaf stage.

Greenhouse method
- Sow the genotypes in nutrient-supplemented peat and place them in a greenhouse at 18ºC, 12 h photoperiod.
- Two weeks after planting (or when the second leaf is fully emerged) the relative humidity in greenhouse should be raised to 100%.
- Inoculate the pots with a conidial suspension at a rate of 0,5 ml per pot.
- Switch the lights off and maintain a high humidity for 24 hours after inoculation - using a humidifier is better than covering the plants with plastic.
- After 24 hours, the humidifier should be on for 2 hours in the evenings.

Assessment methods

Field nursery method
- Carry out visual assessment of disease severity every 2 weeks from the moment that the first symptoms can be observed using the whole plant scale including half points.
- Analysis of variance of the AUDPC calculated on the converted to percentage equivalent scores.

Greenhouse method
- Infection response is recorded for the second leaf 10 days after inoculation using 10 point scale of Tekauz (Fig. 17 colour).
- Several statistical analysing methods available, for ex ANOVA procedure.

Description of rating scale 1-9:

| Rating	Percentage	Description
0	0%	No symptoms
1	0,1%	< 1 small spot / 10 tillers
2	0.5%	< 1 small spot / 5 tillers
3	1%	< 1 small spot / tiller
4	5%	< 2 lowest leaves 25% covered, upper leaves few spots
5	10%	< 2 lowest leaves 25% covered, upper leaves several spots
6	25%	< lower leaves 75-100% covered, upper leaves 10%
7	50%	< lower leaves 75-100% covered, upper leaves 25%
8	75%	< 75% dead, very little green tissue left
9	100%	No green tissue left
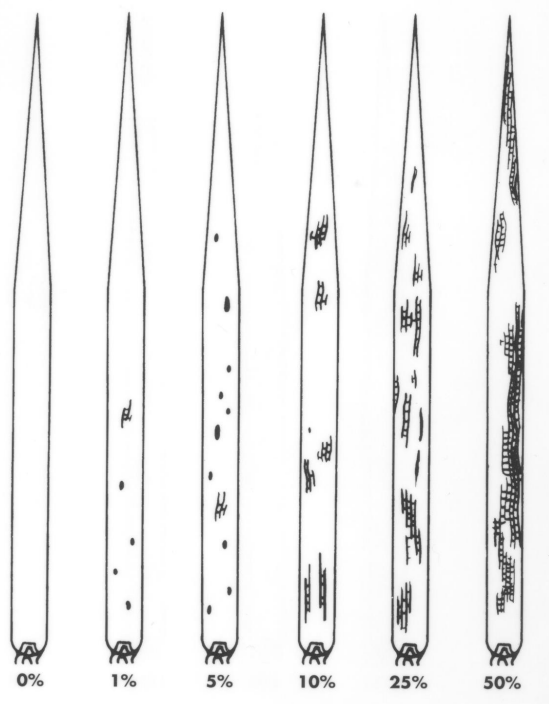
Fig. 16 Rating scale for *Pyrenophora teres*
          (Source: Syngenta Agro)

Literature:

Deadman ML and Cooke BM (1985) A method of spore production for *Drechslera teres* using
     detached barley leaves. Transactions of the British Mycological Society 85: 489-493.
Deadman ML and Cooke BM (1986) A comparison of detached leaf, greenhouse and field
     experiments for screening barley cultivars to *Drechslera teres*. Irish Journal of
     Agricultural Research 25: 63-70.
Tekauz A (1985). A numerical scale to classify reactions of barley to *Pyrenophora teres*. Can J
     Plant Pathol. 7: 181-183.

2.3. General remark

It is obvious that the higher the variability of the agro-ecological conditions, the more locations will be needed for the evaluation of disease infection. Specific diseases do tend to occur in specific locations. In order to use the time available for disease assessment efficiently, these locations should be assessed in the first place. If inoculation can be carried out at a limited number of locations, the locations with the most reliable conditions for occurrence of the disease should be chosen.

3. Specific considerations for organic and low input variety testing compared to conventional variety testing

Timing and frequency of assessments can be different in low input cropping systems compared to conventional cropping systems due to several reasons, related to the nutritional status of the crop, the presence of weeds and intercropping.

The nitrogen level of the crop influences the senescence of the leaves and as a consequence it may be necessary do make assessments in low input and organic trials at an earlier stage than in conventional trials. Moreover, the disease level in organic and low input cropping systems may be under-estimated because abiotic stresses, which enhance a general senescence of leaves, may obscure disease symptoms, in particular for leaf pathogens. An increased variability in nutritional status of organic crops sites may influence the balance between abiotic and biotic stresses (diseases) and interact with disease on individual varieties. The nutritional availability (both absolutely and the release during crop growth) of organic *versus* conventional trials, could interact both with the level and dynamic nature of diseases, as well as symptoms from lack of nutrition (e.g lack of Manganese in early crop growth stages can easily be mistaken for *Rhynchosporium secalis*).

Low-input systems can result in higher weed populations, which may affect the movement of pathogens and hence the rate of development of disease lesions in cereal crops. The same applies to intercropping systems (Bennett & Cooke, 2006 and Bannon & Cooke, 1998). Organic farmers should be aware of the risk that the undercrop may host diseases (e.g. ergot, footrot, barley yellow dwarf virus). In a crop with a heavy weed infestation or with an undersown crop, the diseased leaf area is 'diluted' with healthy green leaves which may need a visual adjustment of the observer.

The occurrence and assessment of seed borne diseases is likely to be more important in organic cropping systems due to limited possibilities for seed disinfection compared to conventional cropping systems with chemical seed treatment. Therefore, strategy concerning origin of seed and aspects of its quality before, during and after germination must be considered carefully.

Revealing susceptibility against diseases can rely on strategies of either 'on site natural infections' or inoculated screening trials. From natural infections local pathogen populations with unknown virulence characteristics, in combination with local weather conditions, determines to what degree individual genotypes are infected by a disease. On the other hand in inoculated trials, both amounts of infectious diseases and virulence characteristics can be controlled (Pinnschmidt et al, 2005). Naturally, in cases where climatic or nutritional conditions

are known not to favour natural infections, no knowledge of disease susceptibility will be revealed, and artificial infections can then be an option. Using artificial inoculation in race specific pathosystems, the choice of virulence should be considered carefully, since genotypes might be exposed to different pathotypes/races in other environments, or in near future. In some cases artificial inoculation might impose a 'worst case scenario', making interpretation of results for practical purposes quite difficult.

4. Populations, varietal mixtures and single varieties

Genetically diverse crops include variety mixtures, multilines and composite cross populations as well as species mixtures (intercropping). Mixtures/populations made up of varieties with different resistance genes to target pathogens, influence disease development in various ways. In general, the disease level in a variety mixture is lower than the average of single varieties when grown in pure stands. The main mechanisms involved in disease reduction are increased distance between plants of the same genotype (susceptible to the same pathotype), resistant plants acting as barriers for spore spread and additional resistance induced by host response to avirulent pathogens. Also the presence of weeds may influence the epidemics.
Most of the methods of sampling, assessment and inoculation used for monocultures apply to mixtures, and the choice of method therefore depends on type of disease, nutrient state and growth stage of the crop and aim of the test. However, as the variation in occurrence of a certain disease is higher in mixtures and populations than in single varieties it is recommended to increase plot size and sample size (destructive or non-destructive) in mixture trials. Interplot interference may be of greater importance in mixture trials and it is therefore recommended to use guard rows around the plots. If a direct assessment method is used the number of assessments per plots per assessment time should be increased. If using indirect methods (e.g monitoring the spore population using spore traps) it is not necessary to distinguish between single varieties and mixtures.
Some argue that mixtures and populations are more difficult to assess than monocultures because of neighbouring plants exhibit different disease levels. From a practical point of view, visual assessments (non-destructive) of disease severity in mixtures or populations, compared to single varieties, is not considered as a problem for trained personnel.

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EVALUATION OF LODGING

Johan van Waes

1. Introduction

Lodging in cereal crops, especially when it occurs in an early stage, can cause considerable losses in yield and quality. Furthermore lodged crops are more difficult to harvest and involve an increased risk for supplementary loss of grains. Therefore resistance to lodging is of essential importance with regard to harvest security and is a basic aim in cereal breeding.
As in organic farming no growth regulators are used, genetic resistance against lodging becomes more important. With the use of tall varieties this problem may increase. On the other hand the problem of lodging is counterbalanced by lower nitrogen inputs in organic systems than in conventional systems.
The evaluation of lodging in variety trials is not easy. Apart from the fact that lodging occurs occasionally, the intensity and distribution in the trials is usually irregular and unpredictable. Furthermore lodging can occur at different stages of maturity of the crop with a variable impact on losses at harvest. Moreover it may happen that nearly the whole trial is lodged (due to storm), with the exception of a few parcels, which makes it difficult to evaluate the genetic differences between the varieties.
There are different ways to evaluate lodging in the field and different ways to calculate the final score of varieties. Different methods are described hereafter. There is not one method that is the best. Every researcher may chose the assessment method that suits best in his or her variety trials.

2. Methods to assess lodging

The assessment of lodging in a variety trial comprises of two elements: which part of the plot is lodged and how serious is this lodging A clear distinction has to made between the extension of lodging (2.1) and the intensity of lodging (2.2). These two parameters may be combined into a final score by using different formulas or indices (2.3).

2.1. Extension of lodging

This is an estimation of the part of a plot that is lodged.
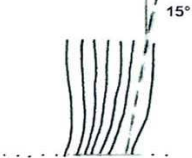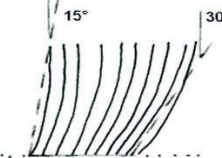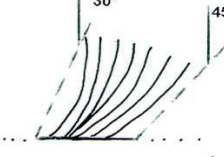It can be expressed as a percentage (0 – 100%) or as a fraction (e.g. 1/4 or 1/3) of the plot.
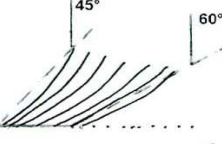
2.2. Intensity of lodging

The intensity of lodging can be described with a score from 1 to 9 or 9 to 1, depending on the system used in various countries. The following description is used in Belgium and the Netherlands:
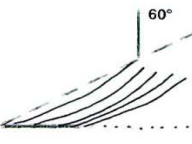
9 : stems right up and vertical
8 : stems lightly sloping (< 15°)
7 : stems sloping between 15° and 30°
6 : stems sloping between 30° and 45 °
5 : stems sloping between 45° and 60°
4 : stems sloping for more than 60° but not touching the ground, except at the basis
3 : straw for less than 50% touching the ground, the rest is upright
2 : straw for more than 50% touching the ground with only the stem ends upright
1 : straw fully down to the ground

An illustration of the scores described above is given in the figure below:

Illustration of lodging scores 9 to 1 (source Van Waes and De Vliegher, 2000)

A similar description is illustrated below, where the lodging intensity is described with a score from 0 to 10. (used in France). Instead of a score, the same description can be expressed on a scale from 0 to 100% (used in Norway).

