BioGreenhouse

Guidelines for Experimental Practice in Organic Greenhouse Horticulture

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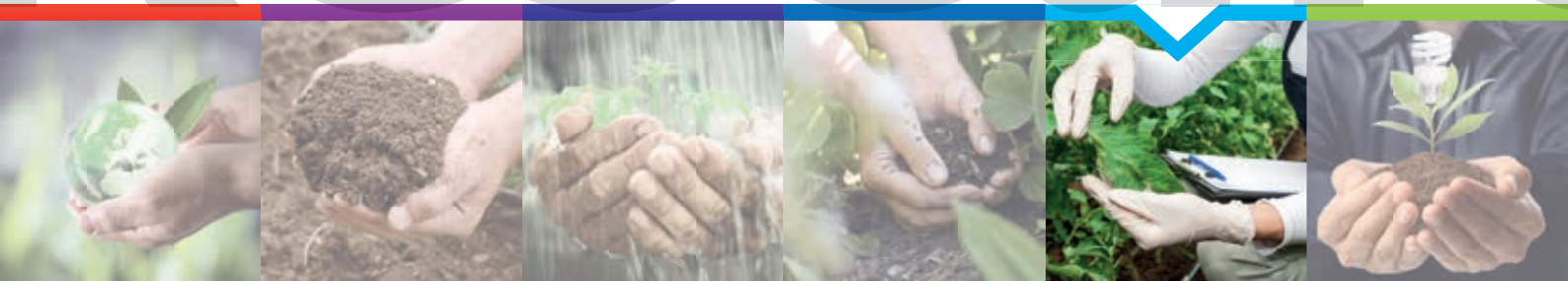

The Editorial Board *This picture was taken at the final meeting to discuss these guidelines, held in Tori, Estonia in September 2015. A commercial organic greenhouse with a tomato crop is shown in the background. Left to Right: Pedro Gomez, Stella Cubison, Wolfgang Palme, Justine Dewitte, Martin Koller, Yüksel Tüzel, Francis Rayns, Ingrid Bender and Ulrich Schmutz.*

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Pictures
All pictures are by members of the Biogreenhouse COST Action FA1105. Contributors to the pictures (in alphabetical order) are: Ingrid Bender, Stella Cubison, Justine Dewitte, Pedro Gomez, Martin Koller, Carolyn Mitchell, Jérôme Lambion, Wolfgang Palme, Virginia Pinillos, Ulrich Schmutz, Yüksel Tüzel and Anja Vieweger.

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The information in these guidelines is based on the expert opinions of the various authors. Neither they, nor their employers, can accept any responsibility for loss or damage occurring as a result of following the information contained in these guidelines.

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2 Trials in Organic Horticulture

By Martin Koller, Francis Rayns and Ulrich Schmutz

A pre-condition for trials concerning organic vegetable and fruit crops is the EU regulation 834/2007 on organic farming, or any other equivalent regulation in countries outside the EU. Inside the EU, in addition to the public (legally binding) EU standard, private standards of certification bodies defining organic or bio-dynamic production are also relevant. Research should always be conducted on well-established organic land (ideally converted for at least 5 years), and the history of conversion should be documented in the Materials and Methods section of any report (for research studying the process of conversion this is obviously not applicable).

If the research involves methods and products which are currently not part of organic standards (public or private) the protocol has to be discussed with the certification body first. In the case of on-farm trials the farmer also needs to be involved. Prior approval from the certification body and written documentation on the extent of the trials and the new methods tested should always be kept.

2.1 Separation from conventional cropping

In any organic research care has to be taken to avoid the influence of any adjacent conventional farming or horticulture - for example spray drift of herbicides or other pesticides. For outside cropping a distance of 10m or a high physical barrier (such as a mature hedge, stone field wall or solid wooden fence is accepted as sufficient). The issue is of less significance in protected horticulture but consideration needs to be given to the danger of contamination through windows open for ventilation etc.

