Project title:	Early detection of stress in strawberry plants using hyperspectral image analysis
Project number:	SF 144
Project leader:	Andrew French University of Nottingham, Jubilee campus, Wollaton Road, Nottingham, NG8 1BB
Report:	Final report, May 2018
Previous report:	Annual report, May 2016
Key staff:	Andrew French (Joint Supervisor) Nicola Harrison (Joint Supervisor) Amy Lowe (Student)
Location of project:	University of Nottingham East Malling Research
Industry Representative:	Marion Regan Hugh Lowe Farms, Barons Place Mereworth, Maidstone, ME18 5NF
Date project commenced:	12 May 2014
Date project completed:	30th April 2018

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Name] Dr Andrew French

[Position] Academic supervisor

[Organisation] University of Nottingham,

Signature ..... A.French ..... Date .....2/5/2018.....

[Name] Dr Nicola Harrison

[Position] Industrial supervisor

[Organisation] NIAB EMR (now AHDB)

Signature ..... Date .....

Name] Amy Lowe

[Position] Ph.D Student

[Organisation] University of Nottingham & East Malling Research

Signature .... Date 2/05/2018.....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headline

- Hyperspectral imaging has been investigated as a means of detecting the onset of plant stress in strawberry.

Background and expected deliverables

The aim of this project was to investigate the use of hyperspectral imaging to analyse and detect the onset of stress in strawberry plants. Such stresses might relate to certain major diseases, common pests or environmental conditions.

Hyperspectral imaging is a challenging technology to work with. It is not a 'point and click' technology: capturing images themselves is a challenge, and the resulting dataset is large and complex. Lighting, calibration and the 3D structure of the plant being imaged can have significant effects on the resulting interpretation of the data.

A hyperspectral image contains both spatial and spectral information, which can be represented in three dimensions. The spatial information is stored in the pixels location along the *x*- and *y*-axes, and the light spectrum reflectance is represented along the z-axis (See Figure 1 in the Science Section of this report).

Unlike a point-source spectroradiometer, a hyperspectral camera measures many reflectance samples throughout a full image of the subject and unlike a *multi*spectral sensor, it is able to record reflectance at many hundreds of wavelength positions, typically extending into at least the visible and near-infrared regions of the spectrum. Hyperspectral data is therefore large in size, especially when multiple plants are imaged for several days. A scan of a single plant could easily be a gigabyte or more in size. If the whole spectrum range is analysed then the process will take considerably longer than selecting several particular wavelengths to analyse. There is a lot of information contained in the data, which could be valuable; the challenge is finding a way to analyse it.

To investigate the potential of hyperspectral imaging to detect stress, a suitable stress to study was first identified. Strawberry plants were subjected to powdery mildew, two-spotted spider mites and drought to capture a time series of how the plants respond to these biotic and abiotic stresses. From these investigations, drought provided the appropriate balance between ease of control of the stress itself over a time series and the resulting nature of the visible/hyperspectral effects seen in the data. The images were collected using hyperspectral cameras, including both the spatial information (the location of the pixels in the image) and spectral information (the narrow bands of contiguous wavelengths from visible light to near

infra-red light). Imaging of the plants took place at NIAB EMR and the University of Nottingham.

Once the images were captured, the strawberry plants needed to be identified in the images using a technique known as ‘segmentation’. This involved labelling objects (leaves, in this case) in the image by finding regions of similar properties such as colour, shape or texture. Once the leaves have been located in the images, the hyperspectral information can be extracted from these regions and analysed over time. This avoids taking measurements from pixels of the background (such as pots or soil) rather than plant material.

In this project, though, hyperspectral reflectance was found to be dependent on certain 3D properties of the plants: factors such as the height and angle of the leaf could affect the hyperspectral reflectance profile. Therefore, 3D information was also collected in order to build a 3D model of the plant. The 2D hyperspectral data was then mapped onto the 3D plant model. To take hyperspectral measures from a plant, the 3D model was used to select a set of leaves on the plant which were likely to produce good quality hyperspectral readings: leaves which are flat, towards the top of the plant, and near the centre of the image.

Readings from these selected leaves were then recorded, and equivalent leaves manually selected from the plant throughout the time series to provide stress response data.

Summary of the project and main conclusions

A new analysis pipeline was developed to automatically take hyperspectral measures from plant leaves. Leaves were selected using information from a 3D plant model which helped remove undesirable leaves from the analysis.

This pipeline was found to produce similar results to the more labour intensive manual approach to data analysis, suggesting such a pipeline could be incorporated in a future hyperspectral imaging system. However, despite the new analysis pipeline, and careful control of experimental and imaging conditions, it was hard to determine clear and robust indicators of the early onset of drought, despite some visual indication in the hyperspectral profiles, suggesting that a difference in NIR reflectance may be present over a period of days. The results are considered in more detail in the Science Section of this report.

For those considering use of a hyperspectral system to determine stress, this study advises:

- To consider the type of hyperspectral camera system carefully, as several systems exist, and they work in different ways.
- To consider if 3D information will need to be taken into account when imaging plants.
- To carefully control lighting during imaging, and additionally calibrate using a reflectance white balance target.

- To consider where spatially on a plant a hyperspectral measure originates, as this can affect the reflectance profile: factors such as shadowing and orientation of the leaves may affect the data.
- Acknowledging that there is unlikely to be an ‘off the shelf’ analysis approach for a particular stress. Methods are still in active development and more complex software packages to support analysis are likely to become available over time.

Financial benefits

No firm financial benefits can be drawn from this early research into hyperspectral imaging in soft fruit. Investment in hyperspectral imaging methods requires outlay in both the hardware technology and suitable, potentially complex, analysis methods. As technology develops, the cost of the hardware drops. However careful use and analysis is still of primary importance when using this technology.

Action points for growers

- There are no direct action points for growers resulting from this work.

SCIENCE SECTION

Introduction

This section overviews the project and the main outcomes and results. Further technical detail is available in the PhD thesis accompanying this work.

Materials and methods

During this project image data was collected at two locations: NIAB East Malling Research (EMR) in Kent, and The University of Nottingham, Sutton Bonington campus. The system set ups are both laboratory-based, with the camera attached to a metal frame for support (Figure 1). The Nottingham system involves moving the camera, whereas the EMR camera is stationary and the object moves on a platform. Therefore, both are pushbroom systems, but implemented in two different ways. The camera captures visible and near infrared (VNIR) wavelengths, which has the range 400 - 1000nm.

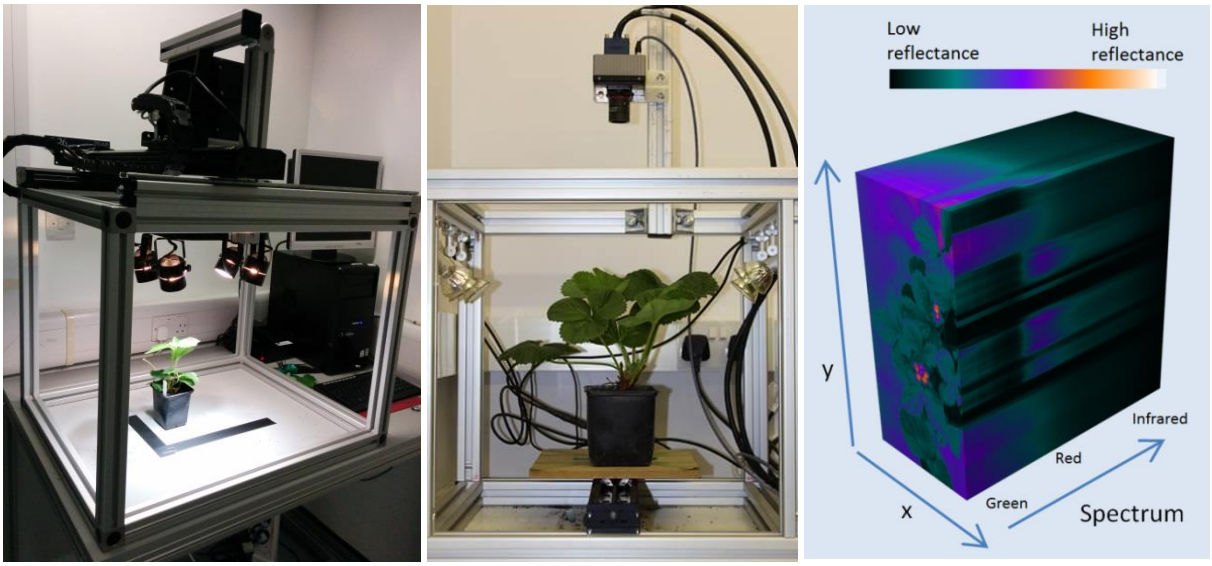

Figure 1. Left: Nottingham camera. Middle: EMR camera. The square frame holds the camera and the cables so the camera can move and capture the plant (left) or the plant itself is moved (middle). The Nottingham system has the capability to move the camera in the x- and y- axis, but for imaging the strawberry plants the camera only needs to move along the -y axis. Right: a hypercube representing hyperspectral data as a 3D image where the (x,y) dimensions are the pixel location and the third dimension is the hyperspectral information.