Score 0 (= 0% lodging): no lodging, all the plants are upright
Score 2 (= 20% lodging): an inclination of 18 degrees
Score 5 (= 50% lodging): an inclination of 45 degrees
Score 10 (= 100% lodging): an inclination of 90 degrees, complete lodging
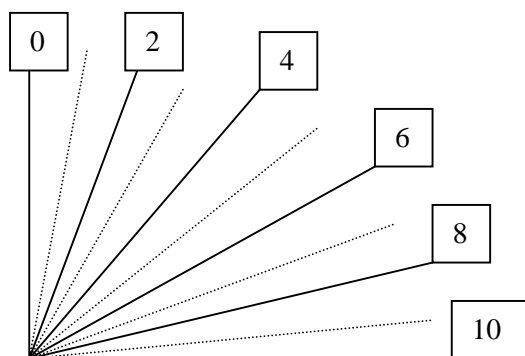
Score 0-10 corresponding to lodging angles of 0-90° (source Arvalis, France)

In case of different angles within one group of plants, an average of these angles is estimated. This is illustrated in the following figures:
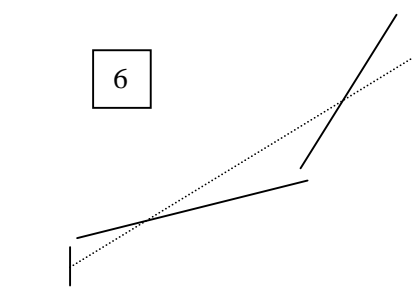
score 6 : average angle in plants that have been partially redressed
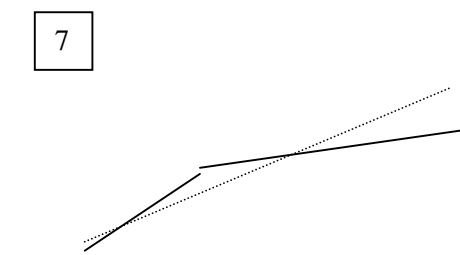
score 7: average angle in plants where the upper part is more lodged than the lower

2.3. The combination of extension and intensity: the calculation of a lodging index

It often happens that different parts of the plot have different inclination angles. The fastest method is to make a visual combination in the field and give one score for the whole plot. A more precise (and more elaborate) method is to register the various parts with different angles separately and put them in a formula or an index in order to calculate one final score per plot.

The visually combined score
The following descriptions are used in Switzerland, Germany and Austria.
The score is a combination of extension and intensity of lodging. [3]

| Description used in Switzerland (FOAG)		Description used in Germany (BSA) and Austria (AGES)
no lodging, rows clearly visible	**1**	no lodging, all stems upright
no lodging, rows not clearly visible (inclination < 30°)	**2**	Intermediate 1-3
slight lodging, a few spots in the plot with < 45° inclination	**3**	all stems have an inclination of ± 30° or some areas (± 25 % of the plot) show more lodging
slight to medium lodging, some spots in the plot with 45° to 60° inclination	**4**	intermediate 3-5
medium lodging, several spots in the plot with > 60° inclination	**5**	all stems have an inclination of ± 45° or some areas (± 50 % of the plot) show more lodging
medium to heavy lodging, up to 1/3 of the plot completely lodged	**6**	intermediate 5-7
heavy lodging, 1/3 to 2/3 of the plot completely lodged	**7**	all stems have an inclination of ± 60° or ± 75 % of the plot is completely lodged
heavy lodging, only border rows not completely lodged	**8**	intermediate 7-9
total lodging, whole plot flat	**9**	complete lodging

Index 1 (used in Belgium)
This index can be applied when part of the plot is not lodged and part of the plot is lodged. It is not possible to combine different degrees of lodging.
The lodging intensity (I) is expressed as a score from 9 to 1 and the extension (E) is expressed as a score from 0 to 10 corresponding with percentages between 0 and 100% (see table 1).

The final score of the plot will be: **9 – {E (9 - I) / 10}**

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[3] *In most German speaking countries a scoring system is used where the score 1 is given for the desired situation and the score 9 for the undesired situation.*

Example
When 40% of a plot (E = 6) has an inclination angle between 45 and 60° (I = 5) the final score for this plot will be: 9 – {6 (9 – 5)/10} = 9 – 2.4 = 6.6

The index for different combinations of E and I can easily be read from table 1.

Table 1: Calculation of the lodging index based on lodging intensity (I = 1-9)
       and lodging extension (E = 0 – 10 corresponding with 0-100% of the plot)

Extension (%)	I⇒ E⇓	9	8	7	6	5	4	3	2	1
96-100	10	9,0	8,0	7,0	6,0	5,0	4,0	3,0	2,0	1,0
81-95	9	9,0	8,1	7,2	6,3	5,4	4,5	3,6	2,7	1,8
76-80	8	9,0	8,2	7,4	6,6	5,8	5,0	4,2	3,4	2,6
51-75	7	9,0	8,3	7,6	6,9	6,2	5,5	4,8	4,1	3,4
31-50	6	9,0	8,4	7,8	7,2	6,6	6,0	5,4	4,8	4,2
21-30	5	9,0	8,5	8,0	7,5	7,0	6,5	6,0	5,5	5,0
11-20	4	9,0	8,6	8,2	7,8	7,4	7,0	6,6	6,2	5,8
6-10	3	9,0	8,7	8,4	8,1	7,8	7,5	7,2	6,9	6,6
1-5	2	9,0	8,8	8,6	8,4	8,2	8,0	7,8	7,6	7,4
< 1	1	9,0	8,9	8,8	8,7	8,6	8,5	8,4	8,3	8,2
0	0	9,0	9,0	9,0	9,0	9,0	9,0	9,0	9,0	9,0

Index 2 (used in France (by Arvalis) and in Norway)
This index can be used when different parts of a plot have different lodging intensities.
The lodging intensity (I) can be expressed as a score between 0 and 10 or as a percentage between 0 and 100%. The lodging extension can be expressed as a percentage between 0 and 100% or as a fraction of the plot.
The final score is the sum of (I x E) in different parts of the field: **Σ (I x E)**

Examples:
When 80 % of the plot is lodged at an angle of 45°(I = 5) and 20 % of the plot is lodged at an angle of 63°(I = 7) the final score will be:
     {(5 x 80) + (7 x 20)} / 100 = 5.4

When 1/5 of the plot is lodged at an angle of 45° (I = 50%) and the rest (4/5) is lodged 60°(I = 67%), the final score will be:
     {(50 x 1) + (67 x 4)} / 5 = 63.6 %

N.B. It is estimated that on average the error made at the level of E is ± 5% and the error at the level of I is ± 10°.

2.4.   The best time to evaluate lodging

The most obvious time to evaluate lodging is:
1. when the first plots are lodging (this is usually around or just after heading, BBCH 60-75)
2. just before harvest
3. any time between these two data when changes in lodging occur.

> The evaluation of lodging has to be carried out in all the replications of the trial.

The arithmetic mean of the different scores should be used as the final score for a variety.
If no more lodging occurs after the first (or second) scoring, the scoring note remains the same as it was in the first (or second) scoring. It is possible that only one score is given e.g. at harvesting time; in that case that is the final score.

2.5.   Special trials

Geves (France) carries out special trials with 2 nitrogen levels: a split plot with two factors (variety and nitrogen level) in four replications. The nitrogen levels used are:
-an average level for an average yield objective (8 T/ha in winter wheat)
-a high level for a high yield objective (10 T /ha in winter wheat)

3. Survey

A survey of the different ways of assessment used by various Susvar partners is given in Table 2 at the end of this chapter.

4. Special considerations for organic and low input variety testing compared to conventional variety testing

The assessment methods for organic trials are not essentially different from the methods used in conventional trials.In spite of the fact that growth regulators are not used in organic trials, the incidence of lodging is often lower than in conventional trials due to lower nitrogen inputs in organic trials. It may therefore be more difficult to obtain reliable lodging figures from organic trials, especially when the varieties are short straw varieties (<120 cm).
When tall straw varieties (often preferred in organic farming) are included in a variety trial it should be taken into account that there may be interference with neighbouring plots with shorter varieties. In that case it should be better to organise the trials in 2 groups, according to the length of the varieties.

5. Special considerations for testing populations and mixtures compared to single varieties

The assessment methods used in variety trials of single varieties can also be used in populations and mixtures. Both in single varieties and in mixtures lodging may show an irregular pattern within a plot. Hence finding statistical significant differences will be difficult.

Mixing a susceptible variety, with a more tolerant variety may reduce the average lodging.

6. Discussion and recommendations

6.1 How to decide on the value of data and datasets?

When making the decision on whether to take a dataset of a certain trial (e.g. a specific location) into account in the overall analysis of data, it should be considered that including a dataset of a trial with too little variation (e.g. a trial with very little lodging) will erase (wipe out) the differences between varieties that have been recorded in other trials or in previous years.

Therefore it is important to define which data should be taken into account for the final judgement of varieties. As a general rule the following conditions should be fulfilled:

> 1. At least one variety is lodging and this must occur in the different replicates.
> 2. Not more than 50 % of the whole trial maybe lodged. It is very likely that in this case other factors than genetic differences are recorded.

6.2 Resistance to lodging or sensibility for lodging

Some countries evaluate the resistance to lodging; other countries evaluate the sensibility for lodging. Some countries give a score from 1 to 9, other countries give a score from 9 to 1. In Germany, Switzerland, Austria, France the desired expression is described with a score 1 and the undesired expression is described with a score 9. In Belgium and The Netherlands the desired expression is described with a 9 and the undesired expression is described with a score 1.

It is beyond the scope of this handbook to give recommendations on what is the best definition or to change the customs that have been used for a long time in various countries.

6.3 Criteria for evaluation of new varieties

Different evaluation systems can be used for a decision on the acceptance of new varieties.

