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Field Scanalyzer: An automated robotic field phenotyping platform for detailed crop monitoring

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Abstract. Current approaches to field phenotyping are laborious or permit the use of only a few sensors at a time. In an effort to overcome this, a fully automated robotic field phenotyping platform with a dedicated sensor array that may be accurately positioned in three dimensions and mounted on fixed rails has been established, to facilitate continual and high-throughput monitoring of crop performance. Employed sensors comprise of high-resolution visible, chlorophyll fluorescence and thermal infrared cameras, two hyperspectral imagers and dual 3D laser scanners. The sensor array facilitates specific growth measurements and identification of key growth stages with dense temporal and spectral resolution. Together, this platform produces a detailed description of canopy development across the crops entire lifecycle, with a high-degree of accuracy and reproducibility.

Additional keywords: data processing, computer vision, field scanalyzer, nitrogen, phenomics, scanalyzer.

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Introduction

The requirement for crop production is projected to double by 2050 to meet the demand represented by the rapidly growing human population and environmental changes (Tilman *et al.* 2011). Furthermore, the increase in crop production must be achieved sustainably, whereby reducing agricultural inputs, especially nitrogenous fertilisers, if we are to reduce environmental degradation caused by our agricultural footprint (Tester and Langridge 2010). In order to meet crop production demands, yields need to increase by 2.4% per annum. However, although breeding and agronomic efforts over the past 50 years have been responsible for tripling cereal yields (Pingali 2012), annual yield increase targets for all three major cereal crops (rice, maize and wheat) are no longer being achieved through traditional breeding programs (Tester and Langridge 2010). This highlights a need to exploit advances in genotyping and phenotyping methods to identify novel traits, and broaden the available genetic diversity of existing traits, in order to accelerate increases in genetic improvement and yield. There has been a rapid progression in functional genomics and genetic technologies over the past 20 years; however, the ability to exploit available genomic tools (sequencing technology, molecular markers) to their full potential are now limited by the ability to phenotype (Araus and Cairns 2014).

Several phenotyping platforms have been developed to increase the precision, resolution, and throughput of phenotyping, including controlled environment-based systems, aerial solutions and various ground-based platforms, each with their own advantages and limitations (Deery *et al.* 2014). Indeed, high-throughput phenotyping platforms (HTPPs) in controlled

environments have enabled detailed non-invasive observations of individual plants in potted soil. However, the resulting quantitative trait loci and candidate genes identified have generally not translated into gains in grain yield in the field, as potted plants experience far different environments to those in the field (Passioura and Angus 2010; Passioura 2012). The current generation of aerial HTPPs, such as unmanned aerial vehicles (UAVs) (Sankaran *et al.* 2015), and blimps (Losos *et al.* 2013) enable rapid data acquisition of numerous plots within a field, simultaneously. Although blimps have a payload of several kilograms, UAVs, such as polycopters, generally only carry up to 2 kg, limiting the number of sensors that can be used at any given time (Sankaran *et al.* 2015). Spatial resolution is also usually limited in such aerial vehicles.

Ground-based HTPPs for the field (often referred to as ‘phenomobiles’) hold an advantage over controlled environment-based platforms, as they operate directly in the field, can be used across multiple sites and have a potential for high spatial resolution. The attachment of cameras and sensors onto a tall-erecting vehicle, such as a cherry-picker (often referred to as ‘phenotowers’), increase the throughput of image acquisition, relative to phenomobiles, however achieve with a lower spatial resolution (Rascher *et al.* 2011). Several different GPS-navigated vehicles, manned or semi-autonomous (Deery *et al.* 2014), have been developed in recent years and are equipped to simultaneously collect detailed phenotypic data of row crops using spectral sensors, as well as thermal and 3D imagers, at plot-level. However, a key caveat with the use of such vehicles is that they still require some level of supervision, and the mass of the vehicles may detrimentally influence the underlying soil

structure, particularly over repeated-measurements, which is critical for the support of plant life (Bronick and Lal 2005). Here, we present the field-based HTPP installed at Rothamsted Research by LemnaTec GmbH, the 'Field Scanalyzer', which is being used for crop phenotyping.

The Field Scanalyzer

Rothamsted's Field Scanalyzer is a fully-automated, high-throughput, fixed-site phenotyping platform, carrying multiple sensors for non-invasive monitoring of plant growth, morphology, physiology and health. Automation enables the Field Scanalyzer to be exploited for detailed large scale screening of germplasm with high temporal resolution. Detailed monitoring of crops in the field will help to (i) quantify traits contributing to crop performance and may serve as yield predictors, (ii) identify novel traits, and (iii) dissect genetic control of complex traits. The information obtained are outputs that may be utilised directly by breeders to generate new elite germplasm.

The platform incorporates an overhead gantry that carries a camera box, which houses eight sensors and moves in three main axes; along rails (x -axis); perpendicular to rails with trolley (y -axis); lifter arm which moves the camera box (z -axis). All three axes have end position safety limiters in three hierarchies, including software and hardware end position, as well as a mechanical rubber end stop (Fig. 1). The Field Scanalyzer is robust enough to cope with harsh environmental conditions, with high sampling frequency and greenhouse accuracy. However, the system has been programmed to secure itself in wind-lock mode (safe position), in the middle of the field, when threshold wind speeds are reached (10 m s^{-1}).

The platform is $125 \text{ m} \times 15 \text{ m} \times 6 \text{ m}$ in size and crops within a specified area of $x_{\text{max}} = 115.8 \text{ m}$; $y_{\text{max}} = 11.3 \text{ m}$;

$z_{\text{max}} = 4.1 \text{ m}$ can be monitored throughout the season with a high degree of resolution (spatial and temporal) and reproducibility. The 6 m height of the platform was chosen to provide sufficient vertical clearance to operate any farm machinery if required. The concept is not restricted to these dimensions. LemnaTec control software is used on a master computer to run the gantry in automatic mode and transfer data to a dedicated database that is accessible remotely via the network. The platform operates through scripts that are written in C# programming language. The scripts select and initialise different cameras and illumination settings for the appropriate application. Once the platform is programmed, the system can operate 24 h day^{-1} , throughout the year, with minimal human supervision. The platform may also be remotely controlled in manual mode.

For any moving system, it is essential that parts work together seamlessly, with high level of accuracy. It is the combination of high-resolution sensors and accurate positioning of these sensors that ensures reproducible measurements, which can then be used to accurately monitor growth and development. In order to achieve precise gantry positioning, barcodes are installed on the rails of the x - and y -axes (Fig. 1), which determines the position and speed relative to a barcode tape. In order to evaluate the repositioning accuracy of the Field Scanalyzer, a test was carried out sending the gantry to the test position, returning to a random position on the field over 100 iterations. At each iteration, the on-board RGB camera imaged a checkerboard, resulting in a series of images that were used to calculate a displacement of the sensor position and presented a high accuracy of 25 pixels as the mean x -axis repositioning error, and 5 pixels for the y -axis. In this case, where the red, green, blue (RGB) camera is 2.5 m above the canopy, a resolution of 1 pixel is equivalent to $\sim 0.15 \text{ mm}$.

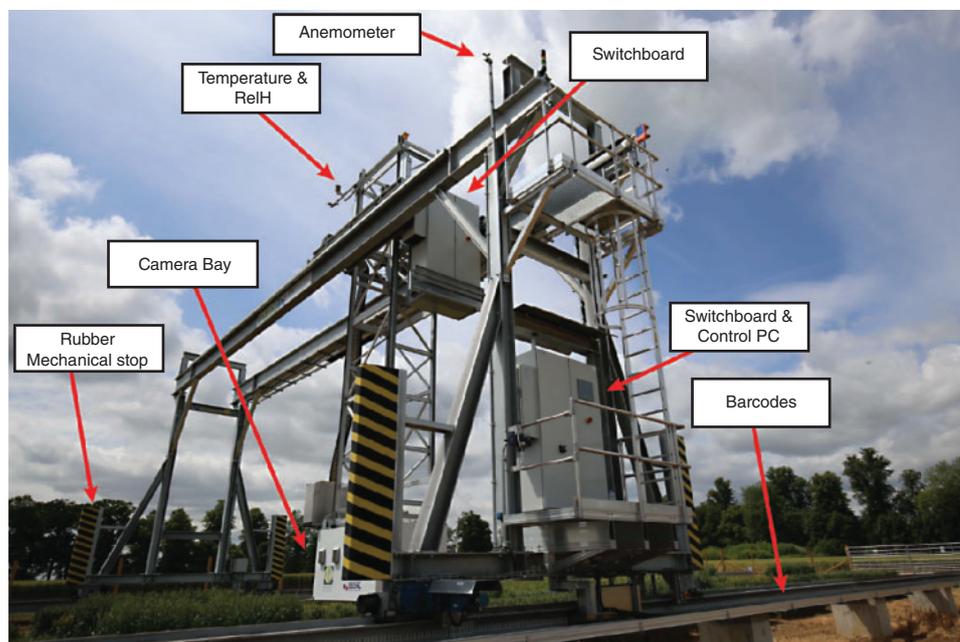


Fig. 1. Components of the Field Scanalyzer.

Sensor array

The camera box is equipped with state of the art cameras, sensors and illumination, as well as a temperature sensors to regulate temperature. All cameras are mounted in separate weatherproof housings, and are moved over the field by the supporting gantry (Fig. 2). The maximum total payload of the camera box is 500 kg, which enables the capacity to carry less portable camera technologies, such as the Field Scanalyzer's chlorophyll fluorescence imager (CFI; 120 kg) and two hyperspectral mirror scanners (30 kg each). The Field Scanalyzer can be programmed to capture images using one or multiple cameras and sensors during a scan, sequentially. However, no two sensors can simultaneously capture images. Fig. 3 illustrates examples taken from five sensors within the Field Scanalyzer's sensor array.

A high-resolution (3296×2472 pixels) 12 colour bit Prosilica GT3300 (Allied Vision), with a maximum frame rate of $14.7 \text{ frames s}^{-1}$ is employed as the visible camera. The lens of camera is directed vertically downward to the ground, and set up in auto-exposure mode to compensate outdoor light effects. Images of multiple plots can be quickly acquired ($\sim 180 \text{ images h}^{-1}$, inclusive of crane positioning time), to monitor canopy closure, by removing the soil background from an RGB image and quantifying the percentage of green pixels (vegetation) within an image (Fig. 4a). Given the autonomous operation of the Field Scanalyzer, images can be acquired with high-temporal resolution, to resolve canopy development in detail. Fig. 4b provides an example of how an RGB image series from the Field Scanalyzer can be used to monitor canopy development of two UK wheat varieties during the 2015–16 winter period. RGB images can also be utilised to automatically detect and quantify plant organs, such as the ears of wheat (Fig. 4c), providing a high-throughput approach to quantifying this important yield component, compared with current labour-intensive methods (P. Sadeghi-Tehrani; K Sabermanesh, N Virlet, MJ Hawkesford, unpubl. data).

A FLIR A645SC (FLIR Systems Inc.) is employed as a thermal infrared camera (TIR; 640×480 pixel matrix) and covers the spectral range of $7.5\text{--}13 \mu\text{m}$. The TIR images acquired have a radiometric resolution of 0.05°C and an absolute precision of 2°C . The TIR camera is directed vertically downward to the ground and can collect $330 \text{ images h}^{-1}$, inclusive of crane positioning time. Data is recorded in raw 16-bit format and the digital number intensities are later converted to radiometric temperature using the RBF equation provided by FLIR Systems.

Twin 3D laser scanners (Fraunhofer Institute) are mounted in the camera bay, opposing each other (Fig. 5a), and are capable of scanning plant canopies with high resolution (0.25 mm) in all three axes, using an NIR laser (840 nm) to ensure high reflectance by plant tissue and minimal physiological interaction. The laser scanners have a throughput of 30 plots h^{-1} and a field of view of $\sim 0.5 \text{ m}$ width and 0.5 m depth. Through the analysis of point cloud images, morphological traits of crop plots, such as plant height may be quantified (Fig. 5b). Fig. 5c represents the comparison of plant height measurements of seedlings of six wheat varieties commonly grown in the UK (*Triticum aestivum* vars. Avalon, Cadenza, Crusoe, Gatsby, Soissons and Maris Widgeon), obtained manually and from the on-board 3D lasers at 90, 196 and 226 days after sowing (DAS). Height measurements obtained from the 3D lasers are comparable to manual measurements ($R^2=0.99$; Root Mean Square Error (RMSE)= 1.88 cm), evidencing the reliability of quantification of a simple morphological trait. The manual height measurements appear, on average, to be slightly lower than those measured from the 3D lasers, especially for the highest values. However, this could likely due to human-error, given that manual measurements are acquired by taking the mean height of only 12 individual plants within an entire 1.5 m^2 plot.

The hyperspectral system comprises two mirror-scanning Hyperspec Inspector (Headwall Photonic) visible and near-infrared (VNIR) and extended VNIR (ExVNIR) cameras,

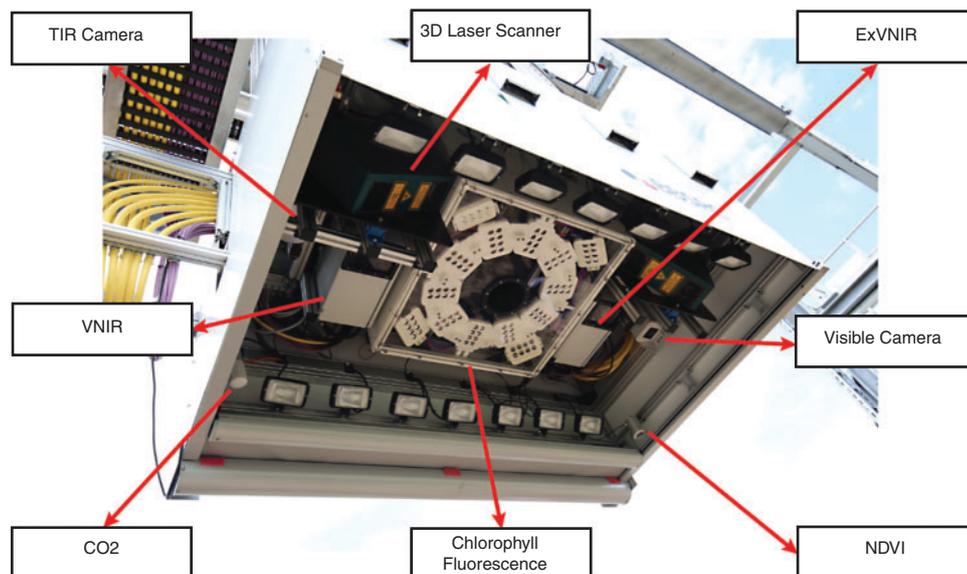


Fig. 2. Camera bay of the Field Scanalyzer.

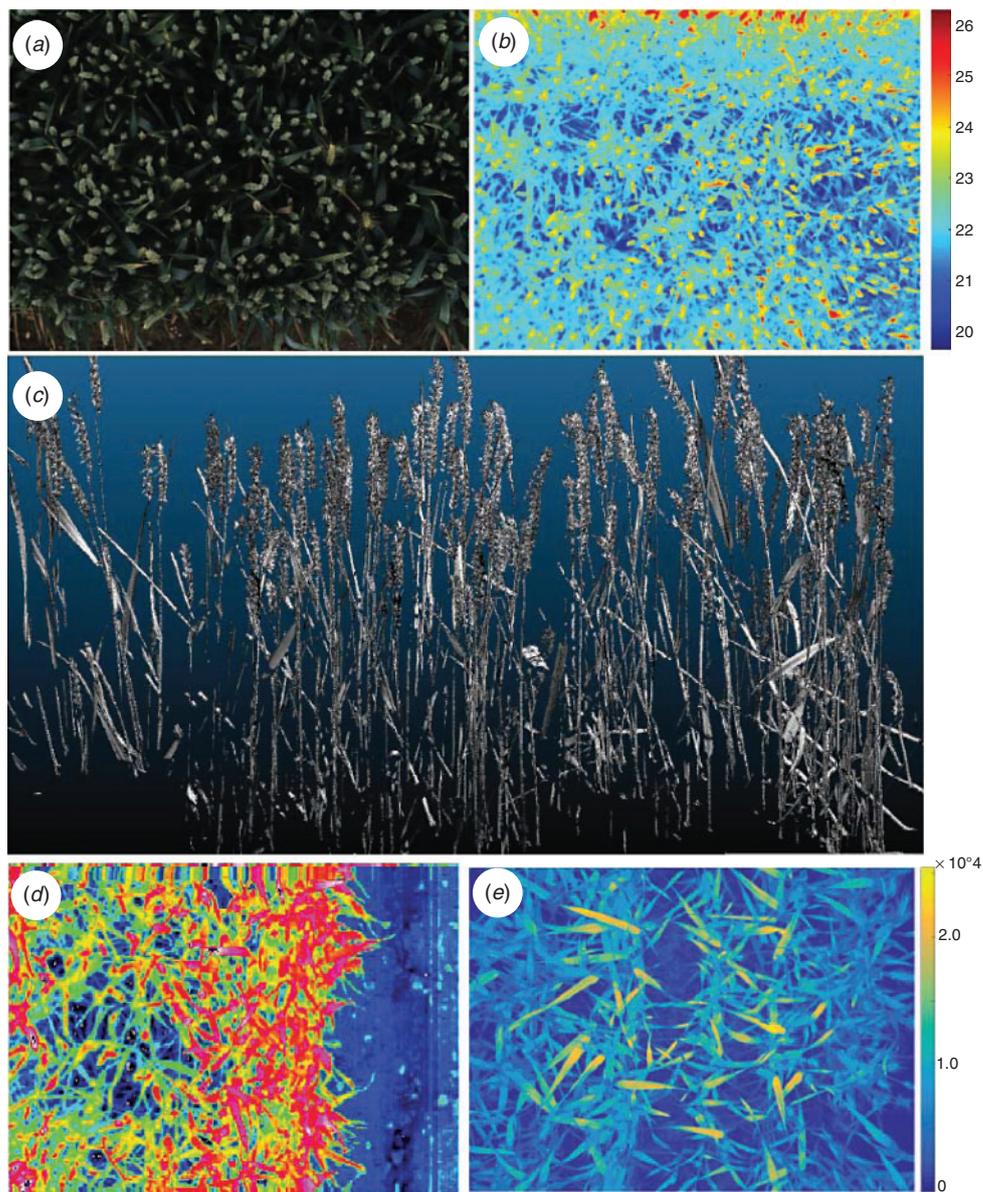


Fig. 3. (a) Red, green, blue (RGB) image taken from a canopy using the visible camera at 2.5 m above the canopy; (b) thermal infrared image (heat scale in °C) taken at 2 m above canopy; (c) 3D image of wheat canopy taken at 3 m above canopy; (d) false colour coded reflectance image at 800 nm taken at 2.5 m above canopy, and (e) false-colour coded fluorescence image (arbitrary units) taken at 0.7 m above canopy.

together covering the 400–1700 nm range. The VNIR and ExVNIR cameras have 1600/923 (0.7 nm step) and 320/229 (4.6 nm step) spatial/spectral resolution, with an $f/2$ optical aperture. Both VNIR and ExVNIR cameras are directed vertically downward to the ground and scan 46 and 80 plots h^{-1} , respectively. During acquisition, the crane remains stationary and the resulting hypercube is collected from the motion of an internal concave mirror. Data is recorded in their original 16-bit format.

A four-channel amplified radiometer normalised difference vegetation index (NDVI) light sensor (Skye Instruments Ltd) is fitted on the Field Scanalyzer, with two channels calibrated for red (633 ± 19 nm) wavebands and the others for NIR (800 ± 17 nm).

One combination of waveband channels is positioned on top of the Field Scanalyzer, pointing at the sky to measure incident solar radiation, whilst the other pair points directly at the ground, to simultaneously measure radiation reflected upwards. A test was performed to compare NDVI values computed from the VNIR sensor (raw data, without radiometric correction) and those from the Skye Instruments sensor (accounts for incoming solar radiation) (Fig. 6). Comparison of NDVI values between the corrected and uncorrected sensors highlighted that, despite the strong linear relationship between NDVI values of the two sensors ($R^2 = 0.89\text{--}0.90$ at 150 and 174 DAS respectively), values generated from the VNIR were consistently lower than

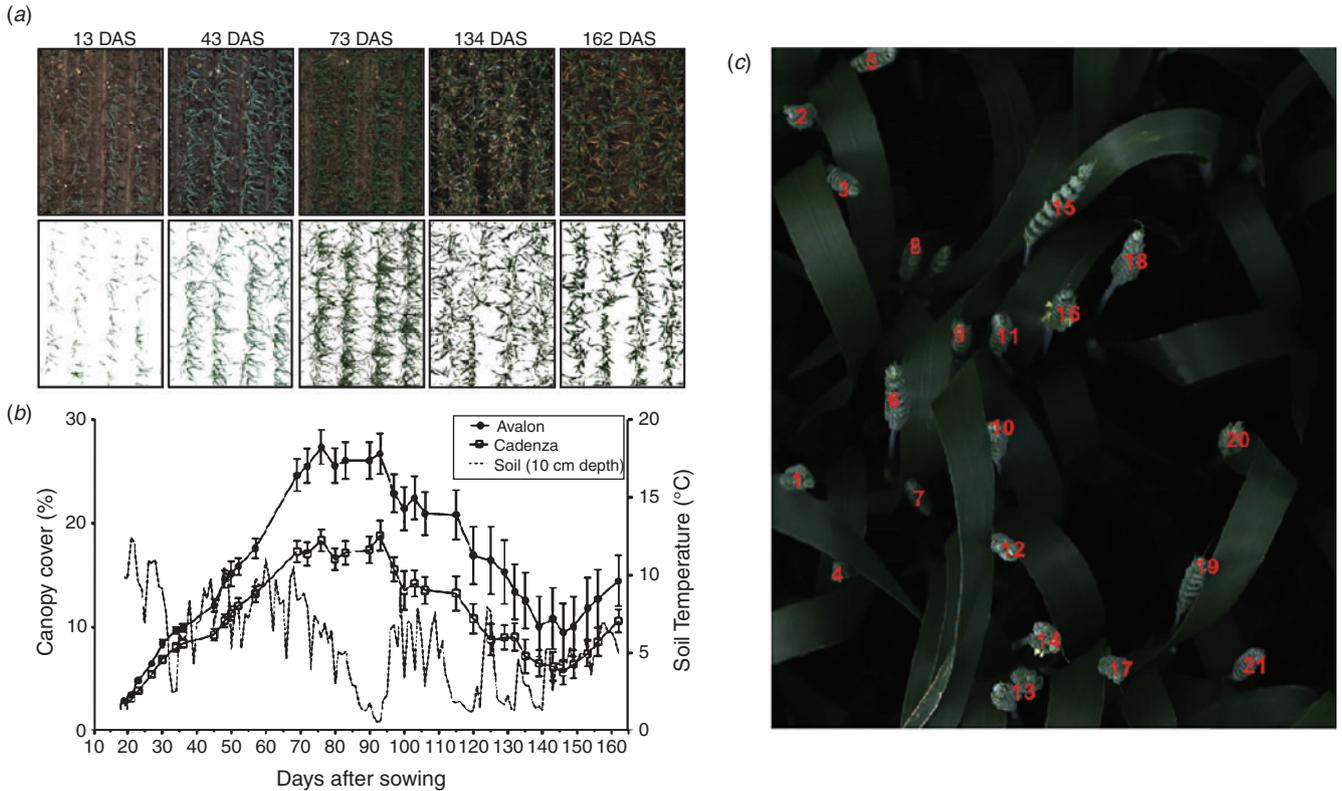


Fig. 4. (a) Images acquired from visible camera of Field Scanzalyzer over one wheat plot (*Triticum aestivum* var. Avalon) between 13–162 days after sowing (DAS). Images on bottom row are of the same corresponding image (raw visible), but with soil background removed, leaving only canopy foreground to quantify number of green pixels as a percent of total pixels within the image. (b) Canopy development of *Triticum aestivum* vars. Avalon (closed circles) and Cadenza (open squares), represented by percentage of green pixels within visible image). Values are means of nine replicate plots \pm s.e. Daily soil temperature at 10 cm depth (dashed line) obtained from the electronic Rothamsted Archive (e-RA; <http://www.era.rothamsted.ac.uk/>, accessed July 2016). (c) An example of the detection and quantification of wheat ears in an RGB image.

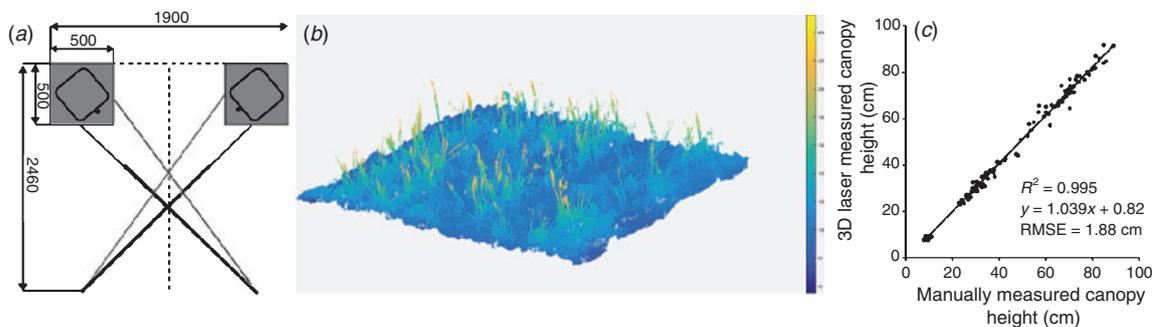


Fig. 5. (a) Orientation of twin 3D lasers within the camera bay of the Field Scanzalyzer. (b) False colour representation of canopy height calculated from point cloud images of a wheat plot at 90, 196 and 226 days after sowing (DAS). (c) Average canopy height of 54 individual wheat plots, calculated from point cloud images acquired from on board 3D laser scanners, compared with manual measurements. Canopy height was manually calculated by the mean height of 12 randomly selected plants within a plot.

the corresponding values of the Skye Instruments sensor, which accounts for incoming solar radiation. The difference in NDVI values may be due to comparing narrowband (VNIR) with broadband (Skye Instruments) measurements. Additionally, the constant within the linear equation differed between 150 and 174 DAS, reinforcing the necessity to apply post-acquisition

radiometric correction, through the use of on-field reflectance standards (Comar *et al.* 2012).

The on-board CFI (CropReporter) is provided by PhenoVation, and enables fluorescence measurements emitted mainly by PSII. The CropReporter is equipped with prime lens, having a fixed wide focal length (18 mm) and a large aperture

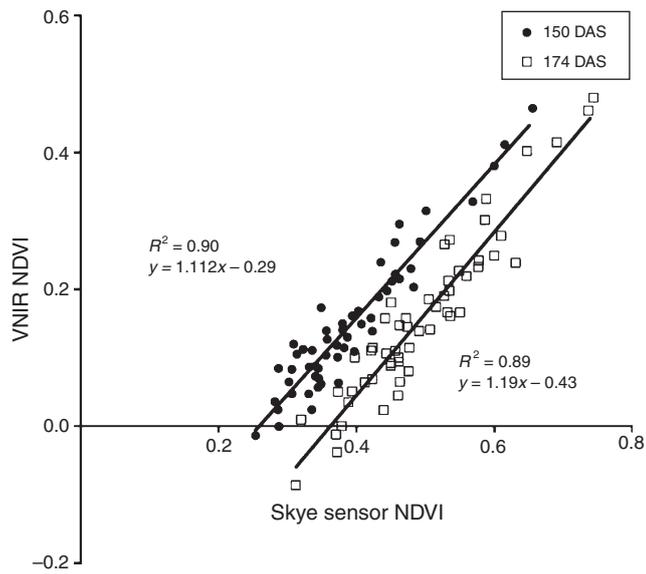


Fig. 6. Comparison of NDVI calculated from VNIR hyperspectral imager (uncorrected for incoming solar radiation) and NDVI sensor (Skye Instruments; corrected for incoming solar radiation) collected from 54 wheat plots at 150 (closed circles) and 174 (open squares) days after sowing (DAS). The resulting relationships between VNIR hyperspectral imager and Skye Instruments NDVI sensor show $R^2=0.90$ and $R^2=0.89$ at 150 and 174 DAS respectively.

($f/2$), with a CCD matrix of 1.4 Mp (1388×1038 pixels). The CFI is a LED (light emitting diode) induced chlorophyll fluorescent transient imager (Jalink and van der Schoor 2011) and the chlorophyll fluorescence (CF) is induced by a flash of red light (620 nm) for 1400 ms, saturating the electron transfer between the two photosystems (PSII and PSI), and records 24 images within that time. At the rising edge of LED illumination, the trigger is being used to start the exposure time of the first image and is called ground fluorescence. The intensity of the flash of light ($0\text{--}4000 \mu\text{mol m}^{-2} \text{s}^{-1}$) is set up according to the distance between the camera and the canopy, and sunlight intensity (for light-adapted measurement). The optimal distance between the sensor and canopy is defined at 70 cm, permitting a final field of view of $\sim 40 \text{ cm} \times 30 \text{ cm}$. The CropReporter generates 14-bit data and can acquire CF measurements at a rate of 90 plots h^{-1} (inclusive of crane repositioning time). Fig. 7a provides an example of *in-situ* CF measurements, which can be used to obtain ground and maximum fluorescence (F_0 and F_m respectively), values of light- and dark-adapted wheat, to subsequently calculate various photosynthetic parameters, such as F_v/F_m , of individual pixels (Fig. 7b).

Data storage and management

Any successful large-scale non-destructive plant analysis requires integrating cultivation systems, automated precise environment monitoring and robust data management system (Araus and Cairns 2014). In order to evaluate and analyse large-scale phenotyping data, it is essential to standardise data storage, as an efficient data management system can improve data accessibility and speed up data evaluation.

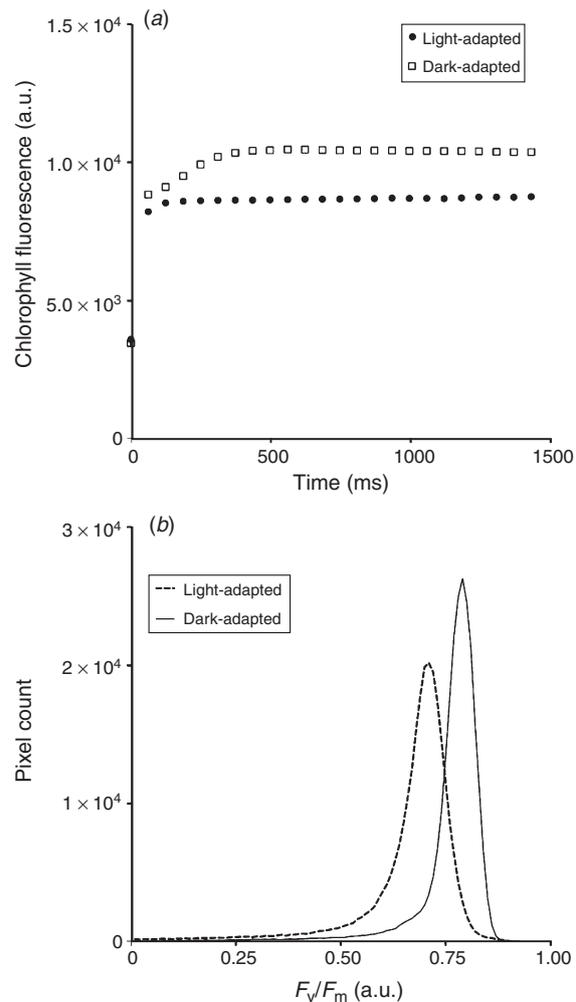


Fig. 7. (a) Chlorophyll fluorescence measurements of a light-adapted (closed circles) and dark-adapted (open squares) wheat plant (*Triticum aestivum* var. Avalon), captured within the field over 24 frames in 1400 ms. (b) Number of pixels within image corresponding to F_v/F_m values calculated from F_0 , F_m values of the same light-adapted (dashed line) and dark-adapted (solid line) wheat in Fig. 7a.

HTPPs such as the Field Scanalyzer produce big datasets, generating gigabytes of data in short amounts of time. For instance, it takes only 7 min to acquire data from all sensors in the camera bay from one plot, which generates 800 MB data. Rothamsted and LemnaTec GmbH developed a comprehensive data solution for the raw data acquired by the Field Scanalyzer to be handled and stored automatically. A high quality data management and data analytic system was created to securely access the data instantly at any time from various workstations and different locations.

Data and meta-information for each sampled plot is acquired and automatically transferred from the Field Scanalyzer to the dedicated database, through optical cables. The meta-information comprises a plot ID tag, timestamp, position of plots, and corresponding images (i.e. N1-Rep1-22-Crusoe_2015-07-13_10-34-46), and facilitates seamless navigation of datasets for quality control, evaluation and analysis. Software provided by

LemnaTec GmbH, such as LemnaControl and LemnaBase, allow users to graphically check the status of the phenotyping equipment and also preview any acquired data.

Raw data itself has little value and needs to be combined and analysed before we can gain knowledge from it. We used a combination of common image processing toolbox from Matlab, Python and R to develop a direct interface for image analysis from the database. Pipelines are developed according to the sensor technology, in order to automatically extract relevant quantitative information concerning the trait of interest. Extracted results (i.e. spread-sheets, figures) are then uploaded to the database and can be used in subsequent years by other partners and researchers. An overview of the data management and data processing workflow is illustrated in Fig. 8.

Advantages and limitations

As the Field Scanalyzer operates on rails, it preserves the below soil structure by avoiding compaction as a result of repeated-measurements (Bronick and Lal 2005). Although the platform was designed to be compatible with farming operations, the use of farming equipment is restricted to sowing and harvest operations, in order to minimise soil compaction. As the experimental plots can be quite condensed, given the area covered by the platform is relatively small compared with standard field experiments, herbicides, pesticides and nutrients are applied manually.

The framework of the platform is also a limiting factor. Unlike the Zürich Field Phenotyping Platform (ETH-Zurich),

which covers ~1 ha and is potentially transferrable to another site, the Field Scanalyzer covers only a 0.12 ha area and does not allow multi-site experiments without investing in duplicate platforms. Moreover, as the main crop of interest is wheat, a three-year rotation is implemented (wheat, oilseed rape and oat), to maintain soil-nutrient balance, as well as to avoid root diseases, such as take-all. In consequence, the effective area for the main experiment is further restricted to one-third of the platform area in any one year.

A key advantage of the Field Scanalyzer comes directly from its framework, which enables the capacity to carry a 500 kg payload within the camera bay. This high payload provides the opportunity to carry together, all the main imagers currently used in field phenotyping, as well as less portable imagers normally used in controlled environment facilities. The volume and the high payload also offers room for the expansion of the sensor array to test and validate new sensor technologies that are not portable, before possible miniaturisation for UAV platforms. Currently, only ~300 kg of the payload is in use.

The Field Scanalyzer was designed for high spatial resolution phenotyping. As screening is generally performed 2–3 m above the canopy, according to the cameras focal-point, with exception of the fluorescence imager (70 cm above canopy), full advantage of the resolution of each camera is exploited to collect high quality images. High resolution images, such as those obtained from the RGB camera and the 3D laser scanner, potentially allow for plant organ recognition and quantification, as shown previously (Fig. 4).

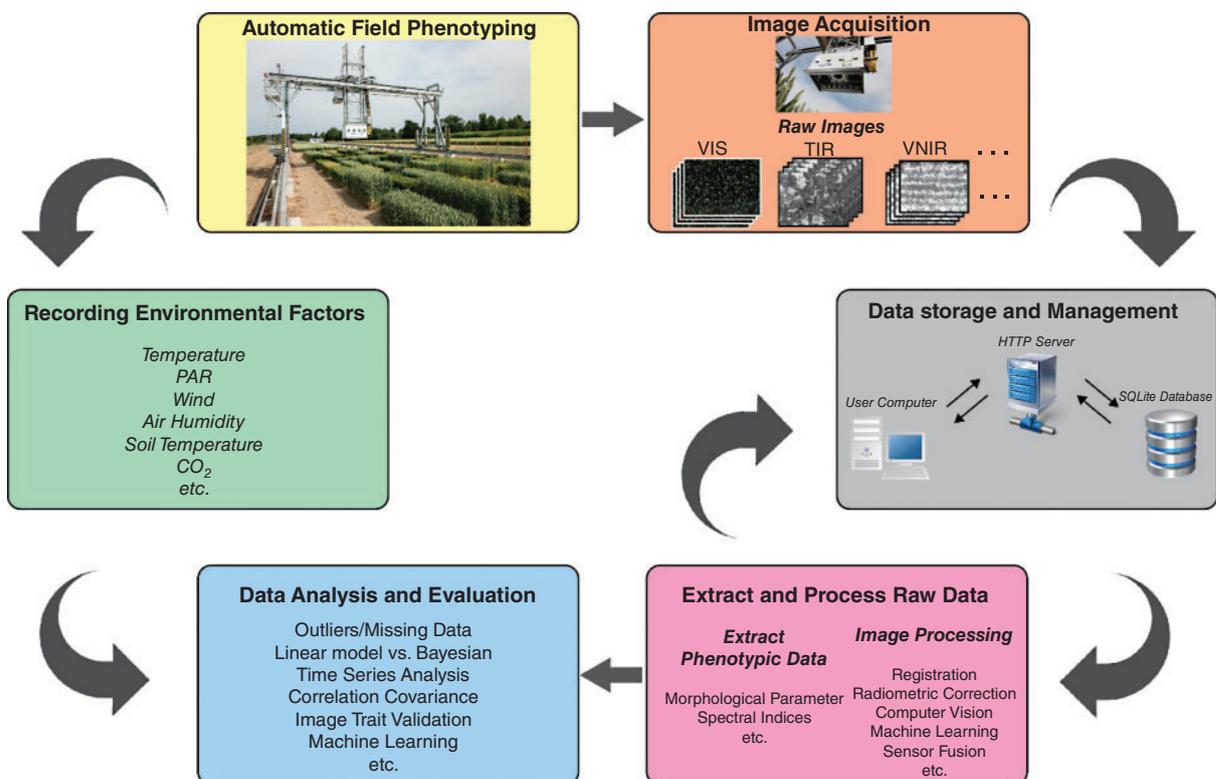


Fig. 8. Phenotyping workflow from image acquisition to obtaining quantitative data.

As a consequence of close range phenotyping, the area covered by each camera is limited by their field of view. Unlike UAVs, which captures multiple plots in a single snapshot, plots are imaged individually, reducing the throughput. Nevertheless, scanning technologies, such as the 3D lasers and hyperspectral imagers require 1–2 min to collect data at each plot. This makes their outputs particularly susceptible to wind and, thus, affecting the image quality. Another limiting factor induced by the close range, common to every ground platform, is the effect of varying ambient illumination, which makes comparison within and/or between days challenging. For example, hyperspectral imagers equipped on the Field Scanalyzer have to be set-up for exposure time before each run, according to the ambient illumination. Whilst clear skies, or homogenous overcast are the best conditions for collecting spectral data (Rundquist *et al.* 2014), variations could likely occur during a 2–3 h scan of a field. This runs the risk of generating under- or overexposed images that cannot be corrected for, particularly when it also occurs on the reflectance standard panel.

The key advantage of the Field Scanalyzer is the implementation as an autonomous system, similar to the Zürich Field Phenotyping Platform (ETH-Zurich). Unlike UAVs and most mobile platforms, which require the supervision of a technician or pilot to ensure safe operation, the Field Scanalyzer requires human interaction only for the initial programming and loading of the script for image acquisition. To prevent any script programming errors, LemnaControl (LemnaTec GmbH), the software controlling the platform, provides the opportunity to simulate the loaded script and track any errors. The only sensor requiring human intervention are the hyperspectral imagers. An initial scan has to be performed before each run on a reflectance standard panel in order to set up an optimal exposure time to avoid saturation of the images when slight changes in illumination occur.

The system was designed for 24 h day⁻¹, 7 days week⁻¹ operation. In practice, sequential sensor runs are performed according to the data needed for the experiment and the forecast weather conditions. Moreover, 3D laser scanning is performed during the night, due to (i) the low throughput of the sensor, freeing up daylight hours for other cameras, and (ii) less windy conditions at night, promoting better image quality. The CFI is also used at night, as it allows collection of fluorescence parameters in the field, simplifying the task of quantifying photosynthetic capacity in dark-adapted conditions, which remains laborious with handheld fluorimeters. Additionally, acquisition of fluorescence, thermal infrared and hyperspectral data throughout 24 h periods can also facilitate a better understanding of crops and plant diurnal rhythm kinetics.

Key challenges

The objective of collecting field data in high throughput is close to being achieved: now the phenotyping community is needed to validate the relevance of the developed approaches for the trait being measured. Although the ability to collect data is now established, challenges remain in terms of dealing with changing ambient illumination, data processing, analysis and the interpretation of the image variables and their potential value as a descriptor of a plant trait.

Illumination conditions

Changes in ambient illumination are inevitable when collecting a data series in the field throughout the growing season. Screening should be performed in the most optimal weather conditions (e.g. minimal wind, even/no cloud cover, no rain), although the temperate climate in some regions remains uncertain, notably in the UK. Acquiring data at the same time interval between days is not always possible because of weather conditions. For RGB cameras, Casadesús *et al.* (2007) suggests that in order to capture well-exposed images, the camera should be set to automatic exposure mode to adapt the aperture, shutter speed and gain, according to the intensity measured by the sensor.

Acquiring data with hyperspectral imagers at close range adds difficulty to the interpretation of data collected from a series of plots. This is because only one plot is captured in a single image and ambient illumination may vary during the acquisition time of multiple plots. To minimise the effect of its variation, exposure time must be manually configured before each run and ensure the run does not exceed 3 h. Additionally, an on-field reflectance standard is systematically added within the data acquisition sequence (every six or 12 plots), allowing radiometric correction to minimise illumination effects between images.

As described by Murchie and Lawson (2013), active CFIs require large LED arrays to ensure even actinic illumination and fully saturating pulses over the entire imaging area, making the instruments relatively large in size and not very portable as the imager equipped on the Field Scanalyzer. Due to its high payload capacity, using this heavy device is not a problem. However, being able to fully saturate a complex canopy in the field remains challenging, especially for the deeper layer of the vegetation as well as the application during the day (Li *et al.* 2014).

Data processing

The ability to efficiently process big datasets collected remains a challenge, as well as the ability to develop robust and fast algorithms according to the sensor used. Recent developments in data processing of RGB images allow the separation of plants from the background in various ambient illumination conditions. Some approaches to achieve this are based on colour spaced transformation, where colour thresholds are applied to differentiate between green and senescent leaves (Casadesús *et al.* 2007; Casadesús and Villegas 2014). Machine learning offers another solution to differentiate vegetation from background (Guo *et al.* 2013), where the model can be trained using plant in various illuminating conditions.

For TIR imaging, different methods have been investigated to extract plant temperature from the image as reviewed in Jones and Sirault (2014). The task is not simple for a heterogeneous canopy, where the presence of mixed pixels (pixel containing signals from both soil and vegetation) is a recurring problem (Jones *et al.* 2009; Hackl *et al.* 2012). It is generally considered that directly using the surface temperature of vegetation is risky, because the weight of mixed or soil pixels in porous plant cover can shift towards the soil surface temperature (Jackson *et al.* 1981). To overcome the problem of mixed pixels, various image pre-processing methods based on both histogram analysis on the raw TIR image or data fusion with RGB image can be applied to exclude mixed pixels (Jones and Sirault 2014). These methods

are time-consuming and can also be subjective, as they depend on the threshold chosen. However, automated segmentation techniques have recently been developed to select pure vegetation pixels in TIR images (Jerbi *et al.* 2015).

Processing images generated from hyperspectral imagers and laser scanners likely remain the most challenging, as they easily generate up to a gigabyte of data from a single scan. Thus, the efficiency of processing tools must be considered when repeated-measurements may generate hundreds of gigabytes to terabytes of data throughout the season.

For hyperspectral imaging, Römer *et al.* (2012) proposed a new method based on unsupervised classification to accelerate the computation of large datasets. This method, which allows computation of how similar spectrum is to observed typical spectra, was suggested to be better than spectral reflectance indices (SRI) for the early detection of drought or nitrogen stress in cereal crops, even in field conditions. Another factor to consider is the pipeline of hyperspectral imaging for field phenotyping, as it should integrate robust and fast algorithms for the various data processing steps before extracting the features of interest: (i) pre-processing of the raw hypercube, (ii) exploration, (iii) segmentation, and (iv) image processing (Amigo 2010).

Processing data generated from 3D laser scanner technology is mainly performed by converting point clouds into (i) distances and angles to generate 2D images or (ii) a voxel image, where a voxel (volume element) is the 3D equivalent to a pixel (Deery *et al.* 2014), which can both be subsequently processed using standard image processing software. However, point clouds converted into the image equivalent adds a few additional steps to data processing and could induce a loss of information. Recently, a new approach based on surface feature classification was developed for high throughput data analysis of 3D data (Paulus *et al.* 2013). In the mentioned study, the authors used the laser scanning outputs directly to establish a reliable and fast technique to differentiate individual plant organs, avoiding any transformation of point clouds.

Data analysis and interpretation

Interpreting a TIR signal is not simple, on account of the high sensitivity of stomatal response to environmental micro-variations. Numerous indices have been developed to assess crop water status from canopy surface temperature (T_s) acquired from aerial or ground platforms (Maes and Steppe 2012). Subtracting air temperature (T_a) from T_s generates a raw variable that is easy to extract from images, but remains sensitive to solar radiation, wind speed and vapour pressure deficit (Maes and Steppe 2012). Temporal comparison of plant transpiration based on this variable ($T_s - T_a$) requires controlled ambient conditions (Li *et al.* 2014), or environmental conditions to be similar on measurement days (Idso *et al.* 1981). The crop water stress index (CWSI) was developed to take into account ambient meteorological conditions, including the vapour pressure deficit, which also influences the canopy temperature (Idso *et al.* 1981). In recent decades, semi-analytical and empirical approaches to facilitate the computation and the use of CWSI have been developed (Maes and Steppe 2012). However, the application of CWSI and its derivatives is limited to full-cover

vegetation and requires calibration from natural (leaf or canopy) or artificial reference surfaces to define the lower and upper baseline necessary to compute those indices. Moreover, the lower baseline (corresponding to a non-water-stressed state) may be genotype dependent, therefore limiting its applications for high-throughput phenotyping where a panel of genotypes is used (Bellvert *et al.* 2015).

Different approaches have been developed to predict crop performance using multi- or hyperspectral data. Statistical methods are most commonly used and are based on univariate and multivariate regression models (Capolupo *et al.* 2015). Although both regression models aim to predict crop performance traits, the univariate approach only uses a limited set of SRI (Gonzalez-Dugo *et al.* 2015), whereas the multivariate approach utilises the entire spectrum to model plant response (Kipp *et al.* 2014). Some authors show that, in comparison to univariate approaches, multivariate approaches were able to provide better results in detection of early stages of biotic stress (Römer *et al.* 2011), or in the predict nitrogen and water content (Kusnierek and Korsaeath 2015). Another approach, based on radiative transfer models, seems promising for field phenotyping, as this takes into account biochemical and structural properties of the leaf and canopy (Thorp *et al.* 2015).

Given that the platform's camera housing is fixed above the canopy, the majority of cameras and sensors are directed vertically downward to the ground. Consequently, sensors relying on quantifying reflected or emitted radiation off the canopy are sensitive to leaf and canopy structure, with areas of the crop canopy directly parallel to the sensor lens reflecting wavelengths at a higher intensity, compared with those not parallel (see Fig. 3e). Moreover, the leaf optical properties have to be taken into account as they affect the directional reflectance. This is because it may become a source of bias in the (i) quantified signal measured, (ii) prediction of leaves biochemical components, and (iii) estimation of canopy cover or greenness using the bidirectional reflectance distribution function (BRDF) (Comar *et al.* 2012). In addition, BRDF varies at different observation angles and also at different solar elevation (Deery *et al.* 2014). This last point must be taken into account when using hyperspectral imaging in field phenotyping through the season. A solution could be to test radiative transfer models, such as PROSAIL (Jacquemoud *et al.* 2009), to allow the simulation of leaf and canopy reflectance from 400–2500 nm (in 1 nm steps), as well as the BRDF. By using the BRDF output, the effects of canopy structure, composition and geometry, as well as illumination on the spectra collected, can be corrected for. Moreover, model inversion techniques and reflectance measurements can be used to obtain input parameters of the PROSAIL model, such as chlorophyll concentration, dry matter content or leaf water thickness (Thorp *et al.* 2015). A challenging approach to overcoming issues caused by canopy geometry may be through the fusion of 3D point cloud images with 2D spectral images, whereby each point cloud within a 3D image is assigned a value corresponding to the same point in an image from a spectral image, however this is an avenue requiring further exploration.

Regardless of the quality and robustness of the phenotyping vector used to acquire data, the data itself is only as valuable as the analyses that can be performed on them. Biological noise

is present even in controlled conditions and must be accounted for in statistical models to assess phenotypic data. Variations in environmental conditions will add further noise and these caveats present a challenge to develop robust statistical models that can be applied to analyse phenotypic data (Cobb *et al.* 2013). However, White *et al.* (2012) indicated that the analysis of data from a temporal series can be difficult because many of the observed traits are auto-correlated and integrate multiple effects from the underlying physiological mechanisms, which each operate on different time scales. Analysis by linear mixed models can be used to detect auto-correlation effects (Piepho *et al.* 2004); however, the collection of data at regular time intervals is required.

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