Handbook for trait assessment in agricultural plant teams

Version 1.0

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* Core traits for intercrop and grassland trials; # core traits for intercrop trials only; + core traits for grassland trials

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Photo: Field trial under Almodovar Castle, Córdoba, Spain (© DR).

I. INTRODUCTION

Background

This handbook was developed to help facilitate coordinated assessments across multiple field trials of plant teams, specifically, legume-cereal intercrops and grassland mixtures. In preparation of the H2020-funded project DIVERSify we identified the need for a handbook combining protocols for assessing plant traits as well as agronomic characteristics, filling a gap that other handbooks are not addressing. With the aim of promoting the generation of 'big data' from a larger set of agricultural field trials, emphasis has also been on the importance of standardised formats through the use of shared data templates and collection of meta-data.

Information included

The protocols in this handbook describe methods for assessing plant and crop stand characteristics (hereafter addressed collectively as 'traits'), yield and post-harvest measurements as well as effects on non-crop biodiversity. The traits have been selected to contribute to the analysis of different types of questions (i.e. 'data stories'), classified here as (1) Plant growth and development, (2) Agronomy, and/or (3) Biotic interactions.

Each protocol provides primary and alternative assessment methods, supplemented by information on classification, target species, scale, timing, repetition, and reporting format. We recommend that multi-partner projects define from the outset which traits are essential and should be assessed by all partners in order to reach project goals. We provide suggestions for a three-level classification of trait priorities. 'Core' traits are considered essential as general performance indicators and as input to any modelling activities, and those to receive priority in all field trials. 'Useful' traits can potentially contribute significantly to the overall value and scope of the data set. These traits could be measured by a number of partners, contingent on resources and expertise, contributing to specific cross-site analyses and data stories. Finally, 'Specific' traits could prove valuable for setting up perspectives for future research and may be measured in one or two sites, depending on local interest and expertise. Trait priorities are provided in each protocol, and core traits are furthermore marked with symbols in the protocol title (* for core traits in both intercrop and grassland trials, $^{\#}$ for core traits in intercrop trials only, and $^{+}$ for core traits in grassland trials only).

Plot and subplot preparation

General criteria for selecting subplots

Whenever possible, use a stratified random sampling technique to select subplots:

(A) Leave out the edges of each plot: Disregard the outermost (1-2) border-rows and do not take measurements and samples from the ends of the plot.
(B) Divide the remainder of each plot into well established (homogenous) and poorly established areas.
(C) Select subplots only within representative areas (if possible, by taking random coordinates).
(D) If a species has a clumped distribution in the plot, make efforts to select areas with a more regular distribution, and/or increase the size of the sampled area (e.g. 1 or 2 m^2 rather than 0.25 m^2).

Preparation of plot harvesting

Plot lengths may deviate from the original plans. Therefore, before harvesting plots, make sure to measure effective plot lengths and recalculate plot size, possibly after running the combine harvester perpendicular to plot lengths (trimming), in a similar way for all plots. Trimming of plot size should also be considered if some areas of the field trial are poorly established.

Trait assessment

General procedures

The methods for trait recording described in the listed protocols rely on mechanical-physical measuring equipment (leaf area index, canopy reflectance etc.), objective quantification (counting, length etc.), or visual scores (flowering rate, disease incidence etc.).

For visual scores, strategies should be taken to standardise or neutralise subjectivity. It is recommended to restrict the work to be conducted to a single person due to subjectivity of the estimates. If this is not possible, cross-calibration of the different observers should be conducted prior to the observations. Alternatively, when multiple people are scoring, allocate blocks/replicates to individuals to ensure person-to-person variability is included within block-level variation, allowing scoring differences among treatments to be standardised. It is important to consider that many visual scores will be relative and only allow ranking of treatments within single trials.

To ensure data consistency in multi-partner projects, and the quality of any subsequent analyses, we recommend that the choice of methods for each trait is coordinated among partners before the beginning of trials and that any methodological differences are recorded.

Planning of coinciding trait measurements

In some cases it is recommended that traits coinciding around specific growth stages are planned to be recorded in a single workflow, whether these are measured on a per-plant basis (of, say, 5-10 plants per plot) or on subplot level. For example, consider assessing the same individual plants for Leaf angle, Nitrogen content (non-destructive), Leaf area, Specific leaf area and Leaf dry matter content, using single leaves for multiple measurements. This allows for inter-trait correlations to be assessed at the plant level rather than the plot level, and so provides more statistical power.

Selective sampling strategy

Some methods are more labour intensive than others, and a selective sampling strategy may be a vital approach to monitor certain traits on a subset of treatments and/or replicates despite limited capacity. This strategy may be particularly useful for traits initially planned for only a limited number of sites, in which case subsequent analyses may profit disproportionately from adding just some data points from additional sites.

Depending on other factors, it may be chosen to sample selectively on a reduced number of replicate plots (with a statistically functional minimum of three), or in some cases just one plot from selected treatments (allowing only qualitative comparisons). Another effective approach is to sample only treatments of particular interest.

Data handling

Data collection and reporting

When doing coordinated assessments across sites, it is recommended to align the formats using a data collection template. This entails alignment of data formats and trait names, suggestions for which are provided under each of the following trait protocols. When reporting per-plot performance (e.g. biomass or grain yield), make sure to adjust the metric in relation to net plot size, taking into account removal of material due to any previous destructive measurements.

Data quality control

Verification of data is essential for ensuring the quality of subsequent outputs and findings. A number of methods are suggested for checking validity of collected data and identify errors in collection and processing:

A. Spot checks on raw data files or hard copies (such as paper) for outliers and errors
B. Spot checks on data entered into the electronic format for outliers and typographical errors
C. Proof reading of printouts of digitised data against raw data files/hard copies by checking off each data point on a printed version
D. Entering of data into electronic format by two different users and the two data sets are compared to identify and correct typographical errors
E. Outlier check conducted on data (e.g. box plot by treatment categories) and outlier values verified and flagged. If justified, remove from the data set.

Archiving of measurements and data

Please note your measurements on pencil and paper (waterproof, or inside weather writers) or (where applicable) electronically. Always keep a second hard copy: photocopy your original sheet(s) regularly. Store electronic data on at least one additional geographic location (e.g. work, home, and cloud-based storage).

Meta-data

The description of the field site is crucial for subsequent data analysis and modelling and can be collected and reported in a metadata sheet in the data collection template. Meta-data includes trial purpose, involved personnel, geolocation, field layout and management, as well as soil and weather characteristics.

Site descriptions should include daily weather characteristics, including standard weather station data such as precipitation, temperature (average, min and/or max temperature), global radiation, relative humidity, and evaporation. If site weather data cannot be collected locally, information on latitude and longitude can be used to access online information from nearby weather stations. Important soil characteristics include soil texture (i.e. the clay, silt, and sand fractions of the soil) of each horizon, contents of soil organic matter (SOM) and plant available nitrogen (alternatively, the humus content and carbon:nitrogen ratio). Management characteristics include standard information on tillage (e.g. ploughing or seed bed preparation), fertilization (date, amount, type), irrigation if any (date and/or rule/threshold, amount), and weed control (date, estimated efficiency/% of kill). Sowing date and harvest date must also be provided.

It is strongly encouraged to take photos of plots and fields on a regular basis for later reference and documentation. Additionally, aerial photographs (by drone, or alternatively, carefully mounting a nearby elevated position) are useful for presentation and dissemination.

II. PROTOCOLS

1 PHENOLOGY

1.1 Growth stage *

Importance Core.

General description The growth stage of plants is important for assessing their phenological development and the relative timing of other measurements.

Type Plant trait.

Plant group All (species-specific).

Scale Plot.

Method It is recommended to record growth stages when assessing any other trait. Note the actual growth stage of each species in each plot or treatment, not the guiding range provided in each protocol.

Use species-specific BBCH scales, e.g. for cereals, peas and beans (see Appendix A). The following table shows the principal growth stages according to the extended BBCH scale (Meier 2001):

0	1	2	3	4	5	6	7	8	9
Germination	Leaf development	Tillering	Stem elongation	Booting	Inflorescence emergence, heading	Flowering, anthesis	Development of fruit	Ripening	Senescence

Crop growth stages should be recorded at least at the level of these principal growth stages (0-9), and preferably to code level (see Appendix A). A monograph helping with determination of BBCH stages is available in a number of languages on the following website (as of November 1, 2020):

https://www.julius-kuehn.de/en/jki-publication-series/bbch-scale/

A smartphone app has been developed to help with determining the correct growth stages of major crops and weeds. It is freely available (in German) on the following website (as of November 1, 2020):

https://apps.agrar.bayer.de/

Timing At each time point when another trait is measured.

Reporting Integer. The average growth stage of each species in each plot or treatment is reported. Abbreviated as 'growth.stage'. Contributes data for assessment of Plant growth and development and Biotic interactions.

References Meier (2001).

Author CS

1.2 Date of flowering *

Importance	Core.
General description	Flowering is an important indicator for plant development, having different progress in cereals and legumes. Should be monitored specifically if other traits (and growth stages with them) are not recorded in the period.
Type	Plant trait.
Plant group	All (species-specific).
Scale	Plot.
Method	Visually inspect on plot level and note the following dates: - Date of 50 % flowering plants (cereals) - Date of first flowering (legumes) - Date of last flowering (all plant groups; in cereals, all spikelets have completed flowering but some dehydrated anthers may remain)
Timing	From [GS61] until [GS69].
Repetition	Monitor regularly in the period.
Reporting	Numeric. Report each of the above as (i) Julian date and (ii) number of days after sowing. Abbreviated as 'flowering.date.numeric'. Contributes data for assessment of Agronomy, Plant growth and development and Biotic interactions
Author	LPK

1.3 Date of pod formation

Importance	Useful.
General description	Pod formation is an important step in plant development, signifying a switch in energy allocation. Can be monitored specifically if other traits (and growth stages with them) are not recorded in the period.
Type	Plant trait.
Plant group	Legumes.
Scale	Plot.
Method	Visually inspect on plot level and note the following dates: - 10 % of pods fully developed - 50 % of pods fully developed
Timing	[GS71] until [GS75], according to definition.
Repetition	Monitor regularly in the period.
Reporting	Numeric. Report each of the above as (i) Julian date and (ii) number of days after sowing. Abbreviated as 'podformation.date.numeric'. Contributes data for assessment of Plant growth and development.
Author	LPK

2 MORPHOLOGY

2.1 Plant count *

Importance Core. Specific.

General description This standard trait enables evaluation of emergence rate, establishment, and survival of the crop in-field. This is commonly lower than the targeted number/density (based on germination rates assessed before sowing, e.g. on filter paper in a petri dish). Allows adaptation of other traits measured on plot or subplot level.

Type Crop performance.

Plant group All (species-specific).

Scale Subplots: defined [1] by area (min. 0.25 m^2) or [2] per row meter (min. 2 x 1 m).

Method Manual counting of each species in each plot. For area-based subplots, use a rectangular frame, preferably open on one side, or set out the area with a ruler (see Appendix H). For row-based subplots, place an object of known length (ruler, stick or similar) along a representative stretch of row. Possibly mark the area for later reference (e.g. with wooden sticks). To counteract spurious estimates in case of uneven establishment, consider increasing the area / number of sampled subplots.

Timing Most important is assessment of field germination shortly after emergence ([GS11]) of [1] each species (separate time points adapted to the germination time of each species) or [2] all species (one time point defined by the slowest emerging species). Later assessments can be used for estimating plant survival rate, e.g. at the beginning of senescence ([GS91]).

Repetition Consider repeating if germination is suspected to be incomplete.

Alternatives Automatised image-based solutions based on UAV sensors and handheld cameras are becoming increasingly available.