1.   the data of a new variety are compared with the standard (= average of the potential standard varieties)
   - Refusal if the value of a new variety is lower than the standard
   - Acceptance if the value of a new variety is at least the level of the standard

2.   the data of a new variety are compared with a reference variety which has some sensibility for lodging. The reference variety must be chosen in such a way that the degree of sensibility is still acceptable for practice.
   - Refusal if the value of a new variety is lower than the reference variety
   - Acceptance if the value of a new variety is at least the level of the reference variety

Table 2   Summary of data for evaluation of lodging by various Susvar partners

	Belgium CLO	Germany BSA	Norway	Spain	Netherlands LBI	France Arvalis	Austria AGES	Switzerland FOAG
Characteristic	Resistance	Sensibility	Sensibility	Sensibility	Resistance	Sensibility	Sensibility	Sensibility
Method for scoring	Combination of extension (0–100%) and intensity (9-1)	Combination of extension (0 – 100 %) and intensity (1 -9)	Extension (0 -100 %)	Extension (0 - 100%)	Combination of extension (0 – 100 %) and intensity (9 – 1)	Combination of extension (0-100%) and intensity (0-10)	Combination of extension (0 – 100 %) and intensity (1-9)	Intensity (1-9)
Use of index	yes	no	no	no	no	yes		
Observation time	1: first lodging 2: just before harvest 3: between 1 and 2	1: after heading (BBCH 60-75) 2: just before harvest	1: after heading 2: just before harvest	1. at first lodging 2. before harvest 3: if necessary more often	1. when lodging occurs or when it increases	no specification	when lodging occurs or when it increases (up to 3 or 4 times)	no specification

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The procedure for evaluation of lodging is described in the national protocols for variety testing.

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NUTRIENT USE EFFICIENCY
Lars Ericson

1. Introduction

In organic cereal production plants have to rely more than in conventional production systems on mineralization from the soil, from green manure and from manure, to fulfil the need of readily available nutrients. In conventional systems it is easier to create high levels of nutrients when the plants need them, by using commercial fertilisers. As a consequence the readily available amount of nutrients is often lower in organic than in conventional systems. Varieties that tolerate low levels of available nutrients in the soil, and nevertheless give high yields, are likely to perform better in organic production systems. The ability of varieties and lines to do so will be an important trait in selection and evaluation.

Different studies have been carried out to determine how breeding has changed the performance of varieties, with respect to nutrient use efficiency (NUE).
(For a definition of the terms, see the Methodologies section below.)
In a recent study in Finland Muurinen et al (2006) evaluated the nitrogen use efficiency of wheat, oat and barley cultivars released between 1909 and 2002. Modern wheat and oat varieties appeared to have a higher nitrogen use efficiency than older varieties. A similar trend was not found in barley. This may be caused by the fact that the old landraces of barley already had a high nitrogen use efficiency while this was not the case for wheat and oats. The authors concluded that the improvement of nitrogen use efficiency in oats and wheat was mainly caused by an improved nitrogen utilization efficiency and not uptake efficiency.

Available nitrogen in the experiments described above was rather high, which may have been an advantage for the modern varieties. Ortiz-Monasterio (1997) showed that also at moderate nutrition levels modern wheat cultivars had higher NUE-values than old cultivars. Another study, however, showed that old landraces and lines bred under low input conditions were more efficient in their nitrogen use than modern European cultivars (Gorny, 2001).

Slafer et al (1990) studied 6 bread wheat cultivars released between 1912 and 1980 and concluded that breeding had not affected total uptake of nitrogen either at anthesis or at maturity. Breeding had improved the grain yield through a changed harvest index (HI), nitrogen harvest index (NHI) and higher grain number. The change in HI, however, is higher than the change in NHI, thus resulting in a dilution of N and a lower nitrogen concentration in the grain.

Baresel et al (2005), studying wheat varieties, concluded that organic farming environments show more variation and differ from those at conventional farms. Varieties adapted to a conventional farming environment therefore are unlikely to be successful in organic farming, especially in environments with low productivity.
As nutrient supply is maintained mainly by mineralization, there is usually a shortage of nitrogen in the later season, whereas in conventional farming addition of nitrogen late in the season can be used to increase protein content in the grain (Barecel et al, 2005).

Genotypes with a high early uptake of nitrogen and a high ability for translocation to the grain will be more adapted to organic farming, whereas a late uptake of N is probably less important in this farming system. The authors found most of the modern varieties to be better performing in high-input environments or to have little genotype-environment interaction. Their uptake of nitrogen under low-input conditions was not high enough to meet quality standards for bread wheat. Older varieties and especially varieties bred for organic farming had higher protein contents, some of them both in high and low input environments. High protein levels were achieved, but in combination with relatively low yields.

Van Ginkel et al (2001) studied a way to segregate populations for improved nitrogen use efficiency in bread wheat, by either using high or low nitrogen conditions or alternating high and low N-conditions during the selection. When tested in low nitrogen environments, all these regimes gave a positive effect on yield, due to higher biomass production and higher harvest index, but there were no differences among regimes. In medium or high nitrogen environments alternating high and low N-regimes during selection, starting with high in F2 gave the best result. All changes were due to higher biomass production with no change in harvest index.

Le Gouis et al (2000) found varietal differences in nitrogen utilisation efficiency at low nitrogen levels. There was no consistent difference between older or more recent varieties in this respect. They also concluded that nitrogen uptake efficiency accounted for more of the variation in nitrogen utilisation efficiency at low nitrogen levels than at high nitrogen levels.

Brancourt-Hulmel et al (2003) concluded that modern varieties in general used nitrogen more efficiently than older ones. In their study recent varieties were also more "stable" in the sense that they produced higher yields both in low and high input environments.

This short literature review shows that there is a genetic variation in nitrogen utilisation efficiency within most cereals. The question about whether old or more recent varieties performs best in low input environments is not answered. Different studies has come to contradicting results and there seems to be more variation within each group then between the groups. Hence, including evaluation of nitrogen utilisation efficiency, or even nutrient utilisation efficiency, is important. It is also important to stress that variety testing ideally should be performed in an environment that is similar to what can be expected in practice.

The studies referred to in this review all deals with nitrogen. The way of expressing use and utilisation can be used for other nutrients as well, but there seems to be little work done on other nutrients than nitrogen.

Root characteristics

Root characteristics can be used as a trait that indirectly indicates differences in nutrient uptake by different varieties. Bertholdsson (2000) has developed a method to select lines with potential high nitrogen use efficiency in aquaculture, using root length in barley and plantlet weight in spring wheat as the primary selection criteria. In barley it is important that the nutrient solution is low in oxygen in order .to obtain a good correlation with field uptake data. Ortiz-Monasterio *et al* (2001), on the other hand, claim that there is a low correlation between the behaviour of varieties in aqua culture and in the field, because a nutrient solution cannot simulate the soil-plant interface.

Manske et al (2000) studied the phosphorus uptake efficiency in wheat in Mexico. They concluded that root length density at anthesis was the single most important root characteristic for phosphorus absorption and it was genetically positively correlated with phosphorus uptake efficiency. Not all varieties showed this correlation. In addition the germplasm had to be adapted to acid conditions and have a high yield potential under favourable conditions.

Other investigations do not confirm a strong connection between root morphology and phosphorus uptake. Løes (2004) found no connection between the studied root traits and the uptake of nutrients in different barley varieties. However, she claims that a high root density is likely to result in a higher nutrient uptake.

2. Methodologies

Yield is the most obvious parameter to measure the performance of different varieties in different environments. However, it may be useful to try to split up the reaction of the plants in different plant characteristics.

The easiest way of estimate the ability of plants to extract nutrients is to measure the amount of the nutrient harvested. Depending on the use of the crop, the concentration of a nutrient (e.g. N-concentration as protein) can also be used as a parameter (e.g. to ensure a good baking quality in bread wheat).

To achieve a realistic testing of the cultivars it is important to choose test sites that have nutrient levels that correspond to those that can be expected in practice on organic farms. The results of Baresel et al (2005) confirm the importance to test new varieties in an environment that resembles as much as possible to the situation that you would expect in practice.

Moll et al (1982) have made a more intrinsic analysis of the efficiency of plants to use nutrients. They define nutrient use efficiency as the grain yield per unit of nutrient supplied, the source of the nutrient being either from applied nutrient in fertilisers /manure or from mineralization of nutrient from the soil.
Nutrient use efficiency can be divided into two components:
- uptake efficiency: the ability of the plant to extract nutrients from the soil
- utilization efficiency: the ability of the plant to convert absorbed nutrients into grain yield.

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1. Nutrient use efficiency = Grain dry weight / Total nutrients supplied
2. Uptake efficiency   = Total nutrients in the plant (above ground)at maturity / Total nutrients supplied
3. Utilization efficiency   = Grain dry weight / Total nutrients in the plant (above ground) at maturity
4. Nutrient use efficiency = Uptake efficiency x Utilization efficiency

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Moll et al also give examples on how to apply a statistical model for data evaluation.

Gerloff (1977) proposes a system to distinguish between cultivars according to their reaction to different phosphorous levels in the soil. This classification can be used as a tool to describe how varieties perform. He proposes the following groups:

- efficient   / responder
- inefficient / responder
- efficient   / non-responder
- inefficient / responder.

Efficient versus inefficient describes the way varieties perform under low nutrient supply. Responder versus non-responder describes the performance at high nutrient supply. This way to classify varieties can be helpful. According to the above discussion one should give priority to efficient-responder varieties; i.e. varieties that perform well under low nutrient supply, but can react positively on increased nutrient levels.

Nutrient uptake efficiency

How and when to sample and analyse the crop depends on what questions are to be answered. Uptake efficiency (Equation 2) is best measured at anthesis or/and at physiological maturity (Ortiz-Monasterio et al, 2001). At anthesis there is no need to separate grains and stems, whereas at maturity this separation is necessary as the concentration in stems and grains at that stage is very different. To calculate nutrient uptake efficiency at maturity one can use the weighted average to calculate the total amount of nutrient in the plant.

Equation 1: $N_t = G_0 \times N_g + B_0 \times N_b$

N_t =   Total above-ground nutrient in the plant at maturity
G_0 =   Grain weight at 0% moisture (g m^{-2})
N_g =   Nutrient concentration in the grain (%)
B_0 =   Non-grain biomass at 0% moisture (g m^{-2})
N_b =   Nutrient concentration in non-grain biomass (%)

Equation 2:
        Nutrient uptake efficiency = N_t /Total nutrient supplied

To do the calculation, the amount of available nitrogen has to be estimated.
In organic farming nutrients usually come from mineralization of organic matter such as manure, preceding crops or from soil organic matter. It is difficult to get an accurate estimation of the total amount of available nutrients during the growing season without intensive sampling. However, it can be justified to simplify this measure by for example sampling for mineral nitrogen in spring. Although the measured value is in fact too low, a relative comparison between cultivars is still possible, assuming that the mineralization is the same in all plots.