2.2 Crop rotation and plant material

The guidance given in the organic standards on crop rotation, the use of green manures and other good practice in organic soil care should also be used for all organic research trials and this includes those in polytunnels and greenhouses. The full crop rotation history for at least 3 years, including any fallow periods should always described in the Materials and Methods of the trial reports. If trials use potted plants or nursery stock details of the origin and composition of the growing media are important. Seeds and transplants should be from an organic source. If none are available a derogation from the certification body is often required and conventional seeds without chemical seed treatments are usually acceptable.

The crop grown immediately before the trial should be as uniform as possible and any plants propagated for the trial should be equally treated under the same standardised nursery conditions as documented in the Materials and Methods. For organic fertiliser trials the land should be cropped for at least 2 years beforehand with high nutrient demanding crops (and the crop residues removed) in order to deplete the fertility.

The microclimate has an important effect on crop performance. This is not only the case in the field but is particularly important in polytunnels and greenhouses. Factors include the effects of walls, heating pipes, position of the plants (in the middle or end of the house), general setting of the house (in a north-south or east-west direction), temperature of the irrigation water and radiation from different surface materials.

2.3 Trial designs

There is normally a distinction between ‘demonstration’ and ‘scientific’ trials. The former are often unreplicated and are therefore smaller, easier (and cheaper) to run and may be linked to open day events aimed at farmers or a pilot study in advance for more detailed work. Results from such trials are not usually acceptable for scientific publication where replication allows statistical analysis to be performed to show that any differences between treatments are not just due to random variations in the experimental area.

Despite care full homogeneity can rarely be achieved and any known gradients should be incorporated into the trial design. This can be done by having randomized blocks with all treatments placed along the gradient or gradients of change (see section 2.3.5). A higher number of replications can also be used if the field or greenhouse is very heterogenic.

Border plants, strips or plots ('guard areas') can be used to separate the research trial area from other land but also to separate different treatments. The appropriate size of the border depends on the research question. Variety trials usually require no borders between treatments unless plants with different harvest times are tested. For fertiliser, plant protection and irrigation trials (especially if these are run for several years) a sufficient border between treatments is necessary to avoid one plot affecting its neighbours (perhaps as a result of mixing of soil during cultivations).

2.3.1 Plots and treatments

A trial plot is the smallest unit of land to which a particular experimental treatment is applied. It can contain many plants, or in case of trees, may only include a few. The plots should be clearly labelled and a plan, with reference to external landmarks, be kept in case the field labels are lost or displaced.

If borders or 'guard areas' are necessary the plot consists of a core-plot (surrounded by the border) and the total-plot, which includes the border. There may be additional uncropped pathways between the plots for access. The plot yield can be measured as the sum of the individual plants in the core-plot or as the total yield of the core-plot without separating individual plants. In some circumstances measuring the yield of the total-plot may also useful to assess the effect the border had on the research design.

The recommendation for the minimum core-plot size (and the number of plants it should contain) is given for each species in Chapter 4. This size is important and if the research effort/funding is limited the number of treatments should be reduced rather the minimum plot size or minimum number of replications

All plots should ideally be the same size. If this is not possible the differences need to be included in the yield calculations and this should be clearly documented in the Material and Methods section of the report. In greenhouses and poly-tunnels plots can be positioned along the main house direction or across it. Usually long narrow plots are easier to work with especially for tomatoes, peppers and cucumbers.

A 'treatment' or 'variant' is the term used to describe each thing that is varied within an experiment. Experimental treatments could, for example, be different varieties or different rates of fertiliser application. Normally there are only one set of treatments in a trial – this is known as a monofactorial experiment. Multifactorial experiments are also possible but are more complicated, larger and so more expensive. An example of a multifactorial experiment would be one to compare both the type and application rate of fertilisers.