At the time of physiological maturity, backwards estimation of overall plant density per species is possible based on other information:

plants/m2 = plot.grain.yield / grain.yield.per.plant / plot.area,

where plot.grain.yield is the grain yield harvested by the combine harvester (see Grain yield), grain.yield.per.plant is the average grain yield per plant that may be calculated from any plant samples already collected for other measurements, and plot.area is the effective area of the harvested plot in m^2. A sufficiently large sample of plants is required for the estimate to be reliable (e.g. 30-40 plants for cereals and 10-15 plants for legumes).

Reporting Integer numbers are extrapolated to plants per m^2 and reported for each species in each plot. Report each time point separately. Can be used to estimate differences or proportions (e.g. survival rate) if measured repeatedly (see Timing). Abbreviated as 'plants.m2'. Contributes data for assessment of Plant growth and development.

Author LPK, ST

2.2 Ground cover *

Importance Core. Specific.

General description Enables evaluation of crop establishment, early vigour, and weed suppression potential. Ground cover at early tillering is strongly correlated with weed suppression throughout the season and can be seen as the result of a range of other plant traits (e.g. growth habit, tillering capacity, rapid early growth, plant height).

Type Crop performance.

Plant group All.

Scale Subplot. Whole plot.

Method Using overhead RGB photos taken with handheld consumer camera (subplot) and/or professional UAV/drone (whole plot or subplot), with subsequent image analysis. Skilled specialists are recommended for drone-based mapping of field trials. Only the handheld method is described here.

Use a camera of 'suitable' quality. Images should be at least 2000 pixels wide and of the high-quality JPG type. Select 2-4 representative subplots, capturing variation in establishment within the plot (number depending on the overall heterogeneity of the plot). Priority is to have similar light conditions in all images, at least within blocks. A light cloud cover provides optimal light conditions, but uniform lighting conditions are more important. Camera height should be kept approximately similar, e.g. aiming for breast height. No date prints should be included in images. With both methods, the camera must be situated right above the target area and point directly downwards (perpendicular to the ground).

Avoid non-target objects such as wheel tracks, shoes/boots and other irrelevant objects in the analysed frames, including the shadow of the photographer. Otherwise, make sure to cut away non-target areas (ex-plot and poorly established areas) from the image prior to image analysis. Consider removing 10-20 % on each side of the photo before further processing to avoid issues of undesirable plant angles, especially in more developed crop stands. A number of freeware solutions can then be used to estimate percentage green in images (e.g. batch code available for ImageJ and R).

For assessments of crop cover, preferably use field plots with chemical and/or mechanical weed control to avoid any contribution of weeds to image green.

Timing Window period is between shortly after plant establishment ([GS15]) and canopy closure (around [GS40] for cereals). The most important growth stage for the evaluation of early coverage (critical for weed suppression) is in the early phases of tillering /side shoot formation ([GS21-GS25]).

Repetition Repeated measurements during the window period allow assessment of canopy closure dynamics (Specific).

Alternatives Visual estimation of percentage ground area within a 1 m^2 or 0.5 m^2 quadrat (wired

	or corner-marked) that is covered by bare soil, crop and/or weed plants. Standing over the quadrat and looking down on the canopy, visually project the area of vegetation to the area of ground below. Conduct this process for each crop species in the plot as well as the combined weed vegetation. Score in 10% intervals (i.e. 0-10 %, 10-20 %, 20-30 % etc.). The bare ground cover percentage is the area of ground without any overlying canopy. Note that the total ground area covered can exceed 100 % due to overlapping canopies of each crop species and the weed layer. Repetition as described above. Visual estimation is independent of weed control and is in fact the preferred method for estimation of weed cover. Smartphone solutions are available, such as Canopeo (canopeoapp.com).
Reporting	Integer (0-100 %). Report means for each plant species per plot. Abbreviated as 'cover.perc'. Contributes data for assessment of Agronomy.
References	Rasmussen *et al*. (2007).
Author	LPK, AJK

2.3 Plant growth habit

Importance	Useful.
General description	Describes cereal plant growth habit based on stem/tiller spatial arrangement of the most representative type of growth habit in the plot.
Type	Crop performance.
Plant group	Cereals and grasses (species-specific).
Scale	Whole plot.
Method	Visual assessment of the whole plot using the 5-point plant growth habit guide (see Appendix B). It is often easier to decide what score it is *not*, e.g. “not 1 / 2 or 4 / 5, so score as a 3”.
Timing	Any growth stage in the period between early tillering ([GS21]) and senescence ([GS92]).
Repetition	Can be measured repeatedly to follow growth habit dynamics during the season.
Alternatives	Measurement of stem angle relative to a vertical line, using a protractor (*clinometer*).
Reporting	Numeric. Integer scores 1 to 5, but 0.5 steps can be used. Abbreviated as 'growth.habit'. Contributes data for assessment of Plant growth and development.
Author	ACN, AJK

2.4 Canopy height *

Importance	Core. Useful.
General description	Describes maximal placement of biomass above ground by each crop species as it stands. Tall varieties appear to be more competitive at moderate to good plant densities (early stem elongation being more important than tall straw at maturity).
Type	Crop performance.
Plant group	All (species-specific).
Scale	Subplot.
Method	Manual measurement with ruler. For measurements during stem elongation, hold a horizontal stick along the average top of the highest plants in the subplot (incl. ears and upper leaves, but excl. spikes), then use a ruler or meter stick to read the distance between the horizontal stick and ground level. Measure and report for each species separately based on 2-5 positions (subplots) in each plot.
Timing	Window period is between beginning of stem elongation ([GS31]) and beginning of senescence ([GS90]). Core is around the end of flowering/anthesis ([GS69]).
Repetition	Repeated measurements during the period allow more detailed assessment of crop development. E.g. Beginning of stem elongation ([GS30]) in cereals; Beginning of flowering/anthesis ([GS61]) in legumes.
Alternatives	Guided measurement based on profile images.
Reporting	Numeric. For each species in each plot, report average of measurements (to nearest centimetre). Abbreviated as 'canopy.height.cm'. Contributes data for assessment of Agronomy and Plant growth and development.
Author	LPK

2.5 Plant length

Importance	Useful.
General description	Describes stem extension of each species (without upper leaves or reproductive organs) as opposed to the physical height of the whole canopy.
Type	Plant trait.
Plant group	All (species-specific).
Scale	Single plants.
Method	Manual measurement with ruler. At least five plants should be measured for each species in a plot. Procedures should be adapted to species: [1] Cereals. Measure the longest shoot (main shoot) from ground level to upper sheath top (early) or ear base (when emerged); [2] Beans. Measure stem length from ground level to the petiole of uppermost visible leaves (app. the growth tip); [3] Peas. Measure stem length from ground level to petiole of uppermost visible leaves (app. the growth tip). Due to a less linear mode of growth, individuals must be disentangled from other plants (if necessary) and slightly stretched before measurement. Consider cutting individuals at ground level and removing them from the plot before stretching.
Timing	Window period is between beginning of stem elongation ([GS30]) and beginning of senescence ([GS90]).
Repetition	Repeated measurements during the period allow more detailed assessment of crop development.
Alternatives	Guided measurement based on profile images.
Reporting	Numeric. Report average height (per species) to nearest centimetre. Abbreviated as 'plant.length.cm'. Contributes data for assessment of Plant growth and development.
Author	LPK

2.6 Internode length

Importance	Specific.
General description	Distance between nodes is a direct measure of plant stretching in different canopy layers.
Type	Plant trait.
Plant group	Cereals and other grasses.
Scale	Single plants.
Method	Manual measurement with ruler. At least five plants should be measured per plot. On each plant, measure the height of each node on the principal stem, as described for the trait Plant height. Report differences between neighbouring node heights, physically tracing the plant from the stem base to the apex with your fingers.
Timing	Window period is between stem elongation ([GS35]) and beginning of senescence ([GS90]), the latter being a measure of cumulative stretching over the season.
Repetition	Repeated measurement (possibly on the same individuals) allows assessment of plant stretching dynamics.
Alternatives	Guided measurement based on profile images.
Reporting	Lengths are reported to nearest centimetre. Abbreviated as 'internode.length.cm'. Contributes data for assessment of Plant growth and development.
Author	LPK

2.7 Tillering *

Importance	Core. Useful.
General description	Tillering is a highly plastic trait in cereals and grasses in general, with more favourable environments (in terms of light and nutrients) generally promoting tiller initiation.
Type	Crop performance. Plant trait.
Plant group	Cereals and other grasses.
Scale	Subplot.
Method	Manual count. Early: Count the number of tiller stems on 5-10 selected plants per plot. Select only plants that are clearly delimited, as to avoid counting two or more plants as one. Late: Harvest 5-10 plants per plot after digging them, separating single plants while using belowground stem parts as guide, and count number of tillers per plant (same sampling used for vegetative biomass dry matter and N content). Possibly use the subplots marked at plant count. At physiologically maturity, distinguishing between fertile and non-fertile tillers will allow the estimation of number of fertile tillers (an important yield component).
Timing	Early (Useful): After the tillering phase ([GS29-GS30]). Late (Core): At physiological maturity ([after GS83]), possibly just before harvest (([GS89])).
Repetition	A comparison of early and late counts allows analysis of in-season tiller production.
Alternatives	Visual assessment of ‘bushiness’ from 1 (simple) to 5 (complex).
Reporting	Numeric. Report the average number of tillers per plant. Abbreviated as 'number.of.tillers' (possibly also 'number.of.fertile.tillers'). Contributes data for assessment of Agronomy and Plant growth and development.
Author	ST, MW, LPK

2.8 Branching

Importance	Useful.
General description	Branching potential in legumes differs between species and cultivar types. Can contribute to other traits such as total grain yield, weed suppression and interspecific competition with a cereal partner.
Type	Crop performance. Plant trait.
Plant group	Legumes.
Scale	Single plants.
Method	Manual count. On each of 5-10 selected plants per plot, physically follow the main stem of individual plants from ground to top and count the number of side shoots.
Timing	Window period is from beginning of stem elongation ([GS31]) till end of ripening ([GS89]). Core is [GS85].
Repetition	Repeated counting allows analysis of dynamics in plant development.
Reporting	Numeric. Report the average number of branches per plant. Abbreviated as 'number.of.branches'. Contributes data for assessment of Plant growth and development.
Author	EA, DR

2.9 Number of nodes

Importance	Specific.
General description	The number of nodes of legume plants is an important indicator of plant growth stage and yield potential. Combined with counts of pods (the mature reproductive organs), it quantifies the proportion of pod-producing nodes (an important yield component).
Type	Plant trait. Crop performance.
Plant group	Legumes.
Scale	Single plants.
Method	Select at least five representative individuals per plot and manually count the number of nodes per plant, possibly restricting counting to the main stem. The fastest way, particularly in tangled canopies such as pea, is to physically trace the plant from the stem base to the apex using hands and fingers.
Timing	From the beginning of stem elongation ([GS30]) till the end of vegetative growth. Highest importance as a yield component is the final number of nodes (assessed around the time of harvest).
Repetition	Repeated measurement until whole-plant maturity allows extended analysis of plant development.
Reporting	Numeric. Report average number per plot. Abbreviated as 'number.nodes'. Contributes data for assessment of Plant growth and development.
Author	LPK

2.10 Leaf angle

Importance	Specific.
General description	Describes relative leaf inclination relative to the sun, thereby correlating with potential photosynthesis, shading, leaf temperature and transpiration. Found to correlate with leaf length in cereals. Allows estimation of light absorption, when combined with correlated measurement of SPAD or leaf absorptance.
Type	Plant trait.
Plant group	All (species-specific).
Scale	Single plants.
Method	Visual assessment of inner leaf angle relative to the stem, using a visual guide (see Appendix F for an example with five intervals). Hold the vertical line parallel to the straw and note the angular interval of the target leaf in the part closest to the stem. Select at least five individuals per species per plot and assess the uppermost, fully developed leaf of each plant. It is suggested to measure on leaves that are (then to be) sampled for other properties such as area, length, and weight.
Timing	Window period is from early stem elongation ([GS31]) till fruit development ([GS75]). Central growth stage is around early heading ([GS51]). Assessment of Leaf angle, Leaf area, Leaf length and Leaf width, SLA and LDMC can be done in the same process, allowing the derivation of additional leaf-specific ratios.
Repetition	Can be repeated for different leaf orders.
Alternatives	Visual assessment without a guide.
Reporting	Numeric. Report the average angle per species per plot from 1 (erect) to 5 (plane). Abbreviated as 'leaf.angle.scale'. Contributes data for assessment of Plant growth and development.
Author	LPK