Another method is to relate available nutrients to the amount that was applied in e.g. manure. It can also be of interest to create different levels of nutrients, either by spreading different amounts of manure or by using different sites that are high or low in nutrients. This can give additional information on how the different varieties perform in different regimes.

There are also different models that estimate available nitrogen during the season. One example is a model developed at the Louis Bolk Institute in the Netherlands. It can be found at their homepage (www.ndicea.nl).

Nutrient utlization efficiency

The crops ability to convert absorbed nutrient into grain yield is defined as nutrient utilization efficiency (see also textbox on page N3).

Equation 3:   **Utilization efficiency = G_w/N_t**

G_w =   grain weight
N_t =   total above ground nutrient at maturity

However utilization efficiency can also be expressed as harvest index (HI) times nutrient biomass production efficiency (BPE) (see equation 4).

Equation 4: $G_w / N_t = G_w / T_w \ x \ T_w / N_t$

G_w / T_w =   harvest index (HI)
T_w / N_t =   nutrient biomass production efficiency (BPE)

At maturity it is necessary to separate grains from other non-grain biomass while measuring nutrients in above ground biomass (see above). That gives the input to calculate both harvest index (HI) and nutrient biomass production efficiency (BPE), which can be valuable as these two parameters can give information on the strategy of different plants to increase nutrient utilization efficiency (Ortiz-Monasterio, pers. comm.).

Differences in root system development are important, especially to explain differences between plants in phosphorous uptake, as most of the phosphorous is absorbed mainly by diffusion. Root studies and especially quantitative analyses of roots in the field are laborious, as they involve digging up roots and washing them clean from soil. As root densities vary quite a lot within space, a rather intensive sampling is needed to get a good estimate of root biomass. They are therefore not well suited for variety trails where we need simple and cheap methods. A review of

different techniques to measure genetic diversity in root development has been made by Manske et al (2001).

Studies in nutrient solutions are only relevant if the results are correlated with field data.
The challenge is to identify crucial factors in the hydroponic system that give results that can be related to field data.
Early vigour (growth) of both shoot and root is of particular interest for high nutrient uptake and grain yield in organic cereal production. It can be evaluated in hydroponics (Bertholdsson 2000) or indirectly by measuring biomass production and nitrogen uptake prior to anthesis. The two parameters can be measured in weed free plots non-destructively by multispectral radiometers (e.g. *Cropscan*).

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PROCESSING QUALITY

Eva Johansson, Heinrich Grausgruber and Stuart Swanston

1.   Introduction – what is processing quality?

Products manufactured from cereals include those derived from flour (bread, biscuits and other bakery products), from semolina (pasta, noodles), from polished and pearled grain (breakfast cereals: flakes, pops, crisps etc.; bulgur, couscous), and from malt (beer, spirits). In this chapter we mainly consider the testing of varieties for baking, pasta, brewing and distilling quality. Marketing cereals with properties that contribute to human nutrition and health is a relatively new area. A brief overview of compounds with possible health promoting effects will also be given.

End-use quality of cereals is generally defined by the requirements for the type of commercial processing for which the crop is commonly used. Thus, growers are provided with recommendations for varieties that are suitable to supply given markets, such as wheat for bread-making or barley for malting (HGCA, 2005).

Quality testing is based initially on tests that mimic, on a laboratory scale, the commercial process e.g. micro-malting (Whitmore and Sparrow, 1957), but this will ultimately be supplemented by data derived from pilot or commercial-scale evaluation. Successful performance at this latter level is generally necessary to achieve recommendation from organisations that represent commercial end-users of cereals. Defined in these terms, quality is essentially a measure of commercial value and standards are set by the end-user, e.g. the quantity of alcohol that a distiller may obtain from a given quantity of grain or malt.

In this type of situation, quality is easy to define, but may be rather more difficult to measure. The expression of quality traits is often influenced by a large number of genetic components and by environmental conditions. In addition, there is likely to be variation in the response of different varieties to environmental changes, a phenomenon generally referred to as genotype by environment interaction (G×E). Consequently, a particular batch of grain may not have a suitable processing quality, despite the fact that it comes from a recommended variety. Also processing equipment, methodology and recipe influence the outcome of quality tests. As end-users usually are a diverse group, with each miller or brewer following its own specific way of processing, it is difficult to define standardized quality testing procedures, which predict the quality for a specific processor.

From the above it becomes clear that the target market of the end product has to be defined before setting up a quality testing system for varieties and that the end-users should be involved in the design of the research. A number of methodologies, which could be included in such research are discussed in the following sections.

2.   Description of methodologies

This section starts with a description of methodologies for evaluating baking quality. This is followed by sub-sections on pasta quality, brewing and distilling quality and finally a number of compounds with possible health promoting effects are presented.

2.1. Baking quality of wheat

Baking quality is an important but complex character (Pomeranz, 1988). For large-scale bread production, the desirable quality criteria are industry driven. Consumer's demands, bread-making technology and cultural traditions differ largely between countries (Mac Ritchie, 1984 and Mesdag, 1985). Application of tests for assessing baking quality must, therefore, reflect this variation. Baking tests are also influenced by the operator and equipment, so any variation can make accurate repetition difficult. Thus, traits and tests to assess baking quality vary from country to country. Only two traits are used worldwide: crude protein content and Hagberg falling number (see below).

In this section we will describe numerous methodologies, which are used in different European countries. These are ordered into four different categories:
- Grain and flour quality traits
- Dough (Rheological) properties
- Baking traits
- Milling quality

Examples of different traits within each category are listed in Table 1. For most traits detailed protocols for assessment have been published by both the International Association for Cereal Science and Technology (ICC, 2001) and the American Association of Cereal Chemists (AACC, 2000).

2.1.1. Grain and flour quality traits

The industry only buys wheat as "baking wheat", at a premium price, if it has reached a minimum percentage of protein. This minimum percentage varies from country to country. Protein content is used as a first prediction for loaf volume. Correlation between protein content and loaf volume is often relatively low. One reason is that besides the total amount of proteins, the quality of the proteins also plays a role. A number of tests and parameters are used to establish protein quality (e.g. Zeleny-sedimentation, gluten index), but, with these parameters, loaf volume can also be only partly predicted.

Hagberg Falling Number predicts α-amylase activity. This enzyme, which usually becomes active when harvesting has been delayed and field conditions have been humid, breaks down starch and this has a negative effect on loaf volume.

Crude protein content
Crude protein is an expression of the total content of nitrogenous compounds in the analysed product, generally calculated from the total nitrogen content by multiplying with a conversion factor (the figure of 5.7 is used for wheat and wheat products for human consumption; values for other commodities range up to 6.25, including wheat used as animal feed). Methods can be roughly subdivided into 'reference' and 'prediction' methods. The reference methods most commonly used in grain analysis are the Kjeldahl method (ICC Standard No. 105) and the Dumas method, or Combustion Nitrogen Analysis (ICC No. 167). The main advantage of the Dumas over the Kjeldahl method include speed per test, improved precision, no corrosive or hazardous chemicals, low cost of installation, and safe operation. The Dumas method is also slightly more efficient than the Kjeldahl test in extraction/conversion of nitrogen (0.15-0.25%

higher protein values than Kjeldahl in wheat) (Williams et al, 1998). The most commonly used prediction methods are Near-infrared Reflectance or Transmittance Spectroscopy (ICC No. 159). These methods are simple, fast, reproducible and accurate enough to be carried out at elevators or during breeding, but require a calibration based on one of the other two methods. Compared to the reference methods there are more sources of error associated with the instrument, the sample or the operator. New generations of instruments and new software, however, have made near-infrared methods consistently more efficient and reliable.

Table 1. Examples of different types of traits assessed in bread-making quality tests

TRAIT/PARAMETER	METHODOLOGY	STANDARD PROTOCOL NUMBER[1]
GRAIN AND FLOUR QUALITY		
Crude Protein Content	Kjeldahl	ICC No 105
	Dumas Combustion	ICC No. 167
	Near Infrared Reflectance (NIR)	ICC No. 159
Protein Quality		
Wet Gluten Content	Glutomatic	ICC No. 137/1
Gluten Index	Glutomatic	ICC No. 155
Zeleny-Sedimentation value	Suspension in lactic acid	ICC No. 116/1
SDS-sedimentation	Suspension in lactic acid-sodium dodecyl sulphate	ICC No. 151
Enzyme (α-amylase) activity	Hagberg falling number	ICC No. 107
Viscosity (Starch quality)	Brabender Amylograph Rapid Visco Analyser	ICC No. 126/1 AACC No. 76-21
DOUGH (RHEOLOGICAL) PROPERTIES		
Mixing behaviour (water absorption, dough development and stability/breakdown)	Brabender Farinograph Chopin Consistograph Swanson Working Mixograph	ICC No 115/1 ICC No. 171 AACC No. 54-40A
Dough properties (extensibility/elasticity)	Brabender Extensograph Chopin Alveograph	ICC No. 114/1 ICC No. 121
Stickiness	Sensory methods, dough profiling with texturometer	
BREAD AND BAKING TRAITS		
Loaf Volume Crumb texture Crust and crumb colour Rising behaviour	Rapeseed displacement Texturometer Spectrophotometer Brabender Maturograph / Oven-Rise Recorder	AACC 10-05 AACC 74-09
MILLING QUALITY		
Flour yield	Experimental milling	AACC 26-ff.
Ash content	Ashing at 900°C (muffle furnace)	ICC No. 104/01
Hectoliter weight	Chondrometer	

[1] Executive summaries of standard protocols of ICC can be found at http://www.icc.or.at/publ.php or in a printed version (ICC, 2001). AACC protocols are published (AACC, 2000).