The six varieties selected for this project, Elsanta, Fenella, Sonatta, BSP 14, Malling Centenary and Hawaii-4, provided a good selection of traditional and modern types of strawberry plants with a variety of architectures and stress susceptibilities.

Three stresses (powdery mildew, spider mites, and drought) were investigated for suitability for further study; three initial experiments were conducted to investigate the development of

these stresses. All were imaged to identify the most suitable to develop methods for the rest of the project (Table 1). Protocols for these image-capture experiments can be found at the end of this report. Following these, drought was selected as an appropriate stress, partially due to a technical issue with the camera at NIAB EMR limiting the utility of the spider mite and mildew images, but also because drought stress signals were found to be relatively simple to control and image in the plants.

Table 1. Table of the data collected comprising four datasets. The plants, time period, number of images, location and extra data are listed for each dataset.

Stress	Plants	Time	Images	Location	Multi view data (for 3D reconstruction)
Spider mite	10 Elsanta,	24 hours -	40 total, 132 bands	NIAB EMR glasshouse	-
	10 Fenella	daily			
Powdery mildew	13 Elsanta,	16 days -	225 (25x9), 132 bands	NIAB EMR Cabinets	-
	12 Fenella	two day interval			
Drought 1	21 Fenella	5 days - daily	105 total, 132 bands	NIAB EMR glasshouse	-
Drought 2	6 Sonata,	6 days,	220 total, 812 bands	UoN Sutton	Pair hyperspectral
	12 BSP14,	6 days,		Bonington campus	3 - 10 SLR images
	8 Malling Centenary,	5 days			per plant
	12 Hawaii4	6 days			

To provide a ground truth analysis, a manual approach to data extraction was explored initially. The image analysis software package 'FIJI' (Schindelin et al, 2011, http://fiji.sc/) was used to manually select sections of leaves from the plants and take an average over three days for the control and drought plants. These graphs are displayed in the results.

There were minimal differences between the reflectance of the control plants (Figure 3), whilst some differences were observed for the drought plants (Figure 4) but remained unclear. More complex analysis was needed to collect the best possible data, which was manually selected from the plants. The next step was to attempt to automate the leaf selection process for analysis, and if possible, develop better quality measures.

To do this, an automated pipeline for segmenting (labelling) the leaves in a plant was created; the centre of the leaves was automatically detected, and the veins of the leaves were located. These two steps were used to locate a leaf shape template in the general vicinity of a leaf,

and then this was incorporated into a level set-based approach to find the actual leaf boundary. Level sets are mathematical models which allow a shape to be fitted around the boundary of an object, in this case, a leaf of a strawberry plant. Final boundaries and accuracies were evaluated and the segmentation approach was found to work well. As a side effect of this approach, poorly visible leaves (for instance, leaves hidden by upper leaves, or leaves poorly lit by the lighting system) were rejected at various stages in this pipeline. This segmentation approach used the image information in 2D hyperspectral images. While this worked well to segment individual leaves, the leaves themselves existed on a live plant. It was hypothesised that knowing information about the structure of the plant would further improve the hyperspectral measures.

Material being imaged for hyperspectral imaging is usually first flattened onto a surface, removing 3D structural effects, where the inclination or overlapping of leaves (or objects) can affect their spectral signatures. (Roscher, R. et al, 2016) showed that accounting for these can improve the classification accuracy of detecting disease. These are challenges need to be taken into consideration when imaging whole plants in situ for good quality hyperspectral measures.

The next challenge, then, was to produce 3D reconstructed models with 2D hyperspectral data mapped onto the 3D model. Digital colour images were captured with an SLR camera from the side of the plant. This data allowed multi-view 3D reconstruction methods to be used. VisualSFM (Wu, 2011), Patch-based Multi View software (Furukawa et al, 2010) and Surface reconstruction (Pound, M et al 2014) were used within the pipeline to build 3D reconstructed models of the plant. The resulting 3D plant model (e.g. Figure 2) then needed registering with the 2D hyperspectral data from the same plant. Once registered, a mapping process was developed to map the 2D hyperspectral data onto 3D surface models.

Figure 2. Example 3D model of a BSP14 plant. Top: side view. Bottom: plan view. The model was created using PMVS and Surface reconstruction. Large areas of leaves are visible; complete reconstruction of the whole plant is not possible due to the limited viewpoint of the SLR images; but a complete plant is not required for further stages of processing used here.

Developing a 3D model enables better quality information to be obtained from the strawberry plant data, for example, calculation of leaf orientation, thereby allowing selection of the leaves presenting the best surface for analysis. Location of the leaves within the plant is also significant; leaves near the top of the plant have optimum light coverage whereas lower leaves are occluded and shadowed by other leaves, as well as suffering light scattering and transmittance effects from surrounding leaves.

The model was used to select leaves for further analysis based on certain criteria. The leaf angle was shown to affect the spectral signature therefore selecting a leaf parallel to the camera imaging plane would be beneficial, likewise leaves at the top of the plant are better-illuminated in these rigs. Leaf material near the centre of the image was also better lit by the lighting rig than leaves at the edge. These properties were measured from the 3D data, and

a weighted scoring model was developed to order the leaves from 'best' to ‘worst'. The final three properties selected were the leaf angle, height and distance from the centre of the plant.

These ‘best’ three leaves per plant were then automatically selected, and then manually matched for the other days in the time series to select the same leaves at each time point. The average of the mapped hyperspectral data for the leaves for each variety for each day was calculated and plotted over the time series.

Results

For the initial manual analysis, several well lit and relatively uniform leaves were selected from each Sonata plant. The control plants’ spectral signatures revealed no obvious change from day 0 to day 5 (Figure 3), whilst droughted plants showed a slight shift in the signatures most visible in the near infra-red range (730 - 1000nm) (Figure 4). Although the shift was small, droughted plants are expected to decrease in reflectance in the near infra-red (and slightly in the green wavelengths) over time, so this observed consistent change, although small, fits with expectations.

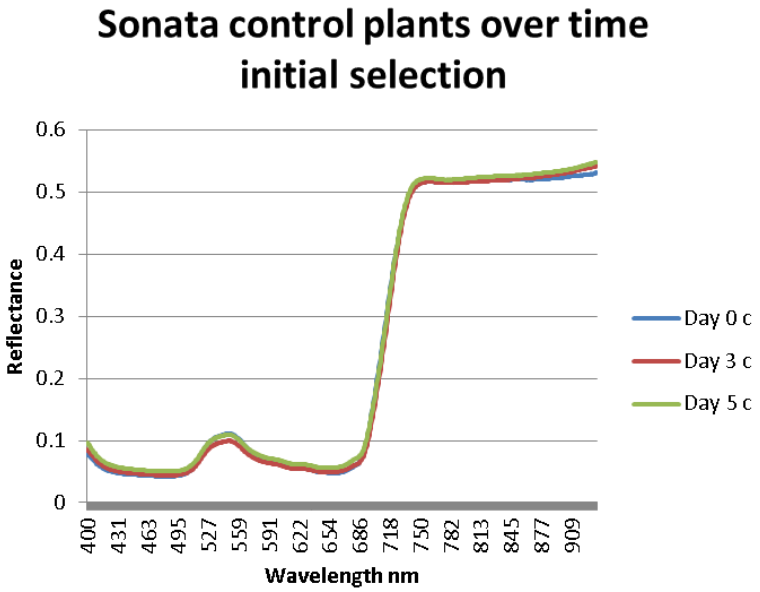

Figure 3. Manual selection of leaves for the Initial Sonata **control** plants over time.