2.3.2 Controls and other comparisons

Controls are usually un-treated variants of a trial – they are important as a reference point and necessary in nearly all trial designs. It must be clearly specified what untreated means in relation to the other treatments. A control can be a standard well-known variety or a standard fertiliser and the measured effects can be calculated and presented in relation to the control. This gives farmers and growers a familiar reference point to relate the result to.

If the test product is applied in water or together with a surfactant or other additive the experiment should include a control with only water and a second control with water and surfactant (without the test product). Any water should also have the same temperature and pre-treatment (e.g. stirring) to make the treatments as comparable as possible.

2.3.3 Replications and randomisation

For statistical validity each variant or treatment should be replicated. A trial with no replications is called 'demonstration trial'; it can have value as a demonstration to farmers and growers and proof-of-concept research; a statistical analysis is not possible. Crop trials have usually 3-6 replications and with more replications more statistically significant results can be measured. However, the cost of the trial also increases as it becomes larger and more time consuming to conduct. For a one factorial trial four replicates may be considered typical. Statistical analysis is still possible with three replicates but more allows more subtle effects to be detected and gives a degree of security in case one of the replicates is unusable (e.g. due to pest damage).

All variants are usually grouped in blocks. A complete block design has each variant once in the block, and the number of blocks is equivalent to the replications of the experiment. Within a block the treatments need to be randomised allocated – this can be done using an electronic random number generator or simply dice. The block location itself can also be randomized. If there is a known gradient across the site (e.g. of soil fertility) then the blocks should be laid out at right angles to this.

2.3.4 Fully randomised design

This is illustrated in Figure 2.1. It is quite possible that two randomly allocated treatments will appear next to one another; this is the case for 3 in the first column of this example. For field trials a completely randomised design is often not useful as known differences are not accounted for in either fields or glasshouses. Only in trials with pot plants in uniform growth chambers is this not an issue.

3 III	5 II	6 IV	5 IV
1 II	4 I	6 I	1 III
4 II	3 IV	2 II	6 II
3 II	4 IV	2 I	5 I
3 I	2 III	1 IV	5 III
4 III	2 IV	6 III	1 I

Figure 2.1 *Fully randomised design with 6 treatments (1-6) and 4 replications (I-IV).*

2.3.5 Block design

The block design is likely to be the best design for most research questions in a greenhouse or poly-tunnel. Each replication block should have the same conditions inside the block and with a two way analysis of variance the 'block-effect' can be removed from the analysis.

The treatments inside the block should ideally be randomized but often 'Block-1' is ordered simply from 1 to 6 as this gives the advantage of having an easily understood demonstration row of plots for visitors (see Figure 2.2). Sometimes a non-randomised block design is used. This is typical of plant variety and breeding trials where there may be a lot of varieties (perhaps as many as 20) in each replication. In such a layout it is easier to keep track of the treatments but statistical analysis of the results is not so robust. Because the same treatments are always adjacent there may be an influence of position (for example in a variety trial if one variety is much taller than the others). An example of this design is shown in Figure 2.3.

6 I	1 II	3 III	6 IV
5 I	3 II	2 III	1 IV
4 I	4 II	5 III	2 IV
3 I	6 II	4 III	5 IV
2 I	2 II	1 III	3 IV
1 I	5 II	6 III	4 IV
Block I	Block II	Block III	Block IV

Figure 2.2 *Block design with 6 treatments (1-6) and 4 replications (I-IV) randomised in Blocks II to IV.*

6 I	5 II	4 III	3 IV
5 I	4 II	3 III	2 IV
4 I	3 II	2 III	1 IV
3 I	2 II	1 III	6 IV
2 I	1 II	6 III	5 IV
1 I	6 II	5 III	4 IV
Block I	Block II	Block III	Block IV

Figure 2.3 *Block design with 6 treatments (1-6) and 4 replications (I-IV) in a fixed order.*

2.3.6 Latin Square design

The Latin square, or rectangle if plots are longer then wide, is a special form of block design. With this design the effect of both block and column can be measured. For a true Latin square the number of treatments should be the same as the number of replications. Then each treatment is found once in each block and each column. If the number of replication is limited to four a 'modified' Latin square is possible, however this also limits the power of the statistical analysis considering block and row effects. An example is shown in Figure 2.4.

Block IV	4 IV	3 IV	2 IV	5 IV
	3 III	2 III	1 IV	6 IV
Block III	6 III	1 III	5 III	4 III
Block II	5 II	4 II	3 II	1 II
	5 I	6 I	6 II	2 II
Block I	1 I	2 I	3 I	4 I
	Column A	Column B	Column C	Column D

Figure 2.4 *'Modified' Latin square with 6 treatments and 4 replications, showing four blocks (I-IV) and four columns (A-D).*

2.3.7 Split plot design

This allows for the testing of two factors in combination. These are known as the main effect and the split effect. An example might be the effect of different fertilisation effects on two different varieties of a glasshouse grown crop (see Figure 2.5). Because all the different treatment combinations are not fully randomised statistical tests require there to be bigger effects before they can be shown to be significant. However, split plot designs can be easier to manage than fully factorial layouts.

Split effect 1	Main effect 1	Main effect 2	Main effect 3	Block 1
Split effect 2	Main effect 1	Main effect 2	Main effect 3	
Split effect 1	Main effect 2	Main effect 1	Main effect 3	Block 2
Split effect 2	Main effect 2	Main effect 1	Main effect 3	
Split effect 1	Main effect 3	Main effect 2	Main effect 1	Block 3
Split effect 2	Main effect 3	Main effect 2	Main effect 1	
Split effect 1	Main effect 1	Main effect 3	Main effect2	Block 4
Split effect 2	Main effect 1	Main effect 3	Main effect2	

Figure 2.5 *Layout of a split plot design with 4 replications, three main effects and two split effects.*

2.3.8 Fully factorial design

In this design all the combinations of two factors are randomised together within each block, allowing straightforward Analysis of Variance to demonstrate the significance of any treatment effects. Statistically this is much more robust than the split plot design. An example layout is given in Figure 2.6.

1: fertiliser 1, variety 1	4: fertiliser 1, variety 2	2: fertiliser 2, variety 1	Block 1
6: fertiliser 3, variety 2	5: fertiliser 2, variety 2	3: fertiliser 3, variety 1	
2: fertiliser 2, variety 1	1: fertiliser 1, variety 1	6: fertiliser 3, variety 2	Block 2
3: fertiliser 3, variety 1	5: fertiliser 2, variety 2	4: fertiliser 1, variety 2	
6: fertiliser 3, variety 2	5: fertiliser 2, variety 2	2: fertiliser 2, variety 1	Block 3
3: fertiliser 3, variety 1	4: fertiliser 1, variety 2	1: fertiliser 1, variety 1	
1: fertiliser 1, variety 1	2: fertiliser 2, variety 1	5: fertiliser 2, variety 2	Block 4
6: fertiliser 3, variety 2	4: fertiliser 1, variety 2	3: fertiliser 3, variety 1	

Figure 2.6 *Layout of a fully factorial design with 4 replications and six treatments in total (two factors).*

2.3.9 Simplified designs for on-farm trials

In on-farm trials (both in the field and in polytunnels and greenhouses) it is often not possible to use the recommended 3 or 4 replications although wherever possible two fully randomised blocks should be used to provide at least some robustness to the findings. Simplified designs can include 'false replications' where e.g. plots are all in the same row to help with large machinery. This can be considered under the following conditions:

- The fields need to be well known and the plots should be placed along any established soil differences to exclude this effect as much as possible.
- A standard treatment is repeated 2, or better 3 times, randomized across the field to assess the trial error.
- The trial error is smaller if the treatments are close together and within an area of known soil homogeneity.
- Differences from prior soil treatments or crops could affect only one treatment; the experiment should therefore be set out and observed closely with the local knowledge of the farmer or grower.

This approach is particularly suited to preliminary screening work, for example to see if a new crop will grow at all in a particular area. If the results are promising a more detailed scientific trial with grater replication can be set up later. During the data analysis the following considerations are important:

- Analyse the repeated treatment separately. If the 'false replications' within one row are not statistically different it can be assumed that no major external influence factors exist and that the differences measured in different treatments are mainly caused by the different treatments.
- If significant differences are found between 'false replications' the interpretation of the whole on-farm experiment has to be careful and descriptive, and all results of the statistical analysis have to be fully disclosed.
- If treatment data are presented in graphs the lack of a statistical analysis should be indicated in the description of the graph. If 'false replications' are used only mean values and standard deviation should be shown.

Additional statistical analysis tools can be used if non-replicated on-farm trials are conducted over many sites and multiple years. This is also the case when treatments are increased in fixed steps, e.g. compost: 1t/ha, 2 t/ha, 3 t/ha, 4 t/ha = four different treatments of compost with increasing tonnage per hectare. There is increasing interest in research work done using a 'citizen science' approach (see section 3.7)

2.4 Recording results

In the Materials and Methods section of any report all general information concerning the research trial must be documented. This can include location, site details (rainfall, altitude, water quality, nitrogen deposition through air, etc.), the organic status of the land, prior use, type of greenhouse, type of glass or plastic, research design, length and exact dates of trial and other relevant information. Soil type and results from soil samples (macro- and micro-nutrients) before and after the trial are also important if relevant to the research question.

Details on the weather conditions before and during the trial should also be provided and these include min/max soil and air temperature, sunshine hours, irradiation, humidity, wind speed. Within the greenhouse additional data on relative humidity, additional lighting type/amount, CO_2 contents, heating type/source, and sun/heat screens usage are required.

For the crop cultivation as much information as possible should be recorded including, variety, rootstock, scion, source of plant, seed-treatments, seeding or planting time, type of grafting, plant density, planting date and further cultivation operations such as leaf pruning, weeding or pest control. Fertiliser additions should be recorded and the source, amount and method of application should be clearly documented. This also includes type of irrigation water used, any fertigation (supply of nutrients in the irrigation water), CO_2 enrichments or climate control to enrich CO_2.

2.4.1 Recording information

Plant records can be made by weighing, measuring heights or lengths, counting (e.g. number of stems of fruits per m² or per plant). They can also be done by rating against an agreed scale (e.g. pest per percentage of leaf area, or percentage leaf area with symptoms). Rating is often done on a scale of 1 - 9 (or 1 -5). Measurements and ratings for a specific record should always be done by the same person for the whole trial, as this reduces the overall measurement error.

In ratings 1 is usually used for the lowest intensity of a trait and 9 for the highest. The optimal rating and the scale used (e.g. if shortened to 1 - 3 - 5 - 7 - 9) must be documented. The Community Plant Variety Office (CPVO, www.cpvo.europa.eu) and the International Union for the Protection of New Varieties of Plants (UPOV, www.upov.int) describes technical protocols for all the important horticultural species that include reference to such scales for a variety of characteristics. Information from both organisations is very similar but that from UPOV is available in more languages. Further details are given in the chapters for individual crops. For statistical analysis quantitative measurements are better than ratings but both give valuable information. However ratings should not be used to record information which could easily be collected by measurement (especially in the case of crop yields. Taking pictures can also help to illustrate differences. In some trials simple assessments of physical parameters (e.g. fruit firmness, colour) or chemical parameters (e.g. sugar content measured by refractometers) are worthwhile.

2.4.2 Missing values

A missing value can be the loss of a plant, a plot or even a whole block of the experiment. All missing values need to be recorded and honestly reported in the research report. The reasons for missing values can be manifold and the most common ones are flooding, animals eating crops, application errors and loss of data. Crop losses due to the actual treatments are not missing values.

If there are too many missing values the statistical analysis of the data is affected and a very large amount of missing values can make the whole experiment useless for statistical analysis. Under certain circumstances border crops outside the core-plot can be used in the analysis as a substitute but this is not good practice and must be clearly documented.

2.4.3 Harvest records

During the growing phase and at harvest all relevant biotic and abiotic stresses and damage to the plants need to be recorded. The harvest should include total yield and marketable yield, with details of the EU or other private retail based trade classification used to make this distinction. The marketable yield depends on the market channel and usually four types of market channel are applicable to organic crops: direct sales, small-scale retail, supermarkets and processing. While for direct sales and processing no trade classification applies (total yield = marketable yield) for other channels there are clear specifications and different prices for Class 1 or Class 2 produce. It is important to record these differences as it is the marketable yield which interests farmers and growers most. Normally the whole trial should be harvested at the same time. There are exceptions however, for example if the harvest date itself is part of the research question.

2.4.4 Time measurement

If new processes or growing techniques are being researched measuring the time needed for application (including preparation time) needs to be recorded. This can be done by using stopwatches or smartphones and data can later be expressed in labour hours per m^2 or hours/ha. It is important that only one person, or one team of people, makes these measurements in all the treatments.

Example 1: Timing tomato harvest (in a trial with different plant densities)
Measurements:
- Harvest time (seconds)
- Total number of harvested fruit
- Shoots per treatment
- Fruits per shoot
- From this data the following can be calculated:
- Harvest time per fruit (seconds)
- Harvested fruit per hour
- Harvested shoots per hour
- Labour hours per hectare or m^2

Example 2: Timing for weed control treatment (in a trial to compare fleece versus uncovered soil)
Measurements:
- Hand weeding time (seconds)
- Number of plants per plot
- Number of weeds per plot
- From this data the following can be calculated:
- Hand weeded plants per hour
- Removed weeds per hour
- Labour hours per hectare or m^2

For further agronomic and economic calculations data from reference material such as farm management handbooks can be used to include machinery costs, fertiliser costs and labour costs for 'what-ifs', i.e. if done on a commercial scale and not in a plot scale. Further information on this can be found in the chapter on the economic evaluation of crop trials.

2.5 Data analysis and statistics

Data should be collected in clear record sheets (on paper or electronically) and all data must be correctly allocated to the particular treatment. Common problems are typing or reading errors, decimal points being accidently moved, double entries, missed records, rounding data or missing digits and a general lack of concentration. Human error in data recording and analysis cannot be eliminated but it can be minimised by good experimental practices.

Data cleaning and checking is best done within a spread-sheet software programme (e.g. Microsoft Excel). Some of the mistakes described above can be spotted in a data screening exercise where extreme values are cross-checked, and data can be sorted to find double entries or other unusual entries. When entering data in a spread-sheet it is useful to immediately calculate mean, median, standard deviation and standard error in predefined formulas, as this can give extra check on typing errors. This descriptive statistics can also give guidance on further statistical methods to be used during the analysis.

2.5.1 Plot values

Yield data from plot areas have to be converted to standard metric area units - usually 1 m^2, 1000 m^2 or 1 ha. It is important to distinguish between the core-plot area and the total-plot area. Borders or pathways specifically associated with an experiment have to be excluded when calculating commercial yields but access ways that are normally part of the cropping system (e.g. tractor wheelings) should be included. Usually yields per ha are appropriate for field crops and some poly-tunnels and yields per m^2 for greenhouses and some polytunnels.

2.5.2 Statistical analysis

The data from experimental trials are usually analysed with the ANalysis Of VAriance (ANOVA) methods. This is a collection of statistical methods to differentiate between treatment means and the variation among and between treatments. The method was developed by Ronald Fisher and first published in the 1920s. The F-test, named after him, is used to find out if the variance of a treatment is different to the variance of the remainder. Other tests exist if the means are not 'normally' distributed (e.g. the Kruskal-Wallis test). It may be necessary to mathematically 'transform' some data sets to make them appropriate before analysis. Following on the F-test, further tests like the Tukey-test can be used to find means that are significantly different from each other. Those differences are marked with different letters in graphs or tables. Further statistical tests, correlation and regression analysis are also possible if the data are suitable for such analysis.

If in doubt about the correct statistical analysis method to use it is worthwhile seeking the advice of a statistician. This should be done before the experiment has been set up to confirm that the design is appropriate to generate the data needed to test the hypothesis.

Further information

Grafen A. And Hails R. (2002).
Modern Statistics for the Life Sciences. Oxford University press. ISBN 978-0-19-925231-2
Zar, J. H. (2010).
Biostatistical Analysis, 5th ed., Pearson Prentice Hall, Upper Saddle River, NJ. ISBN: 978-0-13-100846-5

2.6 Writing up an experimental report

Reports will vary depending on the requirements of the institution and the funding body and the size of the project; a final report of a trial running for several years will clearly contain more information than an interim report. However, most reports will include the following sections that are also usually found in academic papers.

Introduction and background

The focus of the introduction section of the report is the research question and how it is embedded in the background of existing knowledge. What is the framework of the research and which underlining assumptions have been made? What are the overall aims of the research, how can it contribute to new knowledge and who will use this knowledge (e.g. private companies, academic institutions, farmers and growers, consumers)?

Materials and methods

The report should explain the choice of methods used to assess the research question and then describe the materials and methods used in the experimental trial with sufficient detail to enable it to be repeated:
Trial design (e.g. block or Latin square), number of replications (usually called n=4 for 4 replications), plants per plot, plot size, plant density, boarders and size of core-plot, general background on the site in terms of location, climate, soil, cropping history, organic status.
Short but comprehensive summary of all trial details, treatments and operations including dates.
Short, but comprehensive summary of crop data like plant variety, propagation, pre-treatments, pruning, fertiliser etc. which could affect the results of the trial.
Description of all assessment methodology. This is particularly important when there is chemical analysis of soil or plant materials as different techniques will give quite different results.

Results and discussion

The interpretation of results should be written in simple readable text with useful observations and descriptive data from scoring sheets and pictures. This qualitative description should be combined with the presentation of quantitative data from the statistical analysis. It is important to report the quantitative data as un-biased first and then offer an interpretation. The reason for this is that the reader may come to a different interpretation of the data than the author of the report. Authors sometimes have a selective view towards 'good results' they are hoping for. However, any result is a 'good result' if achieved with a pre-defined research method following a rigorous procedure. Graphs and tables are appropriate tools to present results clearly and compactly.

Appropriate and clearly labelled scales are important (e.g. logarithmic for growth functions) and graph types should be chosen to avoid optical illusions. In a discussion sections the personal view and interpretation of the authors (called 'expert knowledge') are valuable information to the reader; they should however be clearly indicated as such and separated from the pure description of the results and this includes personal value judgements in the description of results like "treatment x had an 'a much more important' effect". Methods and experiments that failed should also be described.

Conclusions
Key findings or the research should be presented. This will show how the results can be made use of by growers and identify the need for any further research work.

Acknowledgements
Acknowledgements should include all staff involved, from picture credits to field and greenhouse staff, to lab, statistical and proof reading support. If farmer groups or citizens were involved in setting the research question or collecting results this should also be acknowledged.

References
All academic or 'grey' literature cited in the text should be clearly listed.

Summary or abstract
A short (one or two page) summary of the work that briefly covers what was done and the main findings is useful for people who do not have time to read the full version, especially for commercial producers for whom the results may be relevant. This may be distributed as a leaflet or made available on a website. In such a case every effort should be made to avoid unnecessary jargon.