Protein Quality
The protein quality tests mentioned in Table 1 are based on the fact that different fractions of proteins dissolve (or are suspended) in different chemical solutions. The solutions used thus distinguish the fraction of proteins which can be measured in such tests
Wet gluten is isolated from dough by washing with a solution of sodium chloride, drying and weighing the residue. Usually this test is carried out mechanically using the Glutomatic apparatus. Gluten quality can be determined by centrifuging the wet gluten and forcing it through a specially constructed sieve under standardised conditions. Gluten strength, also known as the Gluten Index (GI), is determined by the proportion of gluten remaining on the centrifuge sieve. If the gluten is very weak all of it may pass through the sieve and the GI is 0; when nothing passes through the sieve, the GI is 100. Gluten quality can also be determined by various swelling tests. The Gluten Swelling Test, also known as Berliner test, determines the volume of gluten swollen in a dilute solution of lactic acid. This test is similar to the Sedimentation tests in which the effect of the swelling capacity of a flour/meal suspension on the rate of sedimentation is determined. The suspension may be in lactic acid (Zeleny test, ICC No. 116/1) or lactic acid-sodium dodecyl sulphate (SDS sedimentation test, ICC No. 151). Better gluten quality (higher gluten strength) leads to higher sedimentation volume.

Enzyme (α-amylase) activity
Activity of α-amylase is determined from the Hagberg falling number which is defined as the time required for a viscometer stirrer to fall a measured distance through a hot aqueous starch gel (ICC No. 107) . As viscosity is reduced by starch hydrolysis, falling number is an indirect measure of α-amylase activity. It is important to note that for this trait the industry requires a minimum standard to be achieved, but higher values may not equate with better quality. Values below 180-200 seconds are undesirable, while, above 250 seconds, wheat is well suited for baking.

Viscosity (Starch quality)
Starch quality is rheologically tested by the Brabender Amylograph (ICC No. 126/1), which determines the gelatinization properties of starch, the method being applicable to wheat and rye flours, meals and wholemeals. The amylograph viscosity is the resistance, measured as torque and expressed in arbitrary units (Amylograph Units, AU) of a flour-water suspension heated at a constant rate of temperature increase and with the bowl rotating at a specified, constant speed. There are other mechanical devices for assessing the viscous properties of starch slurries, one widely used within cereal quality testing being the Rapid Visco Analyser (RVA) (AACC No. 76-21). Both the amylograph and the RVA can also be used for the indirect determination of α-amylase activity (AACC Nos. 22-10 and 22-08, respectively).

2.1.2. Dough properties
Numerous instruments have been devised to obtain objective data on dough properties in order to predict its behaviour in the bakery. The physical dough-testing instruments can be subdivided into recording dough mixers (e.g. Farinograph, Mixograph) and load-extension instruments (e.g. Extensograph, Alveograph). Differences between recording dough mixers rely, for example, on work input, type of mixer, mixing speed and amount of water added at the beginning of mixing. Differences between the load-extension meters rely on preparation and consistency of the dough and direction of extension (i.e. uniaxial or biaxial).

Mixing and water absorption
Water absorption and mixing requirements of flour can be determined by the Brabender Farinograph (ICC No. 115/1), the Chopin Consistograph (ICC No. 171) or the Swanson-

Working Mixograph (AACC No. 54-40A). Water absorption is the volume of water required to produce dough, with a defined consistency, under specified operating conditions. After determining the degree of hydration required, a subsequent test is performed at this hydration level, to evaluate the physical properties of the dough, e.g. dough development time, dough stability, mixing tolerance/dough softening. Various other pieces of equipment are also available to determine the mixing requirements of flours, all of which follow the same basic principles, but differ in factors such as sample size, mixing speed and bowl design.

Extensibility and stickiness
Rheological characteristics of the dough include stickiness and extensibility. Extensibility can be determined either by stretching the dough in a Brabender Extensograph (ICC No. 114/1) or by inflating it in a Chopin Alveograph (ICC No. 121). The force-extension curves are recorded and used to assess the general quality of the flour. Recorded parameters comprise the resistance of the dough to extension, the extent to which the dough can be stretched before breaking (extensibility), the area under the force-extension curves (energy) and ratios between parameters, e.g. resistance/extensibility.

2.1.3. Bread and Baking traits
Flour characteristics such as protein content and, particularly, gluten strength, linked to rheological properties of the dough, may give a good prediction of bread-making quality. However, this can only be confirmed by making bread. The most important baking trait has thus been the loaf volume (MacRitchie, 1984 and Finney et al, 1987), a measure of how well the loaf rises during proving and baking. Baking tests within European countries differ, however, with regard to both dough formulation and process and to bread type and form (pan/tin vs. hearth bread; baguette, buns/rolls, etc.). Consequently, results of loaf (baking) volumes from diverse national variety trials are hardly comparable. Despite this, loaf volume is usually the major characteristic for the classification of varieties with regard to breadmaking quality. Other traits (flour, rheological) are used to either confirm or downgrade the quality classification of a variety. In addition to loaf volume, the texture and colour of bread crumb and crust, and the dough handling qualities are determined in baking tests.

2.1.4. Milling Quality
Another important trait for varieties of bread wheat is milling quality. Unless a variety is exclusively used for whole grain flours the extraction rate (flour yield) is responsible for the final decision as to whether a variety is accepted by millers. Usually the extraction rate is defined as the amount of flour of a defined ash and moisture content obtained from 100 kg wheat ('as received' or after cleaning and tempering). Flour extraction is determined by milling with specified experimental mills, e.g. the Bühler MLU laboratory mill. Flour extraction depends on factors such as kernel morphology and texture.

2.1.5. Classification systems
Traits and methods used to evaluate end-use quality during VCU trials in the European Union are different from country to country, as are the classification systems of wheat baking quality. Intensive testing is carried out e.g. in Austria and Germany. In both countries the main criteria for classification are derived from baking tests, namely loaf volume and dough characteristics (elasticity, stickiness etc.). The baking test method, however, differs between the countries. Performance in the above traits leads to classification of wheats into specific baking quality groups. To remain in a group, however, the respective breeding lines have to reach certain limits

in various other quality traits. In Austria, these other criteria include crude protein content, wet gluten content, Zeleny sedimentation value, Hagberg falling number, Farinograph quality number, Extensograph water absorption and Extensograph energy (area under the curve). If a breeding line fails to achieve the standard in one or more criteria, it will be downgraded one quality group. However, if only one criterion is not fulfilled there is a possibility to compensate if performance in another achieves the standard of an even higher quality group. Finally, the variety is classified into one of 9 quality groups, in which 9 represents the highest baking quality. Trading of wheat follows three market classes: improver wheats ('Qualitätsweizen', quality groups 7-9), bread wheats ('Mahlweizen', quality groups 3-6), and wheat for other uses ('Sonstiger Weizen', quality group 1-2). Wheats with insufficient extraction rate (milling quality) are classified as wheat for other uses independently of their original quality classification. Within improver wheats lots with >15% protein content and >280 sec. Hagberg falling number are traded as 'premium wheats' resulting in extra payments for the farmers.

In Germany, besides baking traits other criteria for quality classification are crude protein content, Zeleny sedimentation value, Hagberg falling number, flour granulation (% particle size >75 µm), flour water absorption, ash content and extraction rate. On the basis of these traits wheats are classified into four quality groups: E ('Eliteweizen'), A ('Qualitätsweizen'), B ('Brotweizen') and C ('Sonstiger Weizen'). Within the last category wheats with a biscuit making quality are indicated by a subscript 'k', e.g. C_k.

2.2. Pasta quality of durum wheat

Durum wheat (T. durum) is an important crop in Mediterranean countries and to a more limited extent in Central and Eastern Europe. Durum is used for a wide range of end-products such as pasta, bulgur, couscous, or bread. In the following, only pasta making quality of durum is considered.

Table 2. Examples of different types of traits assessed in pasta-making quality tests

TRAIT/PARAMETER	METHODOLOGY	STANDARD PROTOCOL NUMBER [1]
GRAIN AND SEMOLINA QUALITY		
Crude Protein Content	Kjeldahl	ICC No 105
	Dumas Combustion	ICC No. 167
	Near Infrared Reflectance (NIR)	ICC No. 159
Protein Quality		
Wet Gluten Content	Glutomatic	ICC No. 137/1
Gluten Index	Glutomatic	ICC No. 155
SDS-sedimentation	Suspension in lactic acid-sodium dodecyl sulphate	ICC No. 151
LMW-glutenin/gliadin analysis	SDS-PAGE	
Enzyme (α-amylase) activity	Hagberg falling number	ICC No. 107
Viscosity (Starch quality)	Brabender Amylograph Rapid Visco Analyser	ICC No. 126/1 AACC No. 76-21
Yellow pigments	Extraction / Spectrophotometer	ICC No. 152
Yellow index	Colorimeter (CIE Lab)	
Kernel vitreousness	Pohl-Farinotom	
Brownness / Brown spots	Visual scoring	
Dough properties (extensibility/elasticity)	Chopin Alveograph	ICC No. 121
PASTA TRAITS (COOKING TEST)		
Texture, stickiness, flavour, taste	Texturometer, Sensory analysis	
Yellowness (before/after cooking)	Colorimeter	
MILLING QUALITY		
Semolina yield (Extraction rate)	Experimental milling	AACC 26-ff.
Ash content	Ashing at 900°C (muffle furnace)	ICC No. 104/01
Hectoliter weight	Chondrometer	
1000 grain mass		
Kernel size >2.8 / >2.5 mm	Sieving machine	
Kernel hardness	Particle Size Index, NIRS, SKCS	

[1] Executive summaries of standard protocols of ICC can be found at http://www.icc.or.at/publ.php or in a printed version (ICC, 2001). AACC protocols are published in AACC, 2000.

2.2.1. Grain and semolina quality traits

Methods to determine grain and flour related quality traits are, in part, similar to those already discussed with regard to baking quality of common wheat. For evaluation of protein quality by sedimentation however, the SDS-sedimentation test is preferred for durum wheat. Grain protein content, protein quality, and colour (yellowness) are the major attributes affecting pasta-making technology characteristics and resistance to overcooking. Generally, high protein durum wheat yields a semolina of uniform particle size with a minimum number of starchy particles, and thus will hydrate evenly during mixing and produce a pasta product that is physically strong, elastic and less sticky. As for bread wheat, protein quality may be determined by the Gluten Index or the electrophoresis of glutenin and gliadin proteins. In durum wheat LMW-2 and gliadin γ-45 express strong gluten, whereas LMW-1 and gliadin γ-42 express weak gluten (Pagnotta et al, 2005). Yellowness of semolina can be measured either directly by the extraction of the yellow pigments with specific solvents, e.g. water-saturated n-butanol (ICC No. 152), and measurement of their concentration in a spectrophotometer calibrated against β-carotene or xanthophyll (lutein), or indirectly using a CIE Lab-system colorimeter, e.g. Minolta chromometer, and registration of the b*-value. Apart from these three traits various others (Table 2) are of greater or lesser importance for breeding or VCU testing in various European countries. Rheological characteristics measured by the Chopin Alveograph are determined for example in Italy, while Falling Number and brownness of kernels are important quality traits in Germany and Austria. Kernel vitreousness, an optical property determined by the degree of translucence of the endosperm, is an important grain trait, especially for trading durum wheat. Vitreous grains have a compact, translucent endosperm whereas mealy grains have a white, opaque endosperm. Kernels with higher vitreousness have a higher protein content and a more continuous protein matrix. Vitreousness is highly influenced by environmental factors, in particular nitrogen fertility.

2.2.2. Pasta traits

Pasta quality traits are mostly evaluated after cooking tests. Cooking quality and appearance are the two most important factors in assessing pasta quality. The appearance of the pasta is mainly determined by the colour. Pasta colour results from a desirable yellow component and an undesirable brown component (Feillet et al, 2000). Yellow and brown indices of the pasta are usually determined before and after cooking by visual inspection (by a trained technician or a panel) or reflectometry. Diverse traits are evaluated on cooked pasta, e.g. stickiness, firmness, resistance to overcooking, texture, flavour and taste, either by sensory analysis by a trained technician/panel or by a texturometer equipped with various test rigs. Pasta-making and cooking tests are usually carried out only if durum wheat plays a major economic role as cereal crop, e.g. in Italy. In most other countries pasta cooking quality is determined indirectly by the evaluation of protein content, gluten strength and yellow pigment content.

2.2.3. Milling quality

Semolina yield (extraction rate) is defined, as for flour yield, by the amount of semolina of a defined ash and moisture content obtained from 100 kg durum wheat. Semolina extraction is determined by milling with specified experimental mills. Extraction depends on factors such as kernel morphology and texture, so several kernel characters, which are correlated with semolina extraction rate, are used as indirect traits for milling quality, e.g. test weight, 1000 grain mass, kernel hardness or percentage of shrunken kernels. The extraction rate of either fine (125-315 µm) or coarse semolina (310-545 µm) is determined, depending on the equipment used. Although the extraction rate of coarse semolina is lower, varietal differences between durum

varieties are similar for both types. Generally, quality durum should yield a high amount of low-ash (0.75-0.85%) containing semolina with a uniform amber colouring.

2.2.4. Durum Quality Index

For several years the European durum wheat quality premium has been paid only if a certain value in a newly created quality index is reached. This index comprises protein content, gluten index, yellow index and specific mass/hectolitre weight (or 1000 grain mass). Varieties have to be tested for at least two years. The mean performance of check varieties (at least the two most important ones according to the multiplication areas) in the respective four traits is set at 100. The performance of the other tested varieties is expressed relatively to the check varieties. Subsequently, the relative values are multiplied with specific weightings, i.e. 0.4 for protein content, 0.3 for gluten index, 0.2 for yellow index and 0.1 for hectolitre weight, and the sum of all four values is calculated. For quality durum varieties a quality index of at least 98 has to be reached.

2.3. Brewing and spirit quality of barley and wheat

Both barley and wheat can be used to produce alcoholic drinks (e.g. beer, whisky). Three processes can be distinguished:
- Brewing (to produce beer)
- Malt distilling (to produce malt whisky)
- Grain distilling (to produce grain whisky, vodka, gin)

In brewing and malt distilling, alcohol is produced by the conversion of barley starch into sugar, which is then fermented by yeast. An initial malting process is required, during which the cell walls and protein surrounding the starch are degraded by enzymes produced as the barley germinates, a process known as modification. In distilling, to produce grain whisky, unmalted cereal, usually soft wheat, is cooked under pressure at high temperature, to gelatinise the starch, before being mashed with a small quantity of malted barley of high diastatic power, i.e. with a high level of starch degrading enzymes. A broadly similar process, though not subject to the same legal restrictions, is used for the production of neutral alcohol, the basis for beverages such as gin and vodka.

For all these procedures, only certain varieties will be accepted, as the quality required for producing alcoholic beverages is a genetic character. However, not all batches of grain from these varieties may be acceptable, due to environmental effects or the varietal response to the environmental conditions (i.e the genotype x environment interaction). Barley grain being offered for malting will thus be subjected to tests based on the appearance, purity and undamaged nature of the sample as well as the grain nitrogen content. Unsatisfactory samples will be rejected. A series of traits can also be tested in the laboratory on grain and malt to assess the probable performance of a grain sample after commercial processing. Similar tests can be applied in breeding programmes.

Examples of different traits, within each category, are listed in Table 3. For some traits, detailed protocols for assessment have been published by both the International Association for Cereal Science and Technology (ICC, 2001) and the American Association of Cereal Chemists (AACC, 2000) . Many traits are also included in recommended methods of analysis produced by the Institute of Brewing (IOB) in the UK, or the European Brewery Convention (EBC).

Table 3 Examples of different traits assessed in brewing and distilling quality tests

CROP	TRAIT/PARAMETER	METHODOLOGY	STANDARD PROTOCOL NUMBER	RECOMMENDED METHOD	
				EBC	IOB
Barley	**GRAIN QUALITY**				
	Thousand grain weight	Counting/weighing		2.4	1.3
	Nitrogen %	Kjeldahl	ICC 105 AACC 46-10	2.3	1.5
	β-glucan %	Enzymic	ICC 166 AACC 32-22		
	Germinative energy	Germination		2.6.3	1.4.2
Barley	**MALTING/BREWING QUALITY**				
	Hot water extract	Specific gravity		3.3	2.3
	Viscosity	Viscometer		3.8	
	Kolbach index / soluble nitrogen	Kjeldahl		3.5.1	2.9
	Apparent attenuation	Specific gravity		7.4	
	Diastatic power	Starch digestion		3.6	2.6.2
Barley	**MALT DISTILLING**				
	Hot water extract	Specific gravity			2.3
	Fermentability	Specific gravity			2.12
	Predicted spirit yield	Equation			
Barley	**GRAIN DISTILLING**				
	Diastatic power	Starch digestion			2.6.2
Wheat	**GRAIN DISTILLING**				
	Spirit yield	Distillation			
	Viscosity	Viscometer			

[1] Executive summaries of standard protocols of ICC can be found at http://www.icc.or.at/publ.php or in a printed version (ICC, 2001). AACC protocols are published in AACC, 2000.
[2] EBC = European Brewery Convention (EBC); IOB = Institute of Brewing (IOB) in the UK

2.3.1   Barley Grain Tests

Grain nitrogen content
Grain protein, usually measured as nitrogen content, is very important. High levels are associated with a significant reduction in extract yield, since the protein forms a strong matrix around the starch granules, restricting access of enzymes (Palmer, 1980). By contrast, if protein levels are too low (i.e. below 7.5%) fermentation may be adversely affected, through inadequate nutrition for the yeast (Swanston et al, 2000). Although there have been several reports in the literature of genes conferring lower levels of protein, through reduced assimilation into the grain (Burger et al, 1979 and Emibiri et al, 2003), protein content is generally most strongly influenced by environmental and agronomic factors, such as rate and timing of fertiliser application.

Thousand Grain Weight
Thousand grain weights are derived by weighing the requisite number of grains and high values are indicative of well-filled grain. Poor grain-fill reduces starch content and extract yield. Maltsters usually sieve grain samples prior to malting to remove thin grain, and will not purchase samples from which significant portions will be lost by sieving.

β-glucan content
Although not used by maltsters, at intake, to determine acceptability, β-glucan content is
an important grain character, and one which has a strong genetic component. As a main constituent of the cell walls in the barley endosperm it is a major barrier to modification during malting (Martin and Bamforth, 1980). β-glucan solubilised during hot water extraction also contributes significantly to viscosity, which can lead to processing problems, particularly during filtration (Bamforth and Barclay, 1993). Routine measurement of β-glucan is now generally carried out by an enzymic method (McCleary and Glennie-Holmes, 1985). It can also be measured in malt as an indication of the extent of modification.

Germinative energy
Grains (100) are germinated, at around 20^oC, on filter paper to which 4ml of water has been added and the percentage of grains germinating is counted at daily intervals. Significant numbers not germinating after 72hrs is generally indicative of residual dormancy. Grain samples will not be malted until dormancy has been shown to have broken.

2.3.2   Malting and Brewing Tests

There is a large number of tests that can be applied to malted barley samples, but the EBC has defined five as particularly important, contributing to a Quality Index. There are many other brewing quality characters, relating to factors like head retention and flavour, but the wide range of brewing styles and products mean that they may only be important in certain cases. Consequently they are not routinely measured in generic assessments of quality. IOB methods are generally similar to those of EBC, but with some important differences, which are noted for the relevant procedures.

Extract Yield
Also referred to as hot water extract, this is regarded as the most important measure of malting quality, with the highest weighting in the EBC quality index. Determined by specific gravity, it is a measure of the percentage of milled malt that can be brought into aqueous solution. In the EBC method, extraction begins at 40^oC, rising to 70^oC. The IOB method uses a uni-thermal extraction at 65^oC (The Inst. of Brewing, 1982), representing the initial temperature used commercially in the UK, and at which cell-wall degrading enzymes are inactivated. Cell-wall degradation must, therefore, occur during malting. However, the measurements made after extraction are similar to those made by the EBC methods (above).

Wort Viscosity
High viscosity is likely to cause processing (especially filtration) problems in brewing. It generally results from β-glucan, not degraded during malting, being solubilised during extraction. Alternatively, β-glucan content can be measured directly.

Kolbach Index

This is the percentage of total malt nitrogen content solubilised during extraction and is measured by the Kjeldahl procedure. It is an indicator of the extent of malt modification. Typical values are around 40%, but can vary depending on the required use for the malt. Soluble nitrogen content (IOB) is measured in the same way as for the Kolbach index, but calculated as a percentage of the total soluble material rather than the total malt nitrogen. Typical values are therefore 0.5 - 0.6%.

Apparent Attenuation

A measure of the alcohol produced after the wort is fermented, this indicates the percentage of the hot water extract that is made up of fermentable sugars.

Diastatic Power

A measure of the total starch degrading enzyme activity, this is particularly important when starch-based adjuncts are added to the mash (see grain distilling, below).

2.3.3. Malt distilling tests

Fermentability

This is a measure of the percentage of the material, solubilised in the wort, that can be fermented to alcohol. However, unlike brewing, the wort is not boiled and cooled prior to fermentation, so starch-degrading activity persists into the fermentation stage, leading to higher levels of fermentability than those of brewing worts.

Predicted spirit yield

For malt distilling, quality is defined by the amount of alcohol obtained from a given quantity of malt. This is predicted, by equation, from the fermentable extract, which is the product of two parameters, extract yield and fermentability. These are determined from specific gravities measured before and after fermentation with yeast, respectively, and indicate the percentage of material in the wort that is both soluble and fermentable.

2.3.4. Grain distilling (Barley)

Diastatic Power

High levels of starch degrading enzymes are required when large quantities of starch-based adjuncts must be broken down by malt enzymes. This occurs in certain types of brewing but, particularly, in grain distilling. Enzyme activity can be measured collectively as diastatic power, using methods from EBC (Analytica EBC, 1998) and IOB (Inst. of Brewing, 1982). Additionally, specific substrates have been developed to allow the enzymes α-amylase (McCleary and Sheenan, 1987), α-amylase (McCleary and Codd, 1989) and limit-dextrinase (McCleary, 1992) to be assayed independently.

Of the enzymes required for starch breakdown, α-amylase is much more heat labile than α-amylase, so activity is lost during hot water extraction. Research in both Japan and Australia has revealed genetic differences in α-amylase thermostability (Kihara et al, 1998 andEglington et al, 1998) and shown low thermostability to have adverse effects on alcohol yield, particularly from EBC extracts (Edney et al, 2005), where temperatures rise to 70°C. As α-amylase activity is highly correlated with grain protein, selection for genotypes that retain a greater proportion of α-amylase activity at extraction temperatures may thus be advisable for low-input cultivation of barleys required for certain types of malt.

2.3.5. Grain distilling (Wheat)

Spirit Yield
The main determinant of quality in distilling wheat is the amount of alcohol obtainable from a tonne of grain. This is, to a large extent, a varietal characteristic and only certain varieties are acceptable to the distillers. Environmental factors are also important, as high levels of grain nitrogen have an adverse effect on spirit yield, so distilling wheat is potentially a crop well suited to low-input cultivation. The Scotch Whisky industry has developed a procedure to determine the spirit yield of wheat samples in the laboratory (Brosnan et al, 1998). This essentially mimics the commercial process, on a much smaller scale, but is too expensive and time-consuming to be applied in breeding programmes. Using a scaled-down version of the IOB method for extract yield of adjuncts, coupled with a measure of grain protein content, has been suggested as a more rapid procedure (Swanston et al, 2005) but this requires testing over more varieties and seasons.

Residue Viscosity
High levels of viscosity can be problematic in a distillery, particularly during the drying and processing of the spent grain, which is often used for feeding to ruminants. Viscosity measurements are therefore made on distilling worts.

2.4. Nutritional/health quality of cereal products

Variety testing for compounds with specific nutritional or health qualities is limited in Europe. Although yellow pigmentation, due to carotenoids, is evaluated in durum wheat, this is not for the antioxidant capacity of carotenoids, but because of its natural colouring effects on the pasta. Bioactive compounds of cereals with potential health effects are micronutrients (e.g. vitamins, trace elements), dietary fibre (e.g. arabinoxylan, β-glucan), starch (e.g. resistant starch), and plant secondary metabolites (e.g. terpenoids, phenolics). Opportunities may result from the recent ruling by the US Food and Drug Administration (FDA) to permit barley products to be marketed as having cholesterol lowering properties. Although β-glucan is undesirable in brewing, it has been identified as a valuable contributor of soluble fibre in human diets (Hecker et al, 1998). Barley also has a low glycemic index (Granfeldt et al, 1994) and is a source of antioxidants such as tocopherols and tocotrienols (Colombo et al, 1996). Hulless (naked) barleys are easier to incorporate into a range of food products (Bhatty, 1996) and breeding of hulless barley with enhanced levels of β-glucan has been achieved in Canada. Although direct human consumption of barley grain is limited, compared to other cereals such as maize, wheat or rice, there is considerable opportunity for inclusion of barley malt, flour or extracted β-glucan in a range of food products. Recent research has considered the addition of hulless barley flour in bread, pasta and noodles (Izydorczyk et al, 2001 and Cavallero et al, 2004). However, because these substances are not included in standard variety testing systems no assessment methodologies will be discussed here.

Table 4. Examples of important compounds in cereals implying nutritional or health qualities

BIOACTIVE COMPOUND	IMPORTANT IN CROP	LITERATURE REFERENCE
MICRONUTRIENTS		
Vitamins		
Trace elements	Rye	Kujala 2006
ANTIOXIDANTS		
Carotenoids	Durum wheat	
Terpenoids	Rye	Kujala 2006
Phenolics	Rye, Oats	Kujala 2006 Dimberg 2005
Tocopherols	Barley	Colombo et al 1996
Tocotrienols	Barley	Colombo et al 1996
DIETARY FIBRE		
Soluble Fibre	Barley, Oat bran and Rye	FAO/WHO 1998
Insoluble Fibre	Wheat bran, Corn bran and Rice bran	FAO/WHO 1998
Arabinoxylans	Barley, Oat and Rye	FAO/WHO 1998
β-glucan	Barley, Oat and Rye	FAO/WHO 1998 Strobel et al 2001
Resistant Starch		
Low Glycemic Index	Barley	Granfeldt et al 1994

3. Special considerations for organic and low input variety testing compared to conventional variety testing

3.1. Choice of methodologies

Organically grown varieties used for large scale industrial processing have to meet the same requirements as conventionally grown varieties. If industries have a special production line for organic material, it may be relevant to adapt the variety assessment, depending on the size of the market and the costs of additional tests.

Conventional quality tests are mainly designed for refined flour and not for whole grain products. Standard methods (AACC, 2000 and ICC, 2001) devised for measuring flour and dough characteristics are primarily based on the use of white flour. Organically grown varieties might be used for a higher proportion of wholemeal products than conventionally grown varieties. However, the quality of bread is not similar when wholemeal and refined flours are compared. The water absorption of wholemeal flour is higher than of refined flour and the loaf volume of whole grain bread is significantly lower.
Assessments that are carried out on whole meal flour are protein content, falling number and SDS sedimentation value. Alternative parameters, such as glutenin/gliadin ratio, high molecular weight (HMW)-, low molecular weight (LMW)- glutenin subunit and gliadin content and composition, percentage of unextractable polymeric protein in total polymeric protein (%UPP) and glutenin macropolymer (GMP) are used in various countries in order to predict loaf volume (Payne et al, 1987, Johansson et al, 2003, Gupta et al, 1993 and Don et al, 2004). However, none of them has been widely applied to determination of wholemeal bread-making quality.

Quality measured on white flour can be used as an estimate of whole wheat performance but the identification of the best genotypes for whole wheat performance may require specific quality analyses. A selection for high quality based on white flour does not necessarily result in genotypes producing high quality wholemeal products. Correlations vary between traits: protein level is highly and consistently correlated with bread quality but, as dough is processed into a final product, the traits measured during this process show a progressive decrease in correlation (Bruckner et al, 2001).

Differences between genotypes or cropping systems are very important in determining the end-use quality in cereals (Johansson et al, 2003, Peterson et al, 1992 and Swanston et al, 2006), although these factors might even be less important than the milling techniques used to produce whole meal flour, such as the use of milling stones instead of industrial roller mills (Finney et al, 1985 and Kihlberg et al, 2004).

3.2. Quality testing and quality level of the raw material

For wheat grown under low input conditions it may be difficult to reach levels required by the conventional baking industry e.g. for protein content and gluten strength (L-Backström et al, 2004 and Andersson et al, 2005). The selection of varieties with improved quality under low input conditions is discussed in the chapter on nitrogen use efficiency.

As fermented beverage production relies on the conversion of starch to sugar, then to alcohol, the problems associated with protein levels in low-input bread wheat production do not occur. There are organic breweries in a number of European countries and it is unlikely that testing for

malting quality in low-input barley will differ significantly from that used for the conventionally produced crop. Many conventional breweries also include one or more organic beers in their portfolio of products. However, most malt sourced by these breweries will come from barley grown with uniform weed and disease control, so there may be concern that organic barley will prove less even in germination or modification during the malting process. Consequently, it may be useful to incorporate tests that are applicable to individual grains (as for populations and variety mixtures, below), so variation within, as well as between, batches can be assessed.

Variety testing for specific nutritional or health quality is not common in conventionally produced grains. Comparisons of the levels of secondary metabolites in plants produced under organic and conventional conditions indicate that they are equal or tend to be higher in organic products. Given the potential to simultaneously exploit health and low-input benefits, variety testing of nutritional properties can be a valuable innovation for low-input and organic cultivation.

3.3. Stability of Quality and the need for multi-environment trials

A very important aspect of variety testing of quality for low input agricultural systems is the use of replicated field trials in various environments, so called multi-environment trials (MET). This is due to the fact that quality is governed not only by genetical factors but also by the environment (Peterson et al, 1992, Johansson and Svensson, 1998 and 1999). Cultivation year and site have been shown to be as important as variety for the bread-making quality (Johansson et al, 2002 and Grausgruber et al, 2000). Nitrogen application is important for protein concentration and bread-making quality (Johansson and Svensson, 1999), but nitrogen availability for the plant is also governed by the soil and the climate as well as the nitrogen application (Johansson et al, 2003). Other factors of relevance for bread-making quality are the earliness of the plant, both in terms of earliness to flowering and early maturation, and, consequently, the length of the grain-filling period is important (Johansson et al, 2005). Earliness to flowering and length of grain-filling period are again a function both of the environment and the variety. Due to the absence of synthetic fertilizers and fungicides in low input systems, both nitrogen availability and grain filling period is more susceptible to especially climatical variations. Thus, in all types of variety testing for bread-making quality the choice of environment is of major importance, and replicated trials in different representative environments are highly recommended.

It is also necessary to have sufficiently diverse sites to cover all possible environments. The determination of varieties which almost never disappoint can be easily done by stability analyses from METs and the application of 'safety-first' rules for the respective traits under investigation (Eskridge, 1990). It is necessary not to focus on environments "below the average", but to have enough diverse sites, seasons etc. to permit a continous and equal distribution of "environmental indices"

For other quality markets such as brewing and distilling, trialling over a range of sites is also important, as is obtaining data from several seasons. Varieties may be susceptible to problems that are potentially serious, but which only occur in certain seasons due to weather conditions. An example is grain splitting (Rajasekeran et al, 2000), in which a gap forms between the palea and the lemma, the tissues that comprise the husk. This can lead to precocious germination, to infection by micro-organisms, or to uneven hydration during malting. For these reasons, grain splitting will lead to samples being rejected by maltsters.

Regarding nutritional/health aspects, MET are also necessary to identify clearly and precisely those genotypes with stable, higher levels of special traits. Plant secondary metabolites, in particular, can be greatly affected not only by the genotype but also by the environment and by G×E, since they are often produced in response to biotic or abiotic stress factors.

3.4. Quality tests and variety ranking

The breadmaking or malting quality of a wheat or barley sample is expressed as a phenotype. It cannot therefore be assumed that varietal classification, generally based on performance under conventional input levels, and a function of the genotype, will accurately predict performance from grain grown under low input conditions.
However, recent publications (Kleijer and Schwarzel, 2006 and Munzing and Lindhauer, 2004) on comparative studies between samples from organic and conventional variety tests find no differences in variety ranking for the traits investigated (e.g. loaf volume, protein content). In the same period similar studies have been carried out or are ongoing e.g. in Austria, France and the Netherlands. It is important to note that interpretation of the results is often difficult because of a limited number of either varieties or trials. Also, only varieties of high baking quality are often compared, which results in too narrow a range of variation to analyse correlations between systems.

4. Populations, varietal mixtures and single varieties

If there is an interest from the industry in using populations and varietal mixtures, similar variety test methods for quality may be used as for conventionally cultivated, individual varieties. Most large-scale industries processing cereals into value added products do not accept populations and/or varietal mixtures, though.

However, while these tests demonstrate variation between grain samples there appear to be certain assumptions made by industry regarding variation within samples. Maltsters frequently process large batches, drawn from several consignments of grain, but as they are from the same variety and of similar nitrogen level, homogeneity is assumed. However, the nitrogen level is effectively a mean value of the grains within the sample tested, around which there may be considerable variation (Palmer, 2000). In addition, there is now some evidence that environmental and GxE effects may be greater in some monocultures than in mixtures of broadly similar varieties (Swanston et al, 2005). For these reasons, the assumption that monocultures will be less heterogeneous than mixtures may not be valid and should be tested by assays that can utilise individual grains.

While maltsters remain sceptical about mixtures, there appear to be fewer objections to the use of wheat mixtures for grain distilling. Some initial data (Swanston and Newton, 2005) suggest that inclusion of a high yielding, but slightly lower quality variety within a mixture improves the grain yield without adversely affecting the spirit yield. At present, however, rapid testing for spirit yield is not readily available, so mixtures should be constructed from varieties acceptable to distillers and grown under conditions that produce samples with low grain nitrogen.

5. General discussion and recommendations

For cereals from low input systems aimed at products produced by large-scale industry, such as bread, pasta, beer, spirits etc, similar variety testing methods for quality, to those normally used for cereals from conventional systems, are recommended. In variety testing for bread-making quality, such methods are for example; total grain protein concentration together with country-specific measurements of gluten strength and baking quality. In variety testing for brewing and spirit quality, the major procedures are those outlined in EBC and IOB methods of analysis, although, for mixtures, testing individual grains for hydration during malting and modification (Swanston et al, 2006), would also be appropriate. Determination of β-glucan content would also be useful as a breeding tool, both to identify potential malting varieties with low levels and as an essential screen for barleys with high levels and, thus, enhanced nutritional quality. Variety testing for nutritional/health aspects could initially be based on some distinctive traits such as carotenoid, anthocyan, dietary fibre/ β-glucan content.

If special production lines for organic or low input agriculture exists standard quality testing procedures may need adaptation. Such may be the case when e.g. a major part of the wheat is used for whole wheat bread instead of white bread. Involvement of key end-users in setting up a quality testing system is important to guarantee that testing follows the needs of the industry.

For cereals included in the Common Catalogue of varieties of agricultural plant species, variety testing for some distinctive characters with potential health benefits, e.g. waxy endosperm, purple/blue grain due to high levels of anthocyans, high levels of β-glucan or carotenoids, should be possible within the national VCU trials. Despite a lower performance than standard varieties in, for example, yield, malting quality etc., such special traits could lead to a successful registration of a variety, if the value for end-use and, therefore, cultivation is of regional economic importance. It would be sensible to test such specialty cereals mainly in organic trials, since production and processing methods could also contribute to the image of a healthy food product. Testing for other bioactive compounds, that are less distinct between genotypes and/or species, but mainly influenced by the environmental factors or agronomy (e.g. soil, climate, cropping system) cannot be recommended for variety testing, particularly if long-term human intervention studies demonstrate no significant health benefit.

Replicated field-trials in various environments are necessary independent of type of quality trait assessed. A high quality variety for low input systems has to perform well in a range of environments. Enough diverse sites are needed in order to reach a continuous and equal distribution of environmental indices for the quality traits. Although, many of the quality traits might perform similarly under low input as under conventional systems it cannot be assumed that varietal classification will be the same under both types of regime.

As most large-scale industrial producers utilising raw materials from low input agriculture will also use material from conventional agriculture, industrial requirements are generally similar across both types of cropping system.

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The extended BBCH-scale for cereals
(Witzenberger et al, 1989; Lancashire et al, 1991)

Principal growth stage 0: Germination
00   Dry seed (caryopsis)
01   Beginning of seed imbibition
03   Seed imbibition complete
05   Radicle emerged from caryopsis
06   Radicle elongated, root hairs and/or side roots visible
07   Coleoptile emerged from caryopsis
09   Emergence: coleoptile penetrates soil surface (cracking stage)

Principal growth stage 1: Leaf development [1,2]
10   First leaf through coleoptile
11   First leaf unfolded
12   2 leaves unfolded
13   3 leaves unfolded
      Stages continuous till . . .
19   9 or more leaves unfolded

Principal growth stage 2: Tillering [3]
20   No tillers
21   Beginning of tillering: first tiller detectable
22   2 tillers detectable
23   3 tillers detectable
      Stages continuous till . . .
29   End of tillering. Maximum no. of tillers detectable

Principal growth stage 3: Stem elongation
30   Beginning of stem elongation: pseudostem and tillers erect, first internode begins to elongate, top of inflorescence at least 1 cm above tillering node
31   First node at least 1 cm above tillering node
32   Node 2 at least 2 cm above node 1
33   Node 3 at least 2 cm above node 2
      Stages continuous till . . .
37   Flag leaf just visible, still rolled
39   Flag leaf stage: flag leaf fully unrolled, ligule just visible

Principal growth stage 4: Booting
41   Early boot stage: flag leaf sheath extending
43   Mid boot stage: flag leaf sheath just visibly swollen
45   Late boot stage: flag leaf sheath swollen
47   Flag leaf sheath opening
49   First awns visible (in awned forms only)

Principal growth stage 5: Inflorescence emergence, heading
51 Beginning of heading: tip of inflorescence emerged from sheath, first spikelet just visible

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[1] A leaf is unfolded when its ligule is visible or the tip of the next leaf is visible
[2] Tillering or stem elongation may occur earlier than stage 13; in this case continue with stage 21
[3] If stem elongation begins before the end of tillering, continue with stage 30

52   20% of inflorescence emerged
53   30% of inflorescence emerged
54   40% of inflorescence emerged
55   Middle of heading: half of inflorescence emerged
56   60% of inflorescence emerged
57   70% of inflorescence emerged
58   80% of inflorescence emerged
59   End of heading: inflorescence fully emerged

Principal growth stage 6: Flowering, anthesis
61   Beginning of flowering: first anthers visible
65   Full flowering: 50% of anthers mature
69   End of flowering: all spikelets have completed flowering but some dehydrated anthers
      may remain

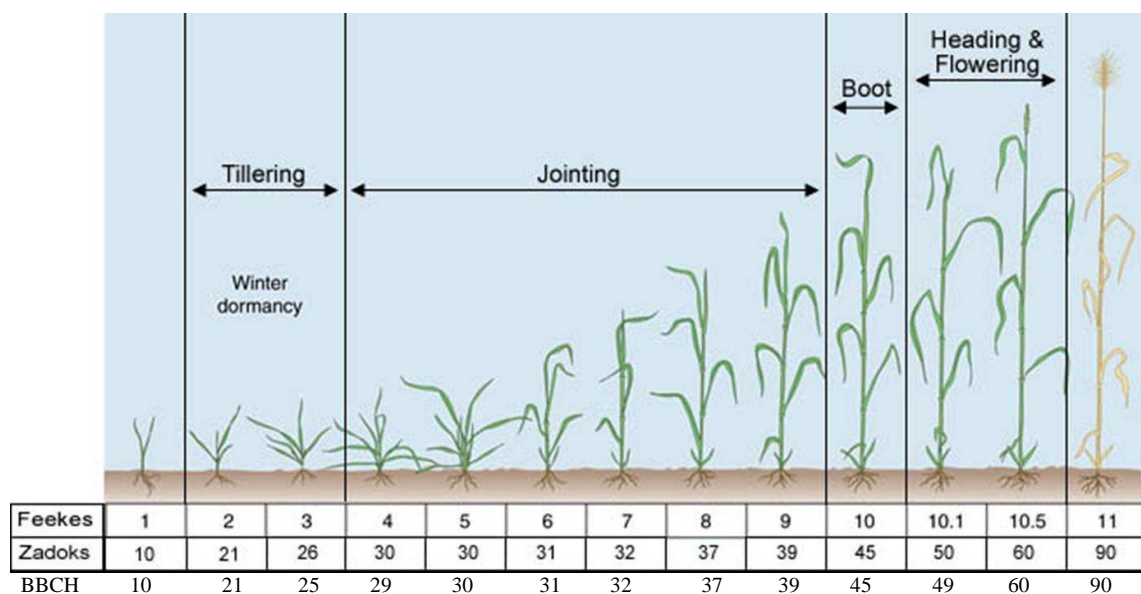**Principal growth stage 7: Development of fruit**
71   Watery ripe: first grains have reached half their final size
73   Early milk
75   Medium milk: grain content milky, grains reached final size, still green
77   Late milk

Principal growth stage 8: Ripening
83   Early dough
85   Soft dough: grain content soft but dry. Fingernail impression not held
87   Hard dough: grain content solid. Fingernail impression held
89   Fully ripe: grain hard, difficult to divide with thumbnail

Principal growth stage 9: Senescence
92   Over-ripe: grain very hard, cannot be dented by thumbnail
93   Grains loosening in day-time
97   Plant dead and collapsing
99   Harvested product

Feekes	1	2	3	4	5	6	7	8	9	10	10.1	10.5	11
Zadoks	10	21	26	30	30	31	32	37	39	45	50	60	90
BBCH	10	21	25	29	30	31	32	37	39	45	49	60	90

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