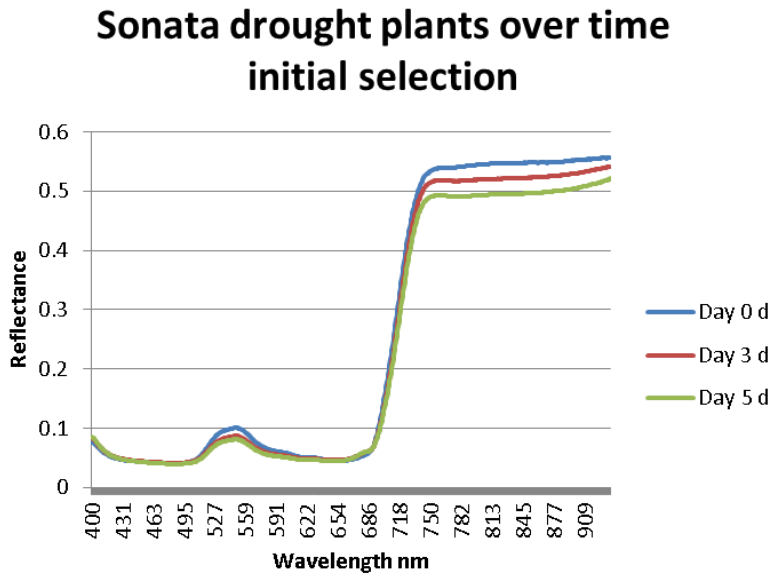

Figure 4. Manual selection of leaves for the initial Sonata **drought** plants over time

The new designed analysis pipeline described above was then used to examine the same dataset. The average hyperspectral reflectance data from the three highest scoring leaves was calculated, initially for Sonata plants (Figure 5 and 6). For Sonata, the control plants' profiles (Figure 5) appear noisy, but broadly similar over the time series compared with the drought plants. The drought plants spectral signatures have consistently decreased over the time series in the near infrared section of the spectrum, as seen in the manual measures (Figure 4).

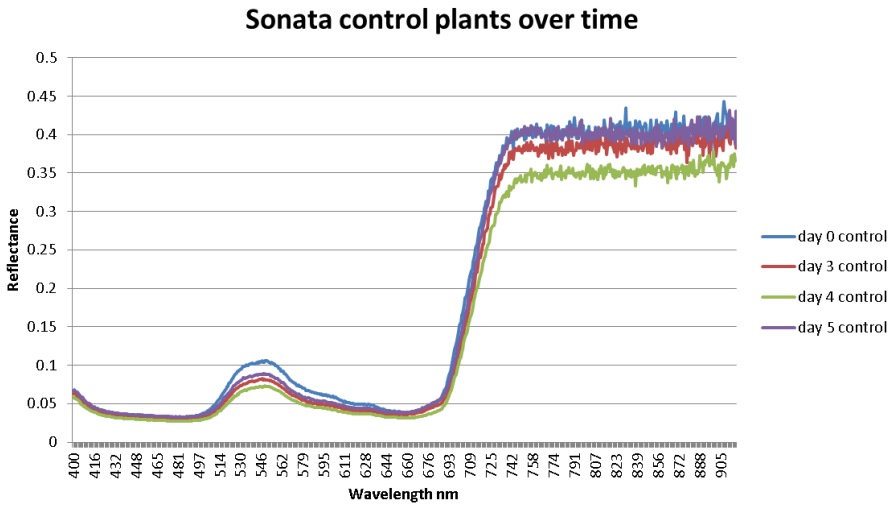

Figure 5. The Sonata control plants' reflectance over the time series, this time measured using the developed pipeline

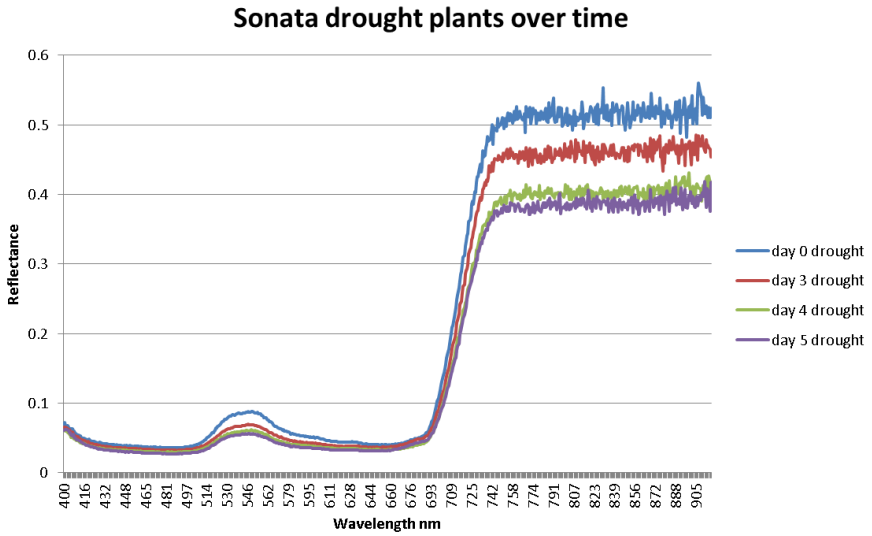

Figure 6. The Sonata drought plants over the time series, this time measured using the developed pipeline.

During the early stage of water drought stress, reflectance appears to decrease in the green and near-infrared sections of the spectrum. It is known that when the leaves dry out the red wavelengths will increase in reflectance because this is where the leaf pigments and chlorophyll affect the spectral signature. However, during the *early* stages of drought the green and near-infrared sections can decrease with little or no change to the red section. A study on maize plants (Manivasagam, 2015) shows signatures similar to the droughted Sonata leaves during different growth stages, suggesting this is not an artefact. BSP14 plants were analysed next, and do not appear to show different reflectance profiles between drought

and control groups in Figures 7 and 8 – both indicate a variation in reflectance in the NIR region of the spectrum especially; although interestingly, *within* the control group there is an apparent *increase* in infrared reflectance over time. One potential explanation is that BSP14 has some drought tolerance. The apparent change in the control group, as it is in a different direction over time to that seen in Sonata, could be caused by an external effect beyond that induced by drought.

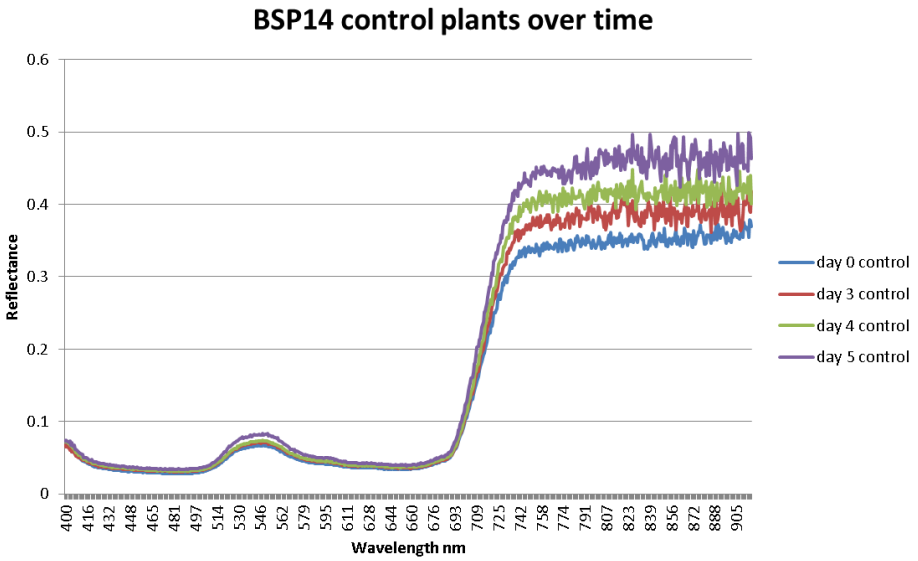

Figure 7. BSP14 **control** plants' reflectance, measured over the time series using the developed approach