2.11 Leaf area

Importance	Useful.
General description	Leaf area is an important indicator of plant growth, vigour and response to competition for light. Essential for estimation of overall leaf photosynthesis, when in combination with non-destructive measurements of nitrogen and chlorophyll content. Leaf area is a key component of canopy architecture that is often correlated with other traits such as Leaf area index, Weed biomass, Disease incidence and severity, Herbivory.
Type	Plant trait.
Plant group	All (species-specific).
Scale	Single plants.
Method	Sample the youngest but fully expanded leaves, avoiding leaves with considerable symptoms of pathogen or herbivore attack. Remove the petiole in species that have this. Place inside a sealable plastic bag with enough water present to be in contact with the leaf, seal, and store in a dark cold room overnight to fully hydrate. Retract from the bag, blot dry, then measure area with either a dedicated leaf area meter or scan on a flatbed scanner (calibrating against a reference) and save the file for later analysis (JPEG files work well). Freeware options for leaf analysis can be found for computers (e.g. www.quantitative-plant.org/software) and smartphones (Müller-Linow et al. 2019).
Timing	Any time whilst leaves are still actively growing. Assessment of Leaf angle, Leaf area, Leaf length and Leaf width, SLA and LDMC can be done in the same process, allowing the derivation of additional leaf-specific ratios.
Repetition	Can be repeated for different leaf orders.
Alternatives	Visual estimation, using scores of 1 (smallest) to 5 (largest) per species per plot. With leaf sizes differing between species and cultivars, a standard size table cannot be devised. Scores will be relative and apply within trials only.
Reporting	Numeric. Report in mm^2. Abbreviated as 'leaf.area.mm2'. Contributes data for assessment of Plant growth and development.
References	Pérez-Harguindeguy *et al*. (2013), Müller-Linow *et al*. (2019).
Author	RJP, LPK, MW

2.12 Leaf length

Importance	Specific.
General description	An important descriptor of leaf morphology and a main component of leaf area.
Type	Plant trait.
Plant group	All (species-specific).
Scale	Single plants.
Method	Assess the leaves sampled for leaf area, using the same image and software. Measure and report the distance from the lamina tip to the point of intersection of the lamina and petiole.
Timing	Any time whilst leaves are still actively growing. Assessment of Leaf angle, Leaf area, Leaf length and Leaf width, SLA and LDMC can be done in the same process, allowing the derivation of additional leaf-specific ratios.
Repetition	Can be repeated for different leaf orders.
Alternatives	Non-destructive measurements in the field, using a millimetre-precision ruler or millimetre paper. Visual assessment on a scale from 1 (narrow) to 5 (wide) - applies within trial only.
Reporting	Numeric. Report the average length (in mm) per species per plot. Abbreviated as 'leaf.length.mm'. Contributes data for assessment of Plant growth and development.
References	Pérez-Harguindeguy *et al*. (2013).
Author	LPK

2.13 Leaf width

Importance	Specific.
General description	An important descriptor of leaf morphology and a main component of leaf area.
Type	Plant trait.
Plant group	All (species-specific).
Scale	Single plants.
Method	Assess the leaves sampled for leaf area, using the same image and software. Measure the widest part of each leaf.
Timing	Any time whilst leaves are still actively growing. Assessment of Leaf angle, Leaf area, Leaf length and Leaf width, SLA and LDMC can be done in the same process, allowing the derivation of additional leaf-specific ratios.
Repetition	Can be repeated for different leaf orders.
Alternatives	Non-destructive measurements in the field, using a millimetre-precision ruler or millimetre paper. Visual assessment on a scale from 1 (narrow) to 5 (wide) - applies within trial only.
Reporting	Numeric. Report the average width (in mm) per species per plot. Abbreviated as 'leaf.width.mm'. Contributes data for assessment of Plant growth and development.
References	Pérez-Harguindeguy *et al*. (2013).
Author	LPK

2.14 Leaf dry matter content (LDMC)

Importance	Useful.
General description	A measure of investment in supporting structure versus metabolic activity.
Type	Plant trait.
Plant group	All (species-specific).
Scale	Single plants.
Method	Sample the youngest but fully expanded leaves, avoiding leaves with considerable symptoms of pathogen or herbivore attack. Remove the petiole in species that have this. Place inside a sealable plastic bag with enough water present to be in contact with the leaf, seal, and place in a dark cold room overnight to fully hydrate. Remove, blot dry, then weigh on a suitable balance. Any measurements of leaf width, length, and area (as part of SLA) are them made. Dry at 70 °C for 24 hours, then re-weigh on the same balance. LDMC is derived by calculating the ratio of dry mass to fresh mass.
Timing	Any time whilst leaves are still actively growing. Assessment of Leaf angle, Leaf area, Leaf length and Leaf width, SLA and LDMC can be done in the same process, allowing the derivation of additional leaf-specific ratios.
Repetition	Can be repeated for different leaf orders.
Reporting	Percentage. Report in mg g^{-1}. Abbreviated as 'LDMC.mg'. Contributes data for assessment of Agronomy and Plant growth and development.
References	Pérez-Harguindeguy *et al*. (2013).
Author	RJP, LPK

2.15 Specific leaf area (SLA)

Importance	Useful.
General description	One of the most widely accepted key leaf characteristics, used to assess plant growth strategy.
Type	Plant trait.
Plant group	All (species-specific).
Scale	Single plants.
Method	Remove the youngest fully expanded leaf, avoiding leaves with obvious symptoms of pathogen or herbivore attack. Exclude the petiole in species where present. Place inside a sealable plastic bag with enough water present to be in contact with the leaf, seal and place in a dark cold room overnight to fully hydrate. Remove, blot dry, then measure area with either a dedicated leaf area meter or scan on a flatbed scanner and save the file for later analysis (JPEG files work well). Dry leaf at 70 °C for 24 hours, then weigh on a balance. SLA is derived by calculating the ratio of leaf area (mm^2) to leaf dry mass (mg). Can be calculated for any leaves sampled in a standardised manner.
Timing	Any time whilst leaves are still actively growing. Assessment of Leaf angle, Leaf area, Leaf length and Leaf width, SLA and LDMC can be done in the same process, allowing the derivation of additional leaf-specific ratios.
Repetition	Can be repeated for different leaf orders and any leaves sampled in a standardised manner.
Reporting	Numeric. Report in $mm^2\ mg^{-1}$. Abbreviated as 'SLA.mm2.mg'. Contributes data for assessment of Plant growth and development.
References	Pérez-Harguindeguy *et al*. (2013).
Author	RJP, LPK

2.16 Vegetative biomass *

Importance Core (intercropping trials only). Useful. Specific.

General description A commonly measured trait used to assess general plant growth and vigour.

Type Crop performance.

Plant group All (species-specific).

Scale Subplot.

Method Core: (a) Clearly demark a rectangular or quadratic subplot of min. 0.25 m^2 parallel to the drilling direction, using one of two methods: (i) a thin frame, preferably a sturdy material such as steel and preferably open on one side (to facilitate placement on ground without damaging or manipulating plants; pea plants can be almost impossible to mark with a closed frame), or (ii) marking the area with ruler and sticks/pins.

(b) Harvest all (crop and weed) plants within the subplot at c. 1.5 cm above ground, carefully separating entangled pea plants (where relevant). Use (i) appropriate scissors, or (ii) handheld battery-powered cutters (such as the Bosch Isio series). In cases where field peas are particularly intertwined, it can be useful to separate the sample area from the rest of the plot using an electric hedge cutter.

(c) Because legumes are often heterogeneously sown/established, count and report the number of legume plants in each harvested area to allow (i) correction for plant number and (ii) calculation of average per-plant biomass.

(d) Place all harvested material in bags suitable for direct drying, each clearly labelled with (date and) unique plot id.

(e) Make sure that samples are not exposed to excess sunlight or heat prior to fractionation and drying. If leaving bags for more than 2-3 hours before further processing, it is recommended to temporarily store samples in the shade or in a cooler bag.

(f) Plant material from multi-species plots should be fractionated per target species shortly after harvest, putting all weed plant parts aside in the process. Store all fractions in separate bags, each clearly labelled.

(g) Dry the harvested (and fractionated) plant material in a drying oven (60-90 ˚C) for 1-2 days.

(h) Weigh plant material on an industrial-grade scale, providing grams with at least two decimals. Consider weighing without bags if sample weight is low, otherwise make sure to subtract average bag weight.

	Useful: Upon sampling of a subplot (either 'Core' or 'Specific'), choose the 5 most representative (and intact!) plant individuals of each crop species (preferably alongside the fractionation, as described above); separate these into spikes (cereals)/pods (legumes), stems and leaves; dry and weigh each fraction (as described above); calculate and report the proportional biomass of each fraction, the reproductive:vegetative ratio, and the leaf:stem ratio; by multiplying each proportion with total subplot biomass, report also estimates of total leaf, stem and reproductive biomass, respectively.
Timing	Core and Useful: During inflorescence emergence / heading phase ([GS51-59]), preferably at 50 % flowering (cereals) or first flowering (legumes). Specific: Additional sampling in the window period between early stem elongation ([GS31]) and end of flowering ([GS69]).
Repetition	Additional sampling dates allow assessment of growth dynamics (facultative).
Alternatives	Visual assessment on a scale from 1 (low biomass) to 9 (high biomass). Indirect indices such as NDVI (see 'Leaf area index' and 'Canopy reflectance').
Reporting	Numeric. Extrapolate biomasses to g per m^2 and report for each species in each plot. Abbreviated as 'biomass.gm2', 'leaf.biomass.gm2', 'stem.biomass.gm2' etc. Contributes data for assessment of Plant growth and development.
Author	LPK, MW

2.17 Leaf area index *

Importance	Core. Specific.
General description	Leaf area index (LAI) is an important characteristic of plant canopies that allows assessment of overall leaf area and PAR interception (shading). Differs from Ground cover by considering all light (incl. diffuse light) at the sensor position(s). Very useful for modelling.
Type	Crop performance.
Plant group	All (combined).
Scale	Subplot.
Method	Use dedicated equipment, e.g. handheld devices such as SunScan SS1, Licor-2000 and AccuPAR LP-80. Carefully follow the instructions for the specific equipment and aim for placing sensor(s) at highly standardised positions within the canopy. Use of overhead sensors to measure background radiation is recommended. Alternatively, use of paired measurements of above-canopy followed by within-canopy PAR levels - LAI can then be estimated based on an extinction coefficient that must be found in the documentation or in the literature. For camera-based devices, measurements should be conducted only under diffuse lighting conditions (no direct sunlight should enter the sensors). Some devices estimate LAI from calibrated equations that include values for plant leaf angle (such as the SunScan SS1) – in these cases, consider assessing plant teams while using a leaf angle value averaged across the species.
Timing	Window period is between early stem elongation ([GS31]) and fruit development ([GS75]). Core is around late booting ([GS48]) or early heading ([GS51]).
Repetition	Repeated sampling allows assessment of canopy closure dynamics.
Alternatives	Visual assessment of total plot leaf area from 1 (low) to 9 (high) or extrapolation of scanned leaves from 5-10 representative plants per species per plot.
Reporting	Numeric, dimensionless (m^2/m^2). Abbreviated as 'LAI.m2m2'. Contributes data for assessment of Plant growth and development.
Author	ACN, ST, CS, LPK

2.18 Canopy reflectance

Importance Useful.

General description Allows calculation of normalised difference vegetation index (NDVI) or normalised difference red edge (NDRE). NDVI is a widely used index of plant biomass. NDRE is sensitive to chlorophyll content and therefore used as a proxy for leaf health - the red edge (RE) band used for calculating NDRE (the part of the spectrum centred around 715 nm) is not as strongly absorbed by the topmost layers of leaves as the red band (R) and therefore may give a better insight at later stage crops, being able to measure further down into the canopy. Hence, NDRE is less prone to saturation in the presence of dense vegetation.

Type Crop performance.

Plant group All (combined).

Scale Subplot (handheld). Plot (drone).

Method Requires dedicated equipment capable of measuring visible (RGB) light as well as near-infrared radiation (NIR). Can be handheld devices (such as RapidScan CS45) or UAV (drone) mounted sensors, the former scanning a delimited area of the plot and the latter enabling estimation on plot level. Calculated as:

NDVI = (NIR - R) / (NIR + R)

NDRE = (NIR - RE) / (NIR + RE)

Timing Early scans reflect ground cover and plant biomass. Late scans reflect morphological features.

Repetition At app. 2-week intervals since difficult to predict useful stages.

Reporting Numeric. Report the calculated NDVI and/or NDRE values. Abbreviated as 'canopy.reflectance.NDVI' or 'canopy.reflectance.NDRE'. Contributes data for assessment of Plant growth and development.

Author ACN, LPK

2.19 Lodging *

Importance Core.

General description Lodging can negatively affect crop growth and harvestable yield, depend on timing. Early lodging (around stem elongation) is rare and does not have significant impact on agronomic traits but may be important for canopy structure. At plant level, lodging could start from the basal internode, and the whole plant is lodged at varying angles from vertical position. If lodging is due to buckling of middle internodes the plant usually bends from these internodes, the basal part of the stem staying erect. More frequently, lodging happens during the later parts of the plant life cycle, mainly because of rain and/or wind. This can impede harvest, cause yield loss, and result in lower grain quality (see Appendix J).

Type Crop performance.

Plant group All (combined; species specific).

Scale Plot.

Method Visual scoring based on the overall main stem angle and proportion of plot affected:

Lodging scale	Lodging development
1	main stems strictly upright
2	main stems incline slightly
3	main stems at 60 degrees angle
4	main stems at 45 degrees angle
5	main stems at 30 degrees angle
6	1/2 of the main stems flat
7	2/3 of the main stems flat
8	4/5 of the main stems flat
9	all main stems flat

Timing During the whole crop cycle. Most importantly, after rain/wind events on well-developed crops from 50 % of flowering until the harvest.

Alternatives A weighted score that includes both the angle of the lodged crop from vertical (e.g. 45/90 for a lodged crop with a 45 degrees angle from the vertical position) and the percentage of surface subjected to lodging:

Lodging Score = (angle of lodged crop / 90) x % area lodged

Also see CIMMYT WheatDoctor (http://www.wheatdoctor.org/lodging).

Reporting Integer. Score from 1 to 9. Abbreviated as 'lodging.scale'. Contributes data for assessment of Agronomy.

References Zhang *et al*. (2006).

Author ST, DR, LPK

2.20 Root biomass and length

Importance	Specific.
General description	Ingrowth core method.
Type	Plant trait.
Plant group	All (species-specific).
Scale	Single plants. Subplot.
Method	Mesh bags filled with root-free soil are buried into the root zone in each plot. At harvest, the mesh bags are pulled out of the soil, the roots inside are washed carefully, dried, weighed and possibly assessed by image analysis to establish root biomass, total root length and root length density.
Timing	After a period shorter than the lifespan of the roots.
Repetition	Periodic sampling of additional bags allows analysis of temporal development.
Reporting	Numeric. Report average weight (g cm^{-3}) and/or length (cm cm^{-3}) across blocks. Abbreviated as 'rootbiomass.gm2' and 'rootlength.cm.cm3', respectively. Contributes data for assessment of Plant growth and development.
Author	LPK, CS

3 PHYSIOLOGY

3.1 Nitrogen content, non-destructive

Importance	Specific.
General description	Nitrogen content of specific leaves can be indirectly assessed in-field with dedicated equipment based on light emission. Used a general indicator of plant health. Correlates well with relative chlorophyll content, transmittance, and reflectance of leaves (Bauerle *et al*. 2004). Allows estimation of light absorption when combined with correlated measurement of leaf angle.
Type	Plant trait. Crop performance.
Plant group	All (species-specific).
Scale	Single plants.
Method	Chlorophyll response varies with a wide range of factors such as leaf order, leaf age, position within leaf, water status, light conditions and time of day. Measurements must therefore be standardised within and among experimental sites and among plant individuals. On each plant (at least five plants per plot), select and measure the uppermost fully developed leaf, disregarding the flag leaf on cereals. Using a chlorophyll meter (e.g. SPAD-502 Plus), measure repeatedly (3-5 times) within the middle region of the leaf, making efforts to avoid veins (middle). Report the mean of all measurements within the plot. All measurements within the trial must be made within a restricted time span (max. two-three hours) under full sunlight within the four-hour period around noon (local time).
Timing	Window period is between beginning of stem elongation ([GS31]) and end of fruit development ([GS79]).
Repetition	Repeated sampling (at the stages indicated above) allows analysis of temporal dynamics in nitrogen content during the growth season (facultative).
Alternatives	Visual estimation of overall leaf greenness per species per plot (scores 1 (light green) – 5 (dark green)).
Reporting	Original measurement is a dimensionless score (SPAD). Can be converted to N content via calibration curves, established separately for each species using C/N elemental analysis. Abbreviated as 'nitrogen.content.SPAD'. Contributes data for assessment of Agronomy and Plant growth and development.
References	Bauerle *et al*. (2004).
Author	LPK, CS

3.2 Nitrogen content, destructive

Importance	Useful.
General description	Assessment of nitrogen (N) content allows the assessment of plant N use efficiency components.
Type	Crop performance.
Plant group	All (species-specific).
Scale	Subplot or Single plants (5 plants per species per plot).
Method	Chemical analysis of harvested biomass by specialised equipment (e.g. CHNS analyser). For use in determination of nitrogen content, it is advised to avoid contaminating the samples (e.g. using latex gloves). Sort freshly harvested material to species before drying. Fractionation into stems, leaves and spikes/pods (of 5 plants per species per plot) allows analysis of N distribution in different plant parts (facultative). Plant material for determination of N content should be dried at a maximum temperature of 70 ˚C. If aligned in plant development, it is possible to analyse the material harvested for assessment of 'Vegetative biomass'.
Timing	Cereals: At tillering ([GS22-]), flowering ([GS61]), physiological maturity ([>GS90]). Legumes: At flowering ([GS65]), physiological maturity ([>GS90]).
Repetition	Repeated sampling (at the stages indicated above) allows analysis of temporal nitrogen dynamics during the growth season (Useful).
Reporting	Percentage N of vegetative biomass (can later be re-calculated to total amount of N in vegetative plant biomass per plot). Abbreviated as 'nitrogen.content.percentage'. Contributes data for assessment of Agronomy and Plant growth and development.
References	Weih *et al*. (2011, 2018).
Author	MW, LPK

3.3 Hydrogen and oxygen isotope ratio, destructive

Importance	Useful.
General description	Assessment of stable isotopes of water (hydrogen and oxygen) allows investigation of water uptake patterns of each species in a mixture (percentage water uptake in specific soil depths).
Type	Crop performance.
Plant group	All (species-specific).
Scale	Subplot or Single plants (1-3 plants per species per plot, depending on plant size).
Method	Chemical analysis of root crown water by specialised equipment (such as an isotope mass spectrometer). Sample plant root crowns (i.e. the top part of the root system from which the stem arises) and soil cores from the same plot to a specified depth (preferably covering most of the root depth of the plants). Free the sampled root crowns from soil. Store in a plastic bag under cool conditions until extraction of crown and soil water in the lab. Extract root crown and soil water through a cryogenic water extraction line prior to analysis in the mass spectrometer. If aligned in plant development, it is possible to analyse the material harvested for assessment of 'Vegetative biomass'.
Timing	From emergence until physiological maturity.
Repetition	Repeated sampling allows analysis of temporal dynamics of water uptake patterns during the growth season (facultative).
Reporting	Hydrogen and oxygen isotope mass ratio of plants along a soil profile (multi-source mixing model). For each soil depth, percentage of water uptake is reported. Abbreviated as 'mixing.model.percentage.[*soil_depth*]'. Contributes data for assessment of Agronomy and Plant growth and development.
References	Freyberg *et al*. (2020).
Author	AS, CSb

3.4 Crop respiration and water status [+]

Importance	Specific.
General description	Used for assessing plant (stomatal) responses to heat load and water stress.
Type	Crop performance.
Plant group	All (combined).
Scale	Subplot (handheld). Plot (drone).
Method	Thermal imaging of plant canopies requires dedicated equipment capable of measuring long-wavelength infrared (LWIR) radiation. Can be handheld devices (such as Snapshot 525) or sensors mounted on an UAV (*drone*), the former scanning a delimited area of the plot, the latter enabling estimation on plot level. Leaf angle and position in the canopy has significant effect on temperature and stomata behaviour, and it is recommended to leave out border areas of the plot. 'Dry' and 'wet' reference surfaces should be included in the image for calibration purposes.
Timing	Window period is between first leaf emergence ([GS11]) and end of senescence ([GS89]).
Repetition	Repeated sampling (before, during or across dry periods) allows analysis of temporal dynamics (facultative).
Alternatives	Calculate leaf water content as part of estimating Leaf dry matter content (LDMC) as described above.
Reporting	Numeric. Contributes data for assessment of Plant growth and development.
References	Jones *et al*. (2009).
Author	LPK

3.5 Gas exchange parameters related to leaf photosynthesis

Importance	Specific.
General description	Infrared gas analysers (IRGA) are among the most accurate and versatile operating system for photosynthesis research. CO_2 assimilation rate, stomatal conductance, transpiration rate, temperature of air and leaf, flow rate, PAR, pressure, are some of the parameters measured with this technique. There are several types of portable equipment available (LICOR, ADC, Walz).
Type	Crop performance.
Plant group	All (species-specific).
Scale	Single plants.
Method	A non-detached young and fully expanded leaf is placed in the equipment chamber. Temperature, CO_2 concentration, saturating light, and chamber flow are adjustable and may be controlled. This control may be at a single point or at multiple, sequential control points. While recording CO_2 assimilation rate (A), internal leaf CO_2 concentration (Ci), stomatal conductance (gs), and transpiration rate values (E), it allows generating automatic experiments such as light response curves and A/Ci curves. It is recommended to measure 3-5 plants per species/genotype.
Timing	Assessed for specific plant development stages.
Repetition	Assessment of multiple growth stages facilitates analysis of plant developmental physiology.
Alternatives	Chlorophyll a fluorescence (see below) requires a less laborious and time-consuming methodology and may be used as a proxy measure of photosynthetic performance.
Reporting	Numeric. Instantaneous and intrinsic water use efficiencies are calculated using the ratios A/E and A/gs, respectively. Abbreviated as ‘A’, ‘E’, ‘gs’, ‘A.E’, and ‘A.gs’. Contributes data for assessment of Plant growth and development.
Author	CVP, STL

3.6 Chlorophyll fluorescence

Importance Specific.

General description Chlorophyll fluorescence describes the light re-emitted by chlorophyll molecules during return from excited to non-excited states. Chlorophyll fluorescence kinetic analysis has become an important tool in basic and applied research on plant physiology and agronomy. The method of OJIP fluorescence transients is based on the fast rise of *in vivo* chlorophyll fluorescence quantum yield after starting actinic (i.e. photosynthesis initiating) light. It is used as an indicator of photosynthetic energy conversion in higher plants. There are several portable chlorophyll fluorometers available (Opti-sciences, Hansatech, LICOR, Walz).

Type Crop performance.

Plant group All (species-specific).

Scale Single plants.

Method Young and fully expanded leaves are dark-adapted for 20 minutes using plant clips and then exposed to a saturating light pulse. This enables the measurement of ratios of fluorescence (Fv/Fm, Fv/Fo) and other JIP-test parameters, such as the PI_{ABS} performance index, which evaluates the structural stability and efficiency of photosystem II (PSII). The Fv/Fm ratio represents the maximum potential quantum efficiency of Photosystem II. Values between 0.79 and 0.83 are considered optimal for most plant species, with lower values indicating plant stress. It is recommended to measure 3-5 plants per species/genotype.

Timing Can be assessed for any plant development stage between unfolding of first true leaf ([GS11]) and end of senescence ([GS89]).

Repetition Assessment of multiple growth stages facilitates analysis of plant developmental physiology.

Alternatives Gas exchange methods.

Reporting Numeric. Abbreviated as ‘Fv.Fm’, ‘Fv.Fo’, ‘PI.ABS’. Contributes data for assessment of Plant growth and development.

Author CVP, STL

3.7 Chlorophylls and total carotenoids concentration

Importance	Specific.
General description	Quantitative determination of chlorophyll a, b, and carotenoids in a whole pigment extract of green plant tissue by UV-VIS spectrophotometer.
Type	Crop performance.
Plant group	All (species-specific).
Scale	Single plants.
Method	Standardised leaf disc samples are placed in tubes with 95 % ethanol. Leaf extract absorbance is measured at different wave lengths (470, 648.6 and 664.2 nm), using a spectrophotometer, and chlorophyll and carotenoid contents are calculated according to Wintermans and De Mots (1965), first constructing a calibration curve for each species under study. It is recommended to measure 3-5 plants per species/genotype.
Timing	Can be assessed for any plant development stage between unfolding of first true leaf ([GS11]) and end of senescence ([GS89]).
Repetition	Assessment of multiple growth stages facilitates analysis of plant developmental physiology.
Alternatives	Other solvents such as diethyl ether, methanol, or acetone are possible.
Reporting	Numeric. Values for chlorophyll a, b, and carotenoids are abbreviated as ‘Ca’, ‘Cb’ and ‘Ccx’, respectively. Contributes data for assessment of Plant growth and development.
References	Wintermans and De Mots (1965).
Author	CVP, STL

4 REPRODUCTION

4.1 Flowering rate

Importance	Specific.
General description	Flowering rate is an important indicator of growth stage, pollination/pollen potential and subsequent grain yield. This trait is particularly useful in combination with monitoring of Flower visitors (e.g. pollinators).
Type	Crop performance.
Plant group	All (species-specific); particularly relevant for legumes.
Scale	Single plants. Subplot. Plot.
Method	Plant-based: Select at least five representative individuals per plot and count the number of flowers per plant. Area-based: Within a representative subplot, count the total number of flowers. On each assessment day, note the growth stage and date of assessment.
Timing	All flowering stages ([GS61] till [GS69]).
Repetition	Regular monitoring within the period allows assessment of flowering dynamics.
Alternatives	Visually assess flower prevalence throughout the plot. Employ a scale from 0 (no flowering), over 1 (very few flowers) to 9 (all individuals flowering at full). All non-extreme values are thus a prone to a subjective combination of the proportion of flowering plants and the number of flowers per plant.
Reporting	Numeric/Integer. Depending on method applied, report as average number of flowers per plant (plant-based), flowers per area (inter- or extrapolate to m^2) or an integer score. Abbreviated as ‘number.flowers.per.plant', ‘number.flowers.m2’ or ‘flowering.rate.score’, respectively. Contributes data for assessment of Plant growth and development.
Author	LPK

4.2 Stability of pods

Importance	Specific.
General description	Entire pods can be lost during pod formation (e.g. in *Phaseolus* beans) due to abiotic stress, e.g. water stress, and can be a considerable source yield loss.
Type	Plant trait.
Plant group	Legumes.
Scale	Single plants.
Method	Select 4 representative plants in each plot. Tag 10 new pods (> 2.5 cm in *Phaseolus*) per plant and note the week of pod formation on the tag (see 'Date of pod formation'). If less than 10 pods were produced, note the number of tags placed on the plant. At each interval, check and collect tags of pods that were lost and note the date of collection. Before harvest, collect the tags of remaining pods that ripened. Calculate average pod stability per plant.
Timing	From flowering phase ([GS61]) until harvest ([GS90]).
Repetition	Repeatedly check for pod drop in 1-week intervals.
Reporting	Percentage. Abbreviated as 'pod.survival.percent'. Contributes data for assessment of Agronomy.
Author	EA

4.3 Mature pods/heads

Importance	Useful.
General description	An important yield component.
Type	Crop performance. Plant trait.
Plant group	All (species-specific).
Scale	Single plants.
Method	Manual count of pods (legumes) and heads (cereals). Select at least five representative individuals per species per plot. Consider using the plants that are used for late counting of tillering. Mature pods are identified as those in which seeds have begun touching each other.
Timing	At physiological maturity ([GS83] till [GS89]), possibly just before harvest.
Repetition	Not relevant.
Alternatives	Measured per area.
Reporting	Numeric. Report average per species per plot. Abbreviated as 'mature.podsheads.per.plant' or 'mature.podsheads.per.area'. Contributes data for assessment of Agronomy and Plant growth and development.
Author	LPK

4.4 Seeds per pod

Importance	Useful.
General description	An important yield component.
Type	Crop performance. Plant trait.
Plant group	Legumes.
Scale	Single plants.
Method	Manual count. Sample 5-10 representative plants per from each plot, possibly the same plants that are used for assessing Mature pods per plant. Thresh the pods manually, count number of seeds and calculate the average number of seeds per pod.
Timing	Before harvesting of plot (pea: [GS89-97], faba bean: [GS89-95]).
Repetition	Not relevant.
Alternatives	In the field, count the number of seeds without threshing or breaking plants or pods: Randomly count the number of visible seed curves on the pods of 5-10 representative plants and calculate the average.
Reporting	Numeric. Abbreviated as 'seeds.per.pod'. Contributes data for assessment of Plant growth and development.
Author	ST, LPK

4.5 Pod shattering

Importance	Specific.
General description	Seed loss from open (dehiscent) mature pods is a potential source of yield loss. Pod shattering due to species differences in maturation time is an important parameter in the evaluation of intercrops and other plant teams grown for grain.
Type	Plant trait.
Plant group	Legumes.
Scale	Single plants. Subplots.
Method	For each legume species in the plot, count the number of shattering pods (missing one or more seeds) and/or the number of missing seeds. At low levels of shattering, count in a representative area of known size within each plot and report per m^2. At higher levels of shattering, count on 10-20 representative individuals and report the average per plant.
Timing	Once before harvest ([GS90]).
Repetition	Not relevant.
Reporting	Number. Abbreviated as pods.shattering.m2[.*SpeciesName*]/ pods.shattering.per.plant[.*SpeciesName*] and seed.loss.m2[.*SpeciesName*]/ seed.loss.per.plant[.*SpeciesName*]. Contributes data for assessment of Agronomy.
Author	LPK

5 YIELD AND POST-HARVEST

5.1 Vegetative yield +

Importance	Core (in some grassland trials; similar to vegetative biomass). Useful (whole crop intercrops for forage and silage).
General description	Straw yield.
Type	Crop performance. Plant trait.
Plant group	All (combined; species-specific).
Scale	Plot. Subplot.
Method	Plot (combined): Make sure to set harvest machine to weigh or keep the non-grain (threshed) fraction of whole-plot harvest if this is an option. Subplot (species-specific): Subplots within each plot can be harvested manually (selecting a representative and well-established area of min. 0.25 m^2). This allows fractionating into species. Include an unidentified fraction if relevant. All samples are dried and weighed at app. 14 % moisture content.
Timing	Dry/senesced stage.
Repetition	Additional sampling dates allow assessment of growth dynamics (facultative).
Alternatives	Manual sampling of senesced single plants can be used to provide rough estimates, following extrapolation to t/ha based on plant counts.
Reporting	Numeric. Extrapolate to t/ha. Abbreviated as 'veg.yield.tha'. Contributes data for assessment of Agronomy.
Author	LPK, MW

5.2 Grain yield

Importance	Core.
General description	Describes the total output from grain crops.
Type	Crop performance.
Plant group	All (species-specific). Not relevant when harvested as whole crop (grassland mixtures and whole crops for forage and silage).
Scale	Whole plot to estimate total production. Subplot to estimate theoretical production.
Method	Whole plots are harvested using an experimental combine. Make sure to measure the final area of the plots prior to harvesting (e.g. subtracting any cut areas). Specific adjustment of combine speed and mechanical settings is required for harvest of intercrop plots and/or one-go harvest of field plot layouts with different sole-crop species. Depending on the species there is likely to be a trade-off, e.g. between obtaining sufficient threshing of cereal spikes and reducing the proportion of split peas. Combine settings should aim to optimise both. Preferably, plan for intercropped border plots to be used in this adjustment process. All samples are cleaned, dried, and weighed at app. 14 % moisture content (see Seed weight). Mixed grain lots are fractionated to species level (next protocol). Moisture content measured directly after/at harvest can provide information of the degree of ripeness of the cultures (especially interesting if different cultivars of a species should be compared).
Timing	Dry/senesced stage ([GS90]).
Alternatives	Subplots within each plot can be harvested (selecting a representative and well-established area of min. 0.25 m2) and threshed manually (see Appendix H). Manual sampling of mature single plants can provide similar information. Preferably, note the scale of harvesting before combining with data from whole plots.
Reporting	Numeric. Extrapolate to t/ha. Abbreviated as 'grain.yield.tha'. Contributes data for assessment of Agronomy.
Author	ST, CS, LPK

5.3 Seed sorting

Importance Core.

General description Sorting of the mixed grain from harvested intercrops is required to estimate the contribution by each component species. While mixed grain can be used directly for feeding, the ability to separate grain is particularly important for food grade grain.

Type Crop performance.

Plant group All (species-specific).

Scale Plot. Subplot.

Method Use screen-based machinery such as a seed sifter or drum machine. Size and shape of screen holes depend on the species. Clean separation of crop grains is harder the closer they are in size. In such cases, a possible strategy is to allow for a mixed middle fraction (aiming to make this as small as possible) and sort this manually or with additional specific settings. Shown below are example settings on a Westrup air screen cleaner (see Appendix I) for separating mixed pea and barley grain:

	Setting	**Purpose**
Screens		
Pre-screen	9 mm (round)	Leave out pod and spike material
Upper-screen	6 mm (round)	Fractionate pea and barley grains
Lower-screen	2.8 mm (round)	Remove dockage *
Mechanics		
Feeding rate	Scale 6 (out of 10)	Entry of material onto first sieve
Vibrator revolutions	330 rpm	Move material across sieves
Air/cyclone rate	Scale 10 (max)	Remove dockage etc.

* material other than target grain (chaff, straw, non-target seeds etc.)

Timing Post-harvest.

Alternatives Manual methods may be used in case no dedicated equipment is available. Select a representative subsample of the mixed grain and manually sort or sieve the seeds. Fractions are then weighed to obtain estimates of their proportion. These are multiplied by the total grain mass to obtain yield estimates of each component species. Please note: this method is more labour-intensive and less precise. Equipment for sorting seeds based on gravity or colour is also available.

Reporting Numeric. Extrapolate to t/ha. Abbreviated as 'grain.yield.tha'. Contributes data for assessment of Agronomy.

Author LPK

5.4 Seed weight

Importance	Core (intercropping trials only).
General description	Seed weight is an important yield component and is directly related to grain quality and seedling vigour. Can be used as an important indicator of plant resource availability and environmental stress during grain filling.
Type	Crop performance.
Plant group	All (species-specific).
Scale	Plot.
Method	Take out a random subsample from each plot harvest, dried to app. 14 % moisture content. Using a dedicated machine (such as the MARViN ProLine), count at least 3 times 100 seeds per species per plot and extrapolate to one thousand seeds.
Timing	Post-harvest.
Repetition	Not relevant.
Alternatives	Manually count a smaller number of seeds (e.g. 3 times 10 seeds per species per plot) and extrapolate to one thousand seeds. Preferably, note the number of seeds counted before combining with machine-counted data.
Reporting	Numeric. Report in grams, extrapolated to thousand-kernel-weight. Abbreviated as 'seed.weight.TKW.g[.SpeciesName]'. Contributes data for assessment of Agronomy and Plant growth and development.
Author	EA, AS, CSb, AJK

5.5 Nitrogen content in seeds (dehulled)

Importance	Useful.
General description	Nitrogen (N) content in seeds is an important indicator of feed quality of legume grains. It is needed for the assessment of the N harvest index and complements the assessment of N use efficiency components in the target plants.
Type	Crop performance.
Plant group	All (legumes in particular). Species-specific.
Scale	Plot.
Method	Chemical analysis of a subsample of seeds (sown seed and harvested seed).
Timing	Sown seed and harvested seed.
Repetition	Not relevant.
Alternatives	Extrapolation from seed protein content if available.
Reporting	Percentage of seed biomass. Abbreviated as 'N.content.seeds.percent' and 'protein.content.percent'. Contributes data for assessment of Agronomy.
References	Weih *et al*. (2011, 2018).
Author	MW

5.6 Whole-crop fodder quality [+]

Importance	Core (grassland trials). Specific (some whole cropped intercropping trials).
General description	Whole crop silage is an essential method for fodder storage and estimating bale quality is essential for fodder planning.
Type	Crop performance.
Plant group	Intercrops. Grasslands.
Scale	Plot. Subplot.
Method	Whole crops are cut, left to wilt for 1-2 days, baled, wrapped in polythene, and stored (e.g. in the field margin). Sample cores for analysis are taken from a set of bales after at least 3 months of storage. Quality assays made by commercial service use 'wet chemistry'. Dry matter, ash, protein, fibre (ADF, ADL, NDF) and several measures of digestibility are among the summary data. In small-plot grassland analysis is also possible to dry the cut samples for 48 h at 65 ˚C and then send these dry samples for the commercial service (fermentation will not occur in these cases, but quality values are comparable between plots).
Timing	Any time before senescence of upper straw.
Repetition	Re-assess any outlier value bales if necessary.
Alternatives	Where calibrations have been validated, non-destructive methods (e.g. near-infrared / NIR based spectrometry) can be faster, less costly, yet valuable measurements.
Reporting	Various. Abbreviated as 'fodder.quality'. Contributes data for assessment of Agronomy.
Author	ACN, CVP

6 NON-CROP BIODIVERSITY

6.1 Weed biomass

Importance	Core (intercropping trials only). Specific.
General description	Weed biomass is an important quantitative measure, being inversely related to the weed suppression ability by the crop stand and potential yield loss due to competition.
Type	Crop performance. Non-crop biodiversity.
Plant group	All (combined).
Scale	Subplot.
Method	Sampled and measured along with the method described for 'Vegetative biomass'. Can be assessed as total biomass (Core), or further fractionated and assessed per weed species or species group (Specific; also see 'Presence and richness of weed species').
Timing	Sampled along with 'Vegetative biomass'.
Repetition	Possibly along with any sampling of 'Vegetative biomass'.
Alternatives	Visual estimation of weed pressure, as described for the trait 'Vegetation cover'.
Reporting	Numeric. Extrapolate to g m^{-2}. Abbreviated as 'weedbiomass.gm2', 'weed.monocot.mass.gm2', 'weed.dicot.mass.gm2' etc. Contributes data for assessment of Agronomy and Biotic interactions.
Author	LPK, MW

6.2 Abundance of weed species

Importance Specific.

General description Describes the abundance of each identified non-crop plant species.

Type Crop performance. Non-crop biodiversity.

Plant group All (combined).

Scale Subplot.

Method Perform a vegetation survey by estimating weed percentage ground cover visually, separately for each weed species. List all species present and record their cover values.

Place quadrat (1 m^2) at a representative area within each plot and record the percentage ground covered by each weed species or category (see below).

Record the size of quadrat used.

Example:

Subplot	**Species**	**Cover**
B1A05	*Poa trivialis*	1 %
B2A06	*Capsella bursa-pastoris*	5 %

Timing At specific crop growth stages (stem elongation, flowering).

Repetition Repetition allows comparison of the weed community in different parts of the growing seasons

Alternatives Percentage cover of monocot and dicot weeds.

Reporting Integer (0-100%). Abbreviated as 'weed.species.abundance(.[*weed species*])' or 'weed.species.percentage(.[*weed species*])'. Contributes data for assessing Biotic interactions.

Author CS, AJK, MW

6.3 Arthropod abundance

Importance Specific.

General description Trapping/collecting methods or on-plant observations are carried out to assess the abundance and diversity of arthropods in the plot, including herbivorous pests and beneficial natural enemies or pollinators.

Type Non-crop biodiversity.

Plant group All (species-specific or community-level).

Scale Plot / single plants.

Method There is a wide range of available methods (see below), usually depending on the target arthropod group and crop plant. Sweep-netting is the preferred method, followed by visual counts as an alternative method.

Briefly, the following methods have proven to perform well in many different cropping systems (see Appendix C for more details):

(a) Pitfall traps [1]
(b) Yellow or colourless pan traps [1]
(c) Yellow sticky traps [1]
(d) Sweep-netting [1]
(e) Dissection of plant parts (e.g. stem, seed pods) [2]
(f) Biocoenometer (suction sampling) – caution: may damage the plants [1]
(g) Visual counts of arthropods feeding on plants [2]
Note: when using the visual count method (g), avoid counting close after rain occurred, as the number of herbivores found might be reduced. It is best to wait some hours before starting to count.

[1] Traps or sampling carried out on per-plot basis
[2] Count on a subset of plants of each species sampled (e.g. 10-20 cereal plants, 5-10 legumes plants, depending on plant size and density per plot)

Timing At specific crop growth stages (e.g. anthesis)

Repetition At least one growth stage (around peak arthropod abundance)

Alternatives Visual counts in dry periods (see above)

Reporting Report as abundance per plot (methods marked as [1]) or abundance per plant/stem (methods marked as [2]) for each arthropod species at each sample locus. Abbreviated as 'abundance.per.plot[.SpeciesName]' or 'abundance.per.plant[.SpeciesName]', respectively. Contributes data for assessment of Biotic interactions.

Author CS, AJK

6.4 Flower visitors

Importance	Specific.
General description	Visitation and activity rates of insects (bees, flies, butterflies etc.) in dicot flowers are important indicators of pollination potential.
Type	Non-crop biodiversity.
Plant group	All plots (weed flower visits) or dicot plots only (crop flower visits).
Scale	Subplot.
Method	In each plot, a 1 m x 1 m area is demarcated clearly (see Appendix D) and observed for a defined period (e.g. six minutes). An observer sits or stands in a comparable position right next to each area and records all insects visiting flowers within it. Observations are assigned to insect groups, and if possible, also the visited crop or weed species (an example Monitoring sheet is found in Appendix D). Preferable synchronise with assessments of flowering rate (described above). If this is not possible, rough assessments of flower cover and/or flower number in the subplot must also be recorded. Record date, time, and weather. Observations should be restricted to calm, wind-less days with warm (>17 ˚C) and sunny (less than 50 % cloud cover) conditions.
Timing	During flowering of the legume species.
Repetition	Repeated observation allows analysis of seasonal development in visitation rate. Sampling before as well as after legume flowering allows for analysis of the effect of legume flowering on visitation rate (if possible, correlate with flower cover, flower number and/or Flowering rate).
Alternatives	Collection by sweep netting (Appendix C), fixation, and subsequent taxonomic classification of individual insects.
Reporting	Abundance/activity per insect group per plot. Abbreviated as 'flower.visitors.m2[.*Plant species*][.*Insect species*]'. Contributes data for assessment of Biotic interactions.
Author	CS, LPK

6.5 Herbivory

Importance	Useful.
General description	Herbivory has considerable negative impact on crops worldwide and results from the feeding of a wide range of herbivores. Herbivory in the form of grazing by arthropods results in full or partial loss of leaf area, which can affect photosynthesis and growth.
Type	Non-crop biodiversity.
Plant group	All (species-specific).
Scale	Plot / single plants.
Method	To obtain a percentage/proportion of herbivory, choose a representative subplot and place a quadrat of known size. Record the number of plants of each crop species in the quadrat. Select a representative subset of plants in the quadrat for damage scoring. Visual estimation of herbivory is preferentially done with reference to graph paper and/or templates of known dimensions (see Appendix E). If relevant, score the proportion of foliage of each crop species showing specific signs of damage: puncture holes, grazed tissue, incised tissue, leaf margin notching (see Damage guide in Appendix E for examples). This can be done for all leaves on each plant or for a selected subset of leaf orders.
Timing	Can be more informative in later growth stages.
Repetition	Can be done at two crop growth stages in order to estimate herbivory rates within a specific growth period.
Alternatives	Leaves (fresh or dried) can be scanned/photographed (see Appendix E) and processed with image processing software to obtain more precise estimates of herbivory. This may in some cases require manual reconstruction of expected original leaf size.
Reporting	Percentage of crop plant damage at each sample locus. Abbreviated as 'herbivory.percentage'. Contributes data for assessment of Biotic interactions.
Author	CS, AJK

6.6 Disease incidence and severity

Importance	Core (intercropping trials only).
General description	Disease or symptoms of pathogen infection quantified by visual assessment.
Type	Non-crop biodiversity.
Plant group	All (species-specific).
Scale	Plot.
Method	Visual assessment (coverage) of each crop species in each plot. Use keys wherever possible, as inexperienced assessors over-estimate low disease severity. Specific assessment keys for common cereal diseases are provided in Appendix G (as used by UK AHDB Recommended List assessors).
Timing	First assessment to be made at first incidence (monitor regularly).
Repetition	Repeat and report assessments every two weeks.
Alternatives	May correlate with NDVI / reflectance measurements.
Reporting	Numeric. Convert individual raw scores (1-9) to percentages before reporting and analysis (conversion equivalents may be non-linear), see Appendix G. Abbreviated as 'disease.percentage.per.plant'. Contributes data for assessment of Agronomy and Biotic interactions.
References	AHDB (2018).
Author	ACN

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IV. APPENDIX

A. Crop growth stages

Principal growth stages

The following table lists **principal growth stages** that can be used across plant taxa (Meier, 2001).

Stage	Description
0	Germination / sprouting / bud development
1	Leaf development (main shoot)
2	Formation of side shoots / tillering
3	Stem elongation or rosette growth / shoot development (main shoot)
4	Development of harvestable vegetative plant parts or vegetatively propagated organs / booting (main shoot)
5	Inflorescence emergence (main shoot) / heading
6	Flowering (main shoot)
7	Development of fruit
8	Ripening or maturity of fruit and seed
9	Senescence, beginning of dormancy

BBCH scale for cereals

Growth stage	Code	Description
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)
1: Leaf development [1], [2]	10	First leaf through coleoptile
	11	First leaf unfolded
	12	2 leaves unfolded
	13	3 leaves unfolded
	1 .	Stages continuous till . . .
	19	9 or more leaves unfolded
2: Tillering [3]	20	No tillers
	21	Beginning of tillering: first tiller detectable
	22	2 tillers detectable
	23	3 tillers detectable
	2 .	Stages continuous till . . .
	29	End of tillering. Maximum no. of tillers detectable
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
	3 .	Stages continuous till . . .
	37	Flag leaf just visible, still rolled
	39	Flag leaf stage: flag leaf fully unrolled, ligule just visible
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)

Growth stage	Code	Description
5: Inflorescence emergence, heading	51	Beginning of heading: tip of inflorescence emerged from sheath, first spikelet just visible
	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
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
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
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
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

[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 [GS13]; in this case continue with [GS21]

[3] If stem elongation begins before the end of tillering continue with [GS30]

BBCH scale for pea

Growth stage	Code	Description
0: Germination	00	Dry seed
	01	Beginning of seed imbibition
	03	Seed imbibition complete
	05	Radicle emerged from seed
	07	Shoot breaking through seed coat
	08	Shoot growing towards soil surface; hypocotyl arch visible
	09	Emergence: shoot breaks through soil surface (“cracking stage”)
1: Leaf development	10	Pair of scale leaves visible
	11	First true leaf (with stipules) unfolded or first tendril developed
	12	2 leaves (with stipules) unfolded or 2 tendrils developed
	13	3 leaves (with stipules) unfolded or 3 tendrils developed
	1 .	Stages continuous till . . .
	19	9 or more leaves (with stipules) unfolded or 9 or more tendrils developed
3: Stem elongation (Main shoot)	30	Beginning of stem elongation
	31	1 visibly extended internode[1]
	32	2 visibly extended internodes[1]
	33	3 visibly extended internodes[1]
	3 .	Stages continuous till . . .
	39	9 or more visibly extended internodes[1]
5: Inflorescence emergence	51	First flower buds visible outside leaves
	55	First separated flower buds visible outside leaves but still closed
	59	First petals visible, flowers still closed
6: Flowering	60	First flowers open (sporadically within the population)
	61	Beginning of flowering: 10% of flowers open
	62	20 % of flowers open
	63	30 % of flowers open
	64	40 % of flowers open
	65	Full flowering: 50 % of flowers open
	67	Flowering declining
	69	End of flowering

Growth stage	Code	Description
7: Development of fruit	71	10 % of pods have reached typical length; juice exudes if pressed
	72	20 % of pods have reached typical length; juice exudes if pressed
	73	30 % of pods have reached typical length; juice exudes if pressed. Tenderometer value: 80 TE
	74	40 % of pods have reached typical length; juice exudes if pressed. Tenderometer value: 95 TE
	75	50 % of pods have reached typical length; juice exudes if pressed. Tenderometer value: 105 TE
	76	60 % of pods have reached typical length; juice exudes if pressed. Tenderometer value: 115 TE
	77	70 % of pods have reached typical length. Tenderometer value: 130 TE
	79	Pods have reached typical size (green ripe); peas fully formed
8: Ripening of fruit and seed	81	10 % of pods ripe, seeds final colour, dry and hard
	82	20 % of pods ripe, seeds final colour, dry and hard
	83	30 % of pods ripe, seeds final colour, dry and hard
	84	40 % of pods ripe, seeds final colour, dry and hard
	85	50 % of pods ripe, seeds final colour, dry and hard
	86	60 % of pods ripe, seeds final colour, dry and hard
	87	70 % of pods ripe, seeds final colour, dry and hard
	88	80 % of pods ripe, seeds final colour, dry and hard
	89	Fully ripe: all pods dry and brown. Seeds dry and hard (dry ripe)
9: Senescence	97	Plants dead and dry
	99	Harvested product

[1] The first internode extends from the scale leaf node to the first true leaf node

BBCH scale for faba bean

Growth stage	Code	Description
0: Germination	00	Dry seed
	01	Beginning of seed imbibition
	03	Seed imbibition complete
	05	Radicle emerged from seed
	07	Shoot emerged from seed (plumule apparent)
	08	Shoot growing towards soil surface
	09	Emergence: shoot emerges through soil surface
1: Leaf development[1]	10	Pair of scale leaves visible (may be eaten or lost)
	11	First leaf unfolded
	12	2 leaves unfolded
	13	3 leaves unfolded
	1 .	Stages continuous till . . .
	19	9 or more leaves unfolded
2: Formation of side shoots	20	No side shoots
	21	Beginning of side shoot development: first side shoot detectable
	22	2 side shoots detectable
	23	3 side shoots detectable
	2 .	Stages continuous till . . .
	29	End of side shoot development: 9 or more side shoots detectable
3: Stem elongation	30	Beginning of stem elongation
	31	One visibly extended internode[2]
	32	2 visibly extended internodes
	33	3 visibly extended internodes
	3 .	Stages continuous till . . .
	39	9 or more visibly extended internodes
5: Inflorescence emergence	50	Flower buds present, still enclosed by leaves
	51	First flower buds visible outside leaves
	55	First individual flower buds visible outside leaves but still closed
	59	First petals visible, many individual flower buds, still closed
6: Flowering	60	First flowers open
	61	Flowers open on first raceme
	63	Flowers open 3 racemes per plant
	65	Full flowering: flowers open on 5 racemes per plant
	67	Flowering declining
	69	End of flowering

Growth stage	Code	Description
7: Development of fruit	70	First pods have reached final length (“flat pod”)
	71	10 % of pods have reached final length
	72	20 % of pods have reached final length
	73	30 % of pods have reached final length
	74	40 % of pods have reached final length
	75	50 % of pods have reached final length
	76	60 % of pods have reached final length
	77	70 % of pods have reached final length
	78	80 % of pods have reached final length
	79	Nearly all pods have reached final length
8: Ripening	80	Beginning of ripening: seed green, filling pod cavity
	81	10 % of pods ripe, seeds dry and hard
	82	20 % of pods ripe, seeds dry and hard
	83	30 % of pods ripe and dark, seeds dry and hard
	84	40 % of pods ripe and dark, seeds dry and hard
	85	50 % of pods ripe and dark, seeds dry and hard
	86	60 % of pods ripe and dark, seeds dry and hard
	87	70 % of pods ripe and dark, seeds dry and hard
	88	80 % of pods ripe and dark, seeds dry and hard
	89	Fully ripe: nearly all pods dark, seeds dry and hard
9: Senescence	93	Stems begin to darken
	95	50 % of stems brown or black
	97	Plant dead and dry
	99	Harvested product

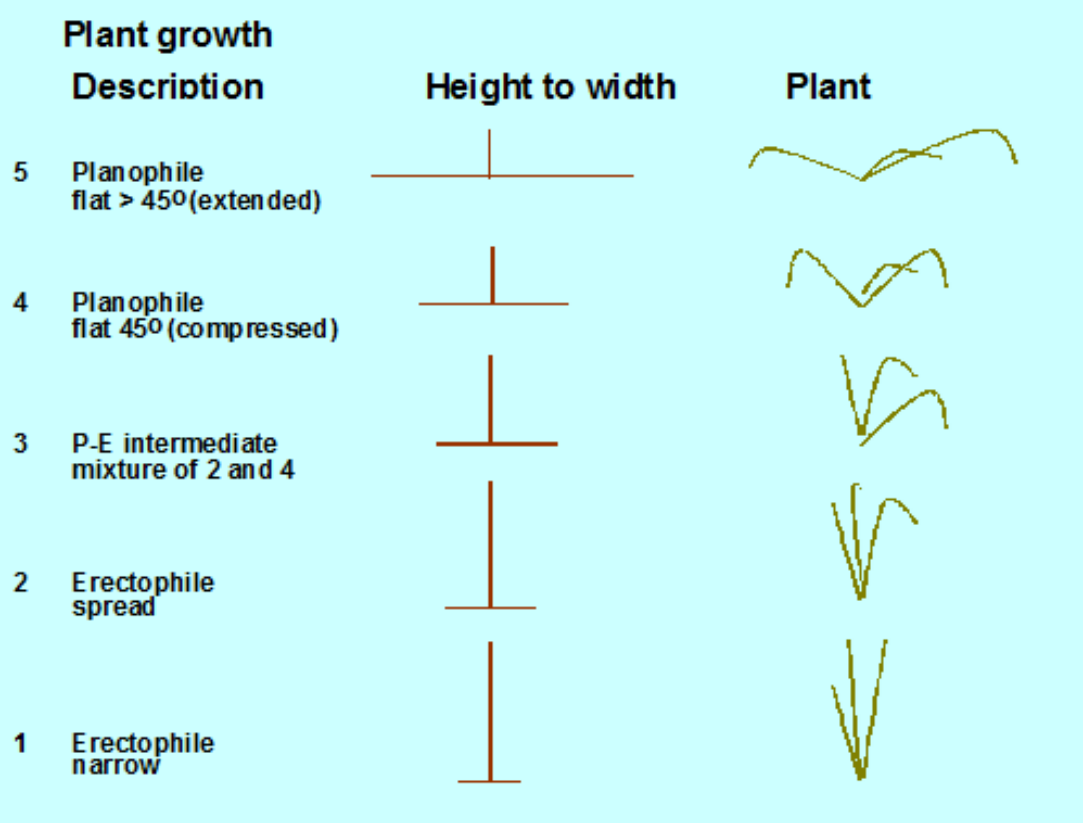
[1] Stem elongation may occur earlier than [GS19]; in this case continue with the principal stage [GS30-]

[2] First internode extends from the scale leaf node to the first true leaf node

Source Enz M. & Dachler C. (1997) Compendium of Growth Stage Identification Keys for Mono- and Dicotyledonous Plants. Extended BBCH scale. 2nd Edition. ISBN 3-9520749-3-4.

B. Cereal growth habit guide

A 5-point guide for measuring plant growth habit in cereals, as defined by the spatial arrangement of the plant stems/tillers. A single measure per plot is reported, based on the most representative type of growth habit.



C. Arthropod sampling

Pitfall trap (funnel trap). View from above. Note that pitfall traps measure activity density (mobile organisms are caught more frequently than immobile ones). [Image: CS]

Pan traps. Left: Yellow pan trap; Right: Colourless version. [Images: CS]

Sweep netting. Sampling is usually not area-specific. [Images: CS]

Biocoenometer (suction sampler). Allows volume-specific sampling but is rather labour-intensive. [Images: CS]

D. Monitoring of flower visitors

Plot demarcation

Demarcation of a 1 m x1 m area for insect observation,
using two a carpenter's rule assembled with adhesive tape
(Photo: Jana Brandmeier)

Monitoring sheet for flower visitors

Example combining information on arthropod species and plant (crop or weed) species:

Plot (e.g. A1):	Date:		Time:			Weather:					
(see plot numbering on Design sheet)											
	Flowers		Visitor groups								
											
Plant species	Cover (%)	Number	*Syrphidae* (Hoverfly)	*Brachycera* (Flies)	*Coleoptera* (Beetles)	*Lepidoptera*	*Heteroptera*	*Vespinae* (Wasps)	*Wild Bee*	*Honey Bee*	*Bumble Bee*
Vicia faba											
Triticum aestiuvm											
Pisum sativum											
Hordeum vulgaris											
Amaranthus hybridus											
Anagallis arvensis											
Atriplex patula											
Brassica napus											
Capsella bursa-pastoris											
Chenopodium album											
Chenopodium polyspermum											
Cirsium arvense											
Daucus carota											
Echinochloa crus-galli											
Equisetum arvense											
Euphorbia exigua											
Fallopia convolvulus											
Fumaria officinali											
Galinsoga ciliata											
Galinsoga parviflora											
Galium aparine											
Lamium purpureum											
Matricaria chamomilla											
Matricaria inodora											
Myosotis arvensis											
Persicaria maculosa											
Phacelia tanacetifolia											
Plantago major											
Poa annua											
Polygonum aviculare											
Polygonum lapathifolium											
Raphanus raphanistrum											
Senecio vulgaris											
Solanum nigrum											
Sonchus asper											
Sonchus oleraceus											
Stellaria media											
Trifolium repens											
Triticum aestivum											
Veronica persica											
Viola arvensis											

E. Herbivore damage

Types of damage

Puncturing

Peppering of holes on the leaf

Leaf miners cause lines in the leaves

Grazing

Feeding damage to one large area of the leaf

Incised

Feeding damage that has not started at the edge of the leaf

Notching

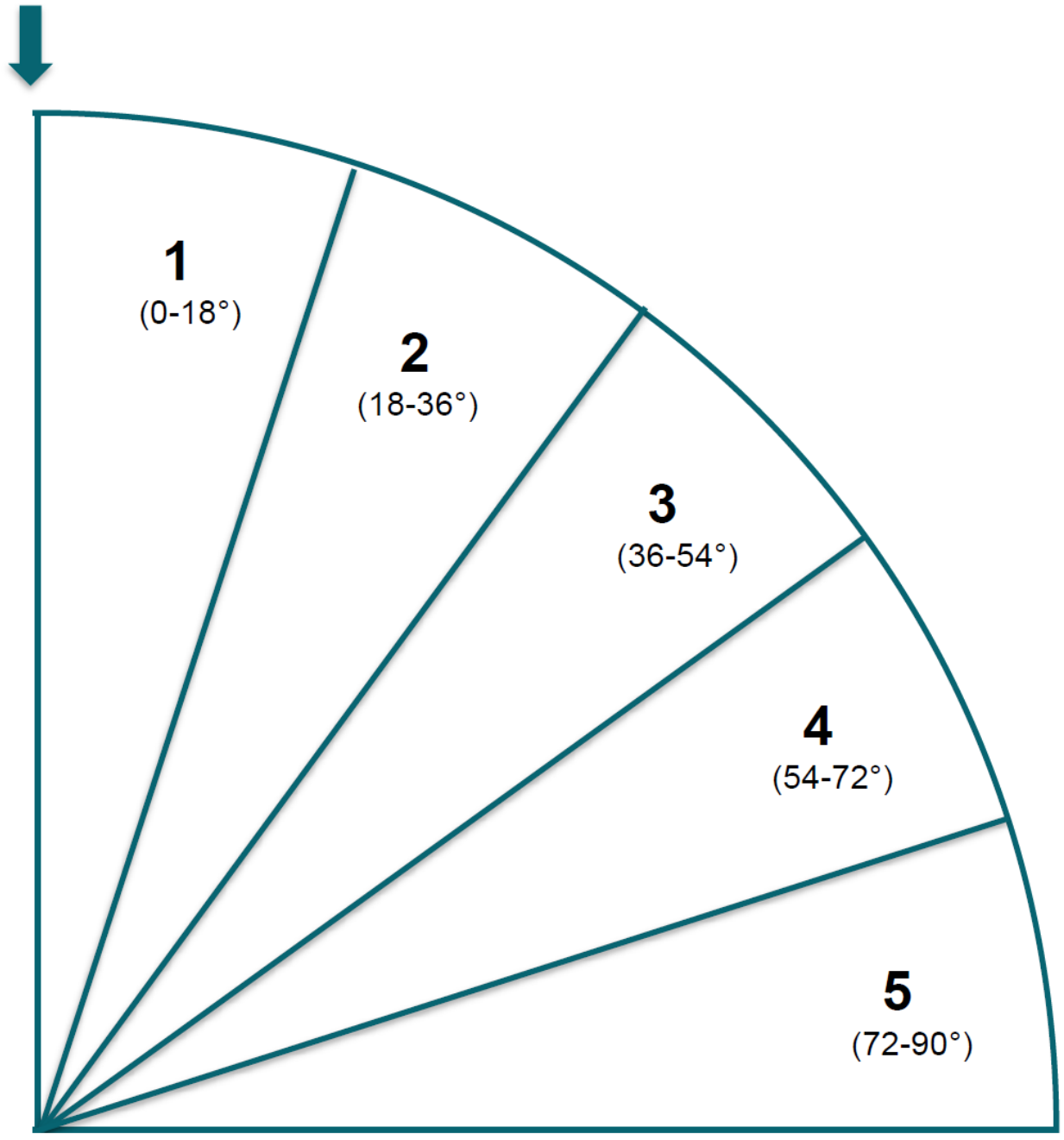
Feeding damage that has started at the edge of the leaf. Usually multiple sites on one leaf.

Estimation of leaf herbivory

Leaves on grid paper (left) and scanned on a flatbed scanner (right) (Photos: CS)

F. Leaf angle visual guide

Straw



G. Disease assessment keys

Foliar diseases

Method

1) Examine top 4 leaves. If top leaf has been fully expanded for less than 14 days, refer to 2nd leaf as 'top leaf'.
2) Ignore all naturally senescent leaf tissue.
3) Include all chlorosis and necrosis attributable to disease.
4) Record % infection; use interpolated values (e.g. 3%) if necessary. Disease may be recorded on a 1-9 scale and then transformed to percentage score. Both scales are given below.
5) If localised outbreaks (foci) are present, record average over the plot as a whole.

1-9 scale	% Infection	POWDERY MILDEW (*Blumeria graminis*)	YELLOW RUST (*Puccinia striiformis*)	BROWN RUST (*Puccinia recondita* & *Puccinia hordei*)
1	0		No infection observed	
2	0.1	3 pustules per tiller	1 stripe per tiller	25 pustules per tiller
3	1	5 pustules per leaf	2 stripes per leaf	100 pustules per leaf
4	5	2 lower leaves appear ¼ infected	Most tillers infected but some top leaves uninfected	Top leaf - numerous pustules but leaves appear green overall
5	10	2 lower leaves appear ½ infected	All leaves infected but leaves appear green overall	Top leaf - pustules sufficiently dense to give brown appearance in patches
6	25	Leaves appear ½ infected ½ green		
7	50	Leaves appear more infected than green		
8	75	Very little green leaf tissue left		
9	100	Leaves dead - no green tissue left		

1-9 scale	% Infection	SEPTORIA TRITICI (*Mycosphaerella graminicola*)	RHYNCHOSPORIUM (*Rhynchosporium commune*, formerly *secalis*)	NET BLOTCH (*Pyrenophora teres*)
1	0		No infection observed	
2	0.1	1 lesion per 10 tiller	1 lesion per 10 tillers	1 small lesion per 10 tillers
3	1	2 small lesions per tiller	1 lesion per tiller	1 small lesion per tiller
4	5	Small lesions beginning to form areas of dead tissue across width of leaf	Discrete lesions on most tillers, about 2 per leaf	2 lower leaves appear ¼ infected. Other leaves - few lesions
5	10	2 lower leaves – large areas of diseased tissue some covering 1/3 of leaf	Lesions coalescing but leaves appear green overall	2 lower leaves appear ½ infected. Other leaves - numerous lesions
6	25	Leaves appear ½ infected ½ green		
7	50	Leaves appear more infected than green		
8	75	Very little green leaf tissue left		
9	100	Leaves dead - no green tissue left		

Source AHDB Encyclopaedia of cereal diseases (2020) Appendix 5 (link).

Ear blight (wheat)

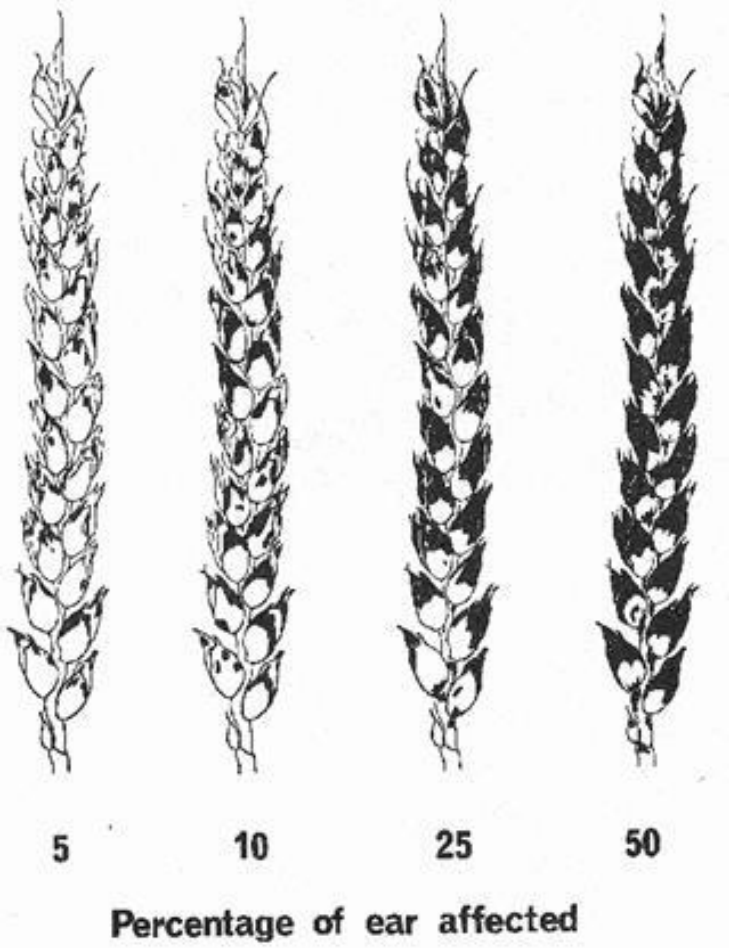
Causal agent	*Fusarium* spp.
Method	1) Carry out the assessment between [GS80] and [GS90]. 2) Select 20 ears at random from each plot. 3) Estimate the percentage area infected on individual ears using the illustrations above as a guide. 4) Record the mean value from the 20 assessments.
References	Parry D W, Bayles R A & Priestley R H (1984) Resistance of winter wheat varieties to Ear Blight (*Fusarium culmorum*). *Journal the National Institute of Agricultural Botany* 16, 465-468.
Source	AHDB Encyclopaedia of cereal diseases (2020) Appendix 6 (link).

Wheat glume blotch

Causal agent *Septoria nodorum* (*Phaeosphaeria nodorum*).

Method 1) Crops may be examined at any growth stage after [GS60] if glume blotch has appeared.
2) Assess 30 ears (selected at random) from each plot.
3) Assess the percentage ear affected on both sides of the ear and record a mean figure for that ear. Interpolate if necessary, e.g., if an ear falls between 10 % and 25 % give it a score in between, say, 15-20 %.

Source AHDB Encyclopaedia of cereal diseases (2020) Appendix 7 (link).



Eyespot of wheat

Causal agent *Oculimacula* yallundae (W type), *Oculimacula* acuformis (R type).

Method

1) Examine 20 tillers per plot.
2) Assign each tiller to one of the infection categories below.
3) Write the number of tillers in each category on the record sheet.
4) Report an index value calculated from the data as follows:

$$\text{Disease index} = \frac{(0 \times a) + (1 \times b) + (2 \times c) + (3 \times d)}{(a + b + c + d)} \times \frac{100}{3}$$

where a, b, c and d are the number of tillers examined which fall into the categories 0, 1, 2, and 3, respectively.

Infection category	Disease severity description
0	Uninfected
1	Slight eyespot (one or more small lesions occupying less than half the circumference of the stem)
2	Moderate eyespot (one or more lesions occupying at least half the circumference of the stem)
3	Severe eyespot (stem completely girdled with lesions; tissue softened so that lodging would readily occur)

References

Scott, P R and Hollins, T W (1975). Effects of eyespot on the yield of winter wheat. *Annals of Applied Biology* 78, 269-279.

Source AHDB Encyclopaedia of cereal diseases (2020) Appendix 8 (link).

H. Harvesting of subplots

Manual harvest of subplots is done by placing a frame with known inner area on the ground and cutting all target plants at a standardised height.

Subplot harvest of wheat to determine grain and straw yield. Here, using a carpenter's rule that is folded to the shape of an open frame and fixing the corners with adhesive tape [Photos: CS].

Harvested subplot of faba bean for mid-season biomass assessment, using an open metal frame, cutting at ground level and also removing all weed plants [Photo: LPK].

I. Equipment for post-harvest processing of small-plot samples

Threshing machine for small-scale threshing of manually harvested material. [Photo: CS]

Sample cleaner to remove debris from seed material. [Photo: CS]

Small-scale air screen cleaner used for fractionation of mixed seed lots [Photo: Westrup A/S]

Example fractionation of mixed pea and barley seeds [Photo: LPK]

J. Lodging

End-of-season lodging in a field pea trial plot [Image: LPK]