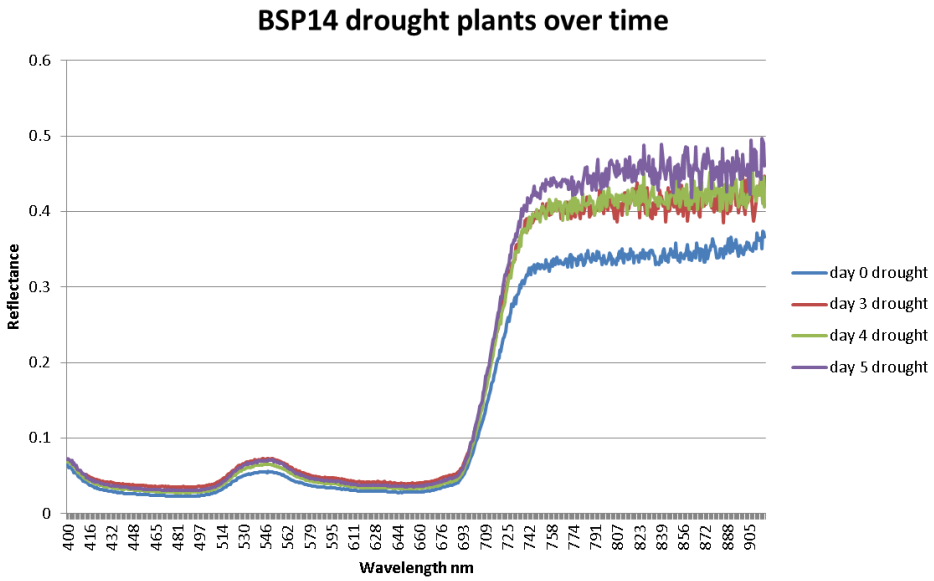

Figure 8. BSP14 **drought** plants' reflectance, measured over the time series using the developed approach

One way to tease out such effects is to 'normalise' the data to remove impacts such as overall brightness changes by adjusting responses to give identical reflectance at a specific reference wavelength, usually chosen for low noise levels and at a point which is not

expected to change between datasets. The first normalisation is at 660nm (Figures 9 & 10) and (Figures 11 & 12). Normalised data range from 1 to a maximum value.

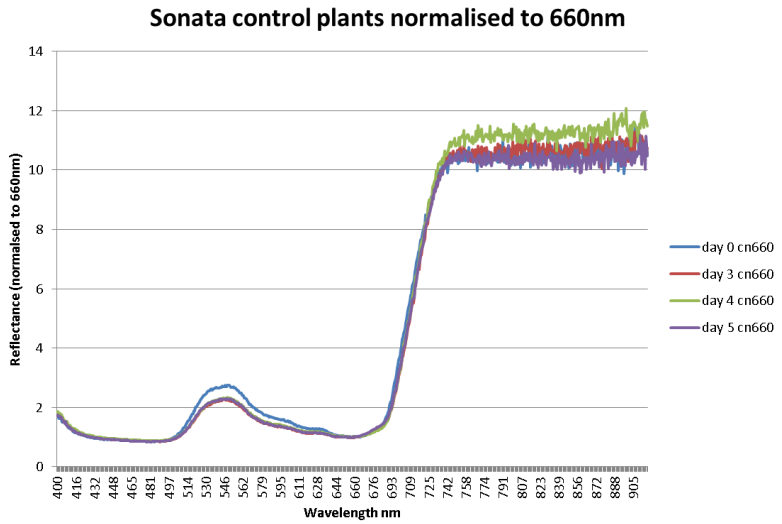

Figure 9. Sonata **control** plants normalised at one wavelength - 660nm.

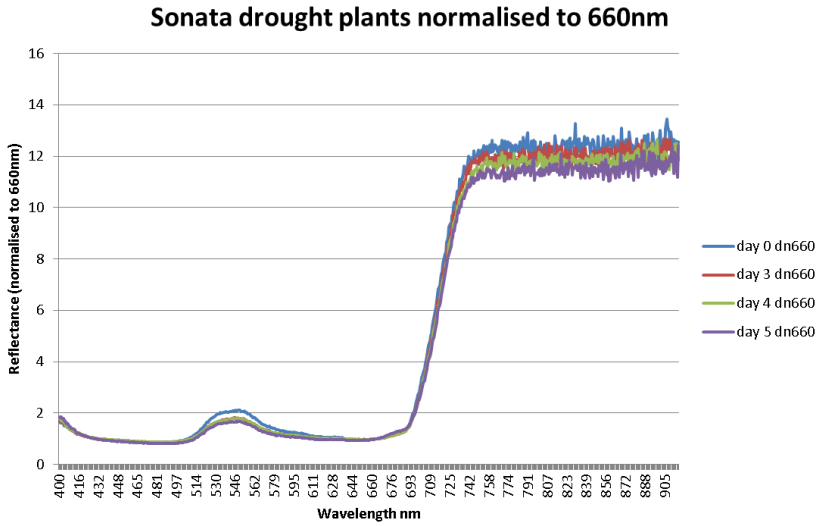

Figure 10. Sonata **drought** plants normalised to a reflectance level of 1 at 660nm. A smaller but consistent separation is still visible in the near infrared.

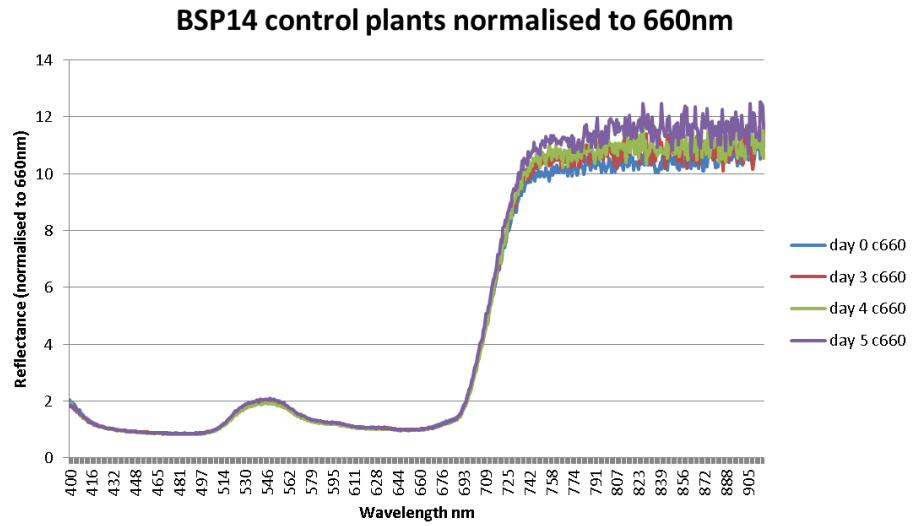

Figure 11. BSP14 **control** plants normalised against at 660nm. No clear change in response is visible over the timeseries.

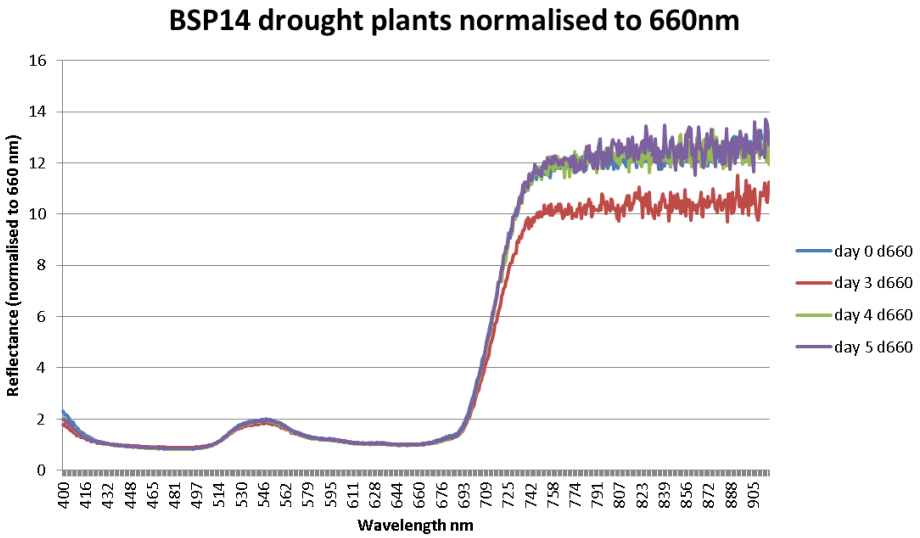

Figure 12. BSP14 **drought** plants normalised at 660nm.

Figure 10 still shows a consistent decrease in the near infrared, but there is a smaller effect. Figures 9, 11 and 12 appear to have no obvious, consistent effect. Day 3 in Figure 12, being in the middle of the time series, would appear to be an outlier.

A second normalisation process is to limit the reflectance values between a maximum and minimum reflectance value, in this case the range 0 and 1, (see Figures 13 – 16). In this case the maximum falls in the near-infrared section of the spectrum, which is noisy and provides poorer differentiation than using a 660nm reference point.

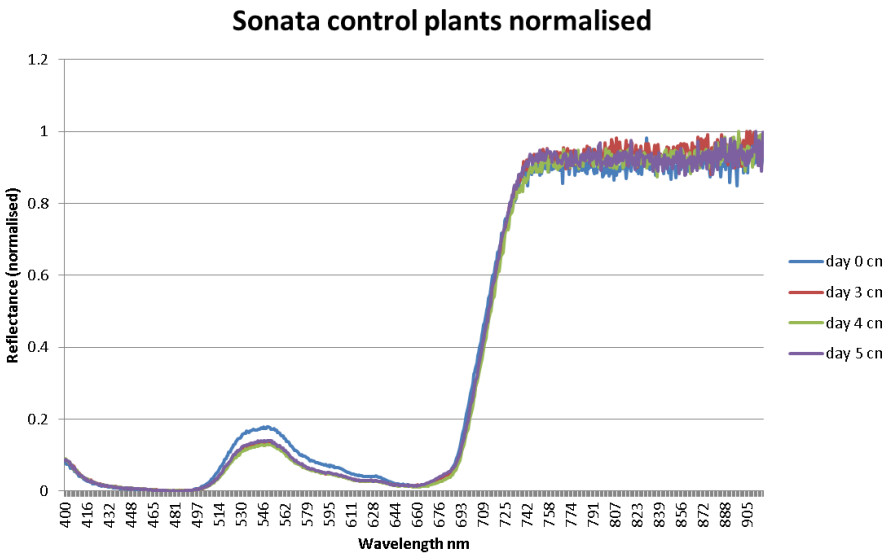

Figure 13. Sonata **control** plants normalised in the 0-1 range.

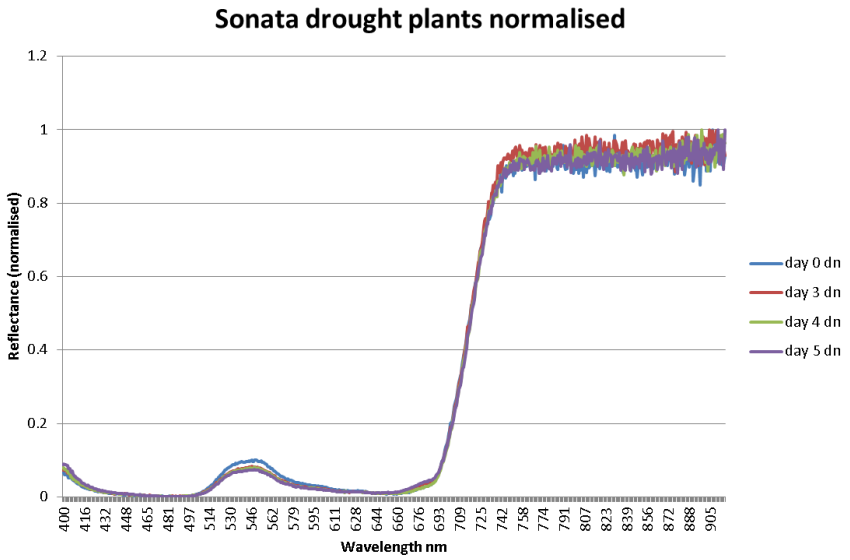

Figure 14. Sonata **drought** plants normalised in the 0-1 range.

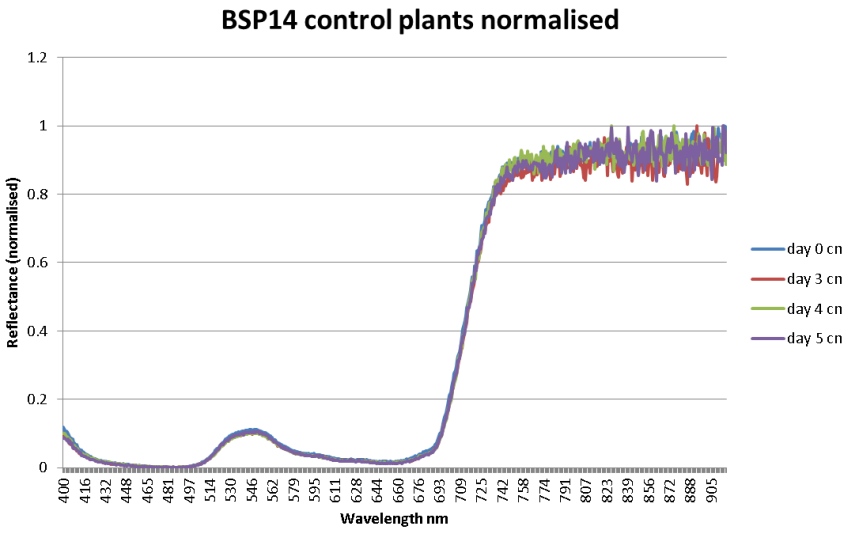

Figure 15. BSP14 control plants normalised in the 0-1 range.

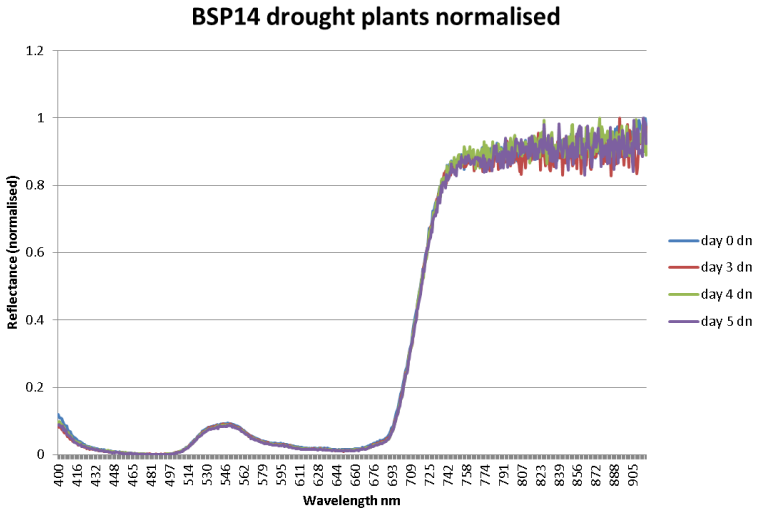

Figure 16. BSP14 drought plants normalised in the 0-1 range. The BSP14 plants have been normalised so the maximum and minimum wavelengths are 1 and 0 respectively.

Figures 4, 6 and 10 suggest that the leaf reflectance for Sonata decreases slightly in the near infrared range of the spectral signatures for the drought plants while the control plants remain almost the same, as was the case for the manual graphs. No clear difference was observed between BSP14 control and drought plants (e.g. Figures 7 & 8), but interestingly, normalising removes some of the previously unexplained variation for BSP14 (Figures 11 & 12).

While there appears to be a slight decrease in the Sonata drought plants this is insufficient to conclude that drought can be detectable on a specific day. Results reveal the challenge in interpreting the datasets when only small reflectance changes are visible in the spectrum.

Normalised Difference Vegetation Index - NDVI

To explore further, a vegetative index which has been used to detect drought, but over much longer time periods, was calculated from the data. NDVI uses two wavelengths from the red and near infrared ranges of the hyperspectral data to produce a ratio 1.0 and -1.0 (Rhew et al, 2011). NDVI has been calculated from the data shown in Figures 5, 6, 7 and 8 to detect changes in the vegetation index. Results in Tables 2 and 3 show that for Sonata there is a less than 0.01 decrease and that BSP14 varies unpredictably over the time series.

Table 2. Sonata drought and control plant data with NDVI.

NDVI	Day 0	Day 3	Day 4	Day 5
Sonata drought	0.8545	0.8534	0.8459	0.8456
Sonata control	0.8254	0.8314	0.8323	0.8229

Table 3. BSP14 drought and control plant data with NDVI.

NDVI	Day 0	Day 3	Day 4	Day 5
BSP14 drought	0.8450	0.8153	0.8464	0.8515
BSP14 control	0.8210	0.8229	0.8294	0.8407

NDVI analysis could not detect a difference during the limited period of observations. Most NDVI drought studies used remote sensing data over long time periods; two separate studies that compared NDVI noted that it took 13 to 16 days to show a difference in the plants (Römer et al, 2012, Behmann et al, 2014), so not finding a positive result here confirms that NDVI is insensitive to such early-stage drought effects.

Summary

Figure 6 indicates that Sonata leaf reflectance decreases slightly in the near infrared range for the droughted plants while the control plants remain almost the same, as with the manual graphs. No difference between BSP14 control and drought plants was observed (Figures 7 and 8). Normalising removes some of the variation for BSP14 (Figures 11, 12, 15, 16), which could indicate that the spread in reflectance is from a brightness change or other external factor, rather than colour change due to drought. Previous studies found that BSP14 is more resistant to drought than varieties NCC85-13V and Elvira, and other studies also describe BSP14 as drought resistant. (Zhang and Archbold, 1993, Johnson, A et al, 2014)

While there appears to be a slight decrease in the NIR reflectance for the Sonata drought plants, this is insufficient to conclude that drought can be detectable on a specific day. It is still challenging to identify any reliable difference despite measuring from the same leaves over the time series, and reducing the effects of leaf height and leaf angle changes.

The resulting reflectance profiles for the Sonata drought-condition plants showed visually similar results to that obtained by more laborious and error-prone manual methods. However, by visual inspection alone it was noted there was too much variability in the results to be able to derive reliable indicator wavelengths, although it was noted that most consistent differences were in the near infrared, followed by the green regions of the spectrum. If these effects are real, however, it may be possible to detect drought as early as 3 days after withdrawal of water (Figure 4). Despite controlled growth and imaging conditions, and a dedicated analysis pipeline, some variability was also seen in the control datasets. Normalisation was explored as a way to further remove artefacts from the data; however normalisation can itself introduce misleading interpretations.

Discussion

It is clear there are many challenges that arise with hyperspectral imaging. Leaf orientation relative to the camera plane can change hyperspectral profiles, limiting the ability of the technology to detect subtle changes caused by early symptoms of stress. Therefore the orientation of the leaves must be determined using 3D imaging. Leaf height also needs to be taken into consideration since illumination properties change with distance from the camera.

The hyperspectral data here was calibrated with a white balance target to account for the variance in lighting; however there was also a question of whether to normalise the data post-capture. There are many methods to normalise data but are they helpful for hyperspectral data analysis, and which one is the most appropriate? The choice of normalisation method can affect the interpretation of the results. This must be considered in future work. Care must be also taken when reducing a hyperspectral dataset to the specific wavelengths necessary to ensure optimal resolution of changes under examination.

Many factors must be considered with hyperspectral imaging and overcoming its challenges. Here, a complete, automated pipeline has been developed incorporating hyperspectral and 3D data. Results suggest a *possible* difference in reflectance for some varieties in early stages of drought, but no firm conclusion may be made at this stage because of the many variables inherent in hyperspectral imaging.

Milestones

The initial objectives for the project are as below. Comments on addressing the milestones follow each.

Milestone 1:

An automated hyperspectral image acquisition system will be designed and a prototype installed at East Mailing Research (EMR) in a glasshouse, to enable the capture of time series image data of multiple strawberry plants. This is potentially as simple as installing the available camera within the glasshouse at EMR at particular times, but could evolve into developing an automated, robotic imaging approach, to move the camera at set time points.

Two hyperspectral cameras were used, one at NIAB EMR and one at the University of Nottingham. Both were pushbroom-type cameras, meaning they scan over a sample, building up an image line by line. The EMR camera moves the sample during this scanning process; the Nottingham camera moves the camera unit itself. Images of both are presented in the sections above.

It was clear that due to the impractical nature of moving these heavy units by hand, and the precision required in the scanning process, imaging was only possible on the image rigs the cameras were built into. That said, future automation is certainly possible: the sample moving/camera unit moving was handled by robotic actuators, so each of these system could be used e.g. a glasshouse, and either moving plants under the camera system (such as on a conveyer belt), or passing the camera of a length of plants, given a suitable length actuator. The cost and engineering complexities of a full automation approach were beyond the scope of this project.

Milestone 2:

Registering the hyperspectral datasets in space and time. Aligning the data from the acquisition system is an important and non-trivial first step in the analysis of the data. Registration will ensure a particular plant can be monitored over time, by aligning the multiple camera images of the plant as well as possible, ensuring data captured at different times can be compared

Registering data during the project was a challenge, but rather than over time, the challenge evolved to registering 2D hyperspectral image data onto the surfaces of a 3D plant model. To take into account prevalent 3D effects such as lighting variation, shadowing, occlusion, and inclination angle of the leaf, it became desirable to register the hyperspectral data onto a 3D plant model in order to account for these effects.

Milestone 3:

a) Segmenting leaves and fruit. Segmentation is the process of identifying areas of the image which represent objects of interest: for example, picking out leaves from the background.

b) Once segmented, measures can be taken of the area of interest (in the case of leaves, this could be leaf size, colour measures (such as greenness, or more generally in the case of hyperspectral data, the spectral response across the leaf)

a) A novel segmentation approach was developed to segment (label) leaves in 2D hyperspectral images. This consisted of first approximately locating the centres of the leaves, then using the vein patterning on a leaf to place and orientate a leaf shape model. Then a Level Set approach was used to deform this template until it matched the leaf boundary.

b) Before a hyperspectral measure was taken, a 3D model of the plant was built using multi-view stereo to combine digital-SLR camera images collected around the plant into a 3D model. This 3D model revealed surfaces of leaves: where they sit in 3D space, and which direction they face was then registered with the hyperspectral data (see Milestone 3). From this, a quality metric was devised to determine a good quality leaf from which to take measurements (in fact, a set of three such leaves was automatically determined for each plant in the study). Hyperspectral reflectance measures could then be taken across a leaf surface area and recorded.

Milestone 4:

Identifying the most significant wavelengths as predictors or indicators of specific growth events, such as stress responses. Once segmentation and registration have been used to produce time series hyperspectral datasets of the crop growing and responding to environmental effects, data mining techniques can be used to identify statistically significant "key" data points, which can be used to best predict certain events (drought, disease onset, fruit ready for harvest etc.) Predictions can be compared to ground truth measures of the same factors, to assess the accuracy of the models.

The segmentation, registration and 3D reconstruction pipelines were used to take automated hyperspectral measures from suitable leaves in the drought experiments. The resulting reflectance profiles for the Sonata drought-condition plants showed visually similar results to that obtained by more laborious and error prone manual methods. However, by visual inspection alone it was noted there was too much variability in the results to be able to derive reliable indicator wavelengths, although it was noted that most notable differences were in the near infrared region, followed by the green region of the spectrum. If these effects are real, it may be possible to detect drought as early as 3 days after withdrawal of water (Figure 4). However, it is impossible to determine the exact nature of this effect. Despite controlled growth and imaging conditions, some variability was also seen in the control datasets.

Normalisation was explored as a way to further remove artefacts from the data; however normalisation can itself introduce misleading interpretations.

Conclusions

The final conclusion of the project can therefore be summarised as the points below.

- Differences in spectral response were observed between drought and control plants in an initial manual analysis. This effect was subtle, and challenges of hyperspectral imaging practices affecting reflectance were encountered. Therefore, a new pipeline was proposed to take 3D effects into account in the hyperspectral data.
- Leaf segmentation approaches were first developed to successfully identify and locate individual leaf boundaries within the hyperspectral datasets. This allowed individual leaf measurements to be taken automatically from hyperspectral data; we extended the approach further to also incorporate 3D plant structure information.
- Using the 3D model, these individually-segmented leaves (and their corresponding hyperspectral data) can be mapped into 3D space, and their 3D location and orientation properties used to select a set of “good quality” leaves from which to derive hyperspectral measures from the dataset. Leaves which were well lit near the centre of the plant, facing the camera, and on the upper layer of the plant canopy (i.e. not occluded by other leaves or in shadow) were automatically selected.
- Measurements were retaken from the experimental datasets using the above pipeline.
    - The pipeline was found to produce similar measurements to the initial manual approach, demonstrating that it is possible to automate the time-consuming manual inspection process of hyperspectral data.
    - The apparent differences between drought and control plants for Sonata variety were still apparent, but the effect still subtle (Figures 5 and 6). Some variation was also observed in the control group (Figure 5), though not as pronounced as the drought group.
    - Data normalisation approaches were investigated; data interpretation was sensitive to choice of approach and caution is advised when selecting methods.
- Finally, a small difference was observed in the spectral response curves processed using the developed pipeline between drought and control conditions for one particular drought sensitive variety (Sonata). The difference was apparent as early as 3 days after the withdrawal of water, before obvious visual signs appeared. However, despite being

consistent with results from literature examining drought-induced reflectance changes over longer time periods, due to the complexities and challenges of the hyperspectral datasets, we do not feel able to draw firm conclusions about the interpretation of the data. This is in spite of strict experimental conditions, lab-based imaging of the plants, careful calibration, and dedicated analysis development.

- In summary, when working with hyperspectral data, extreme care must be taken to 1) take the best quality source images, calibrating for lighting effects 2) and ideally 3D information regarding the subject should be taken into account, or, if possible the sample should be flattened to present a 2D target to simplify interpretation of the data; 3) care must be taken when post processing (e.g. normalising) hyperspectral data as this can affect interpretation.

Knowledge and Technology Transfer

- Hyperspectral imaging with strawberry plants - Mapping hyperspectral data onto a 3D plant model - *Image Analysis Methods for the Plant Sciences Workshop, Nottingham, UK, 2018* - Presentation/Poster
- Journal literature review - Lowe, A., Harrison, N., & French, A. P. (2017). Hyperspectral image analysis techniques for the detection and classification of the early onset of plant disease and stress. *Plant methods*, *13*(1), 80.
- HyperPatches - a pipeline for reconstructing 3D plant models with registered hyperspectral data - *ICCV conference workshop on CVPPP, Venice, Italy, 2017* - poster
- Meaningful region segmentation using hyperspectral imaging - *Image Analysis Methods for the Plant Sciences Workshop, Angers, France, 2016* - Presentation/Poster
- Study visit to EMR May-July 2015 to collect data and transfer strawberry plants for a repeat drought experiment in Nottingham.
- EMR/AHDB Horticulture Soft Fruit Day, 22 July 2015 – Discussing the project at a stand.
- AHDB Horticulture conference 16-17th September 2015, poster session, tour and annual review.
- International workshop on Image Analysis Methods for the Plant Sciences in Belgium 20-21st September 2015, presentation.
- Study visit to EMR October 2015 to collect data and transfer strawberry plants for the drought experiment. However the strawberry plants on site were quarantined.

- Biotechnology YES competition with a group of PhD students from EMR. Not directly related to the project but it involved networking and improved presenting skills. Workshop - November 11-13th and final 10th December 2015.
- Study visit to EMR June-July 2014 to collect data and transfer strawberry plants for the drought experiment.
- Visit to Hugh Lowe Farms, June 2014, with Marion Regan, industry representative for this studentship.
- HDC studentship conference, York, 16-17th September 2014, poster session and tour.
- Poster presented at the International Workshop on Image Analysis Methods for the Plant Sciences (IAMPS, Aberystwyth, September 2014)
- EMRA/HDC Soft Fruit Day 26th November 2014 at EMR. Dissemination of progress in this studentship through a poster exhibition to both industry and academia.

Glossary

Endmember – An endmember refers to a material such as plant, background, soil etc.

Endmember signature – the material's reflected light over a selected range of the spectrum, for this project the range is from visible light to near-infrared light.

Hypercube – All of the hyperspectral bands collected together to represent a 3D image where the first two dimensions are the pixel location and the third dimension is the hyperspectral information (wavelength bands over the spectrum).

Hyperspectral – narrow contiguous bands of light reflectance responses recorded over a spectrum

References

Behmann, J., Steinrücken, J., & Plümer, L. (2014). Detection of early plant stress responses in hyperspectral images. *ISPRS Journal of Photogrammetry and Remote Sensing*, *93*, 98-111.

Furukawa, Y., & Ponce, J. (2010). Accurate, dense, and robust multiview stereopsis. *IEEE transactions on pattern analysis and machine intelligence*, *32*(8), 1362-1376.

Johnson, A. W., Davies, M. J., Grant, O. M., & Simpson, D. W. (2014). Developing strawberry cultivars with improved water use efficiency. *Acta Hortic*, *1049*, 853-858.

Manivasagam, V. S., & Nagarajan, R. (2015). Water stress assessment in maize crop using field hyperspectral data.

Mishra, P., Asaari, M. S. M., Herrero-Langreo, A., Lohumi, S., Diezma, B., & Scheunders, P. (2017). Close range hyperspectral imaging of plants: A review. *Biosystems Engineering*, *164*, 49-67.

Pound, M. P., French, A. P., Murchie, E. H., & Pridmore, T. P. (2014, September). Surface reconstruction of plant shoots from multiple views. In *European Conference on Computer Vision* (pp. 158-173). Springer, Cham.

Rhew, I. C., Vander Stoep, A., Kearney, A., Smith, N. L., & Dunbar, M. D. (2011). Validation of the normalized difference vegetation index as a measure of neighborhood greenness. *Annals of epidemiology*, *21*(12), 946-952.

Römer, C., Wahabzada, M., Ballvora, A., Pinto, F., Rossini, M., Panigada, C., & Kersting, K. (2012). Early drought stress detection in cereals: simplex volume maximisation for hyperspectral image analysis. *Functional Plant Biology*, *39*(11), 878-890.

Roscher, R., Behmann, J., Mahlein, A. K., Dupuis, J., Kuhlmann, H., & Plümer, L. (2016). Detection of disease symptoms on hyperspectral 3D plant models. *ISPRS Annals of the Photogrammetry, Remote Sensing and Spatial Information Sciences*, *3*, 89.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ... & Tinevez, J. Y. (2012). Fiji: an open-source platform for biological-image analysis. *Nature methods*, *9*(7), 676.

Wu, C. (2011). VisualSFM: A visual structure from motion system.

Zhang, B., & Archbold, D. D. (1993). Water relations of a Fragaria chiloensis and a F. virginiana selection during and after water deficit stress. *Journal of the American Society for Horticultural Science*, *118*(2), 274-279.

Appendices

Appendix A Protocol – Strawberry Plants - adapted for the Mildew experiment

Plant Material Preparation

Dormant strawberry plants were purchased from strawberry propagators and kept in a cold store at 2°C until required. The dormant strawberry plants were taken out of the cold store the night before potting and left at +2 °C to ensure plants were not damaged due to a sudden change in temperature. The following day, the strawberry plants were potted into FP9 pots using standard compost and grown for 2-3 weeks until they have 2-3 leaves.

When plants have 2-3 leaves, a thorough inspection of the leaves was undertaken and any marks/signs on the leaves of any other diseases/pests were noted. A dissecting microscope is used to check the leaves on 10% of the plants at random to ensure they were pest-free.

Equipment:

- FP9 pots
- Standard compost
- Cold store plants
- Glasshouse for the plants to grow in

- Number the plants for the experiment (1 – 5 Elsanta, 11-odd number-23 Fenella) for the control and (6 – 10 Elsanta and 12 – even number – 24 Fenella) for the infected plants.

Location for the plants during the experiment

The experimental strawberry plants were grown in CE cabinets at NIAB EMR. The CE cabinets were cleaned thoroughly with 70% ethanol the day before the experiment to make sure no disease/pests were present that would infect the control/infected plants during the experiment. After the experiment was finished, the cabinets were cleaned again using 70% ethanol.

Mildew Infection process

Two upside-down plant saucers were placed into the CE cabinet ready for the large mildew infected plants to be placed upon. Previously infected mildew plants were transferred from the glasshouse to the CE cabinets. The plants were transferred in a completely covered box to prevent spreading of mildew spores to any nearby strawberry plants. The two large mildew plants were placed into the middle of cabinet but evenly spaced. The test strawberry plants, cv. 'Elsanta' and 'Fenella' were placed in a circle around the two plants to evenly expose them to the mildew spores.

Equipment:

- Mildew infected strawberry plants (x2)
- Box with lid and box
- 25 plants (11 Elsanta plants and 14 Fenella plants)
  (7 Fenella plants and 5 Elsanta plants will be placed in Cabinet 6 as control plants. 7 Fenella plants and 6 Elsanta plants will be placed in Cabinet 7 with the two mildew plants.)

Imaging Process

The strawberry plants were imaged before they were placed into the cabinets (day 0). They were then imaged every other day until there were visible signs on all infected plants. To transport the plants from the cabinets for imaging, a box with a lid was used to contain the mildew spores. Water the plants every other day. Also after each imaging session the plants are placed back into random positions.

Imaging method

- Clean the area around the camera with 70% ethanol
- Turn on the three switches at the plugs and turn the PC on.
- Open the HSI software and select 'start pipeline'
- Switch the lights on at the voltage box, bottom left and then bottom right.
- Move the board using the arrow keys on the screen if it is needed.

- Turn the main light off.
- Place the white balance on the board.
- Select the white balance calibration button
- Cover lens with hand and select dark (white) calibration button
- (make sure the two steps above have the same exposure rate, ie 18)
- Cover lens with hand and select dark (object) calibration button
- Now check the box that says ‘toggle calibration settings’
- Place the plant on the board under the lens.
- Make sure the lens is in focus due to the different plant heights.
- Move the board to the desired start position using the arrow keys on the screen
- Select the start position
- Move the board to the desired end position using the arrow keys on the screen.
- Select the end position.

(Repeat the steps below for each plant)

- Take a digital image of the plant
- Place the plant on the board
- Click the right arrow key on screen to take the board to the start position
- Select the translation box
- Select automatic speed button
- (select right arrow key to let the plants speed be tested, the image on the screen needs to match on the left and right sides)
- Change the number of the speed slightly and repeat the last three steps until the image matches on the screen.
- Select the right arrow key on the screen.
- Name the file a relevant name ie. ‘Day0_Elsanta1_Cal’
- Select frame arrow right key on screen to finally capture the images.

Equipment:

- Camera
- Lighting
- Frame
- Board
- Software – HSI
- PC
- Plants
- Ethanol 70% and paper towels
- Box (to carry the plants in)

<u>Data Collection and Recording</u>

For each experiment and imaging session, the following information was recorded:

- plant variety, number, date, time and time after infection
- Position of the plant during imaging
- Speed, start and stop position, lighting, exposure rate and frame rate
- Any visible marks/signs (including before infection to discount it)
- General health

Appendix B Protocol – Strawberry Plants - adapted for the Spidermite-1 experiment

Plant Material Preparation

Dormant strawberry plants were purchased from strawberry propagators and kept in a cold store at 2°C until required. The dormant strawberry plants were taken out of the cold store the night before potting and left at +2 °C to ensure plants were not damaged due to a sudden change in temperature. The following day, the strawberry plants were potted into FP9 pots using standard compost and grown for 2-3 weeks until they have 2-3 leaves.

When plants have 2-3 leaves, a thorough inspection of the leaves was undertaken and any marks/signs on the leaves of any other diseases/pests were noted. A dissecting microscope is used to check the leaves on 10% of the plants at random to ensure they were pest-free.

Equipment:

- FP9 pots
- Standard compost
- Cold store plants
- Glasshouse for the plants to grow in
- Number the plants for the experiment 1- 10 for the control and 11 – 20 for the inoculated.

Location for the plants during the experiment

The plants were kept in a contained glasshouse that limits exposure of spider mites. The area was cleaned before and after the experiment using 70% ethanol.

Spider mite preparation

Use spider mite infected plant. Remove and cut the leaf into sections using a microscope and put the sections into a sealed container. Do this on the day that the plants will be infected.

Set up the area in the glasshouse with a tray filled with water and place 10 saucers upside down onto the tray then put the plants on these saucers. This creates a barrier and keeps the spider mites location as controlled as possible.

Infection process

Place an infected leaf section on the bottom of the stem of each plant. This will allow an even distribution and also a natural infection compared to placing the infected leaf on the top of a leaf.

Equipment:

- Spider mite leaf sections (x10)
- 10 plants (5 per variety) that will be infected.
- 2 (or 3 if possible) varieties.

Imaging Process

Image before the plants are placed into the glasshouse (day 0). Then image every day (10am-2pm) until there are visible signs on all/most of the plants. (8/10).

Imaging method

- Clean the area around the camera with 70% ethanol
- Turn on the three switches at the plugs and turn the PC on.
- Open the HSI software and select ‘start pipeline’
- Switch the lights on at the voltage box, bottom left and then bottom right.
- Move the board using the arrow keys on the screen if it is needed.
- Turn the main light off.
- Place the white balance on the board.
- Select the white balance calibration button
- Cover lens with hand and select dark (white) calibration button
- (make sure the two steps above have the same exposure rate, ie 18)
- Cover lens with hand and select dark (object) calibration button
- Now check the box that says ‘toggle calibration settings’
- Place the plant on the board under the lens.
- Change the focus on the lens.
- Move the board to the desired start position using the arrow keys on the screen
- Select the start position
- Move the board to the desired end position using the arrow keys on the screen.
- Select the end position.

(Repeat the steps below for each plant)

- Take a digital image of the plant
- Place the plant on the board
- Click the right arrow key on screen to take the board to the start position
- Select the translation box
- Select automatic speed button
- (select right arrow key to let the plants speed be tested, the image on the screen needs to match on the left and right sides)
- Change the number of the speed slightly and repeat the last three steps until the image matches on the screen.
- Select the right arrow key on the screen.
- Name the file a relevant name ie. ‘Day0_Elsanta1_Cal’
- Select frame arrow right key on screen to finally capture the images.

Equipment:

- Camera
- Lighting
- Frame
- Board
- Software – HSI
- PC
- Plants
- Ethanol 70% and paper towels
- Box (to carry the plants in)

Recording data

During imaging write down

- plant variety, number, date, time and time after infection
- Position of the plant during imaging
- Speed, start and stop position, lighting, exposure rate and frame rate
- Any visible marks/signs (including before infection to discount it)
- General health
- Grading 1-4 where 1 is no signs, 2 is slight marks or discoloration, 3 is a few light green speckles and 4 is obvious light green speckles.

Appendix C Protocol – Strawberry Plants - adapted for drought experiment

Plant Material Preparation

Dormant strawberry plants were purchased from strawberry propagators and kept in a cold store at 2°C until required. The dormant strawberry plants were taken out of the cold store the night before potting and left at +2 °C to ensure plants were not damaged due to a sudden change in temperature. The following day, the strawberry plants were potted into FP9 pots using standard compost and grown for 2-3 weeks until they have 2-3 leaves.

When plants have 2-3 leaves, a thorough inspection of the leaves was undertaken and any marks/signs on the leaves of any other diseases/pests were noted. A dissecting microscope is used to check the leaves on 10% of the plants at random to ensure they were pest-free.

Equipment:

- FP9 pots
- Standard compost
- Cold store plants
- Glasshouse for the plants to grow in
- Number the plants for the experiment 1- 10 for the control and 11 – 20 for the inoculated.

Location for the plants during the experiment

The plants were kept in a heated glasshouse.

Drought preparation

Before starting the drought experiment make sure the plants are controlled with the watering.

Drought process

Stop watering the drought plants on day zero.

Equipment:

- X plants (minimum of 3 per variety that will be infected but aim for more).
- 2 (or 3 if possible) varieties.

Imaging Process

Image the plants on day 0. Then image every day (10am-2pm) until there are visible signs on all/most of the plants. (8/10).

Imaging method

- Clean the area around the camera with 70% ethanol
- Turn on the three switches at the plugs and turn the PC on.
- Open the HSI software and select ‘start pipeline’
- Switch the lights on at the voltage box, bottom left and then bottom right.
- Move the board using the arrow keys on the screen if it is needed.
- Turn the main light off.
- Place the white balance on the board.
- Select the white balance calibration button
- Cover lens with hand and select dark (white) calibration button
- (make sure the two steps above have the same exposure rate, ie 18)
- Cover lens with hand and select dark (object) calibration button
- Now check the box that says ‘toggle calibration settings’
- Place the plant on the board under the lens.
- Change the focus on the lens.
- Move the board to the desired start position using the arrow keys on the screen
- Select the start position
- Move the board to the desired end position using the arrow keys on the screen.
- Select the end position.

(Repeat the steps below for each plant)

- Take a digital image of the plant
- Place the plant on the board
- Click the right arrow key on screen to take the board to the start position
- Select the translation box
- Select automatic speed button
- (select right arrow key to let the plants speed be tested, the image on the screen needs to match on the left and right sides)
- Change the number of the speed slightly and repeat the last three steps until the image matches on the screen.
- Select the right arrow key on the screen.
- Name the file a relevant name ie. ‘Day0_Elsanta1_Cal’
- Select frame arrow right key on screen to finally capture the images.

Equipment:

- Camera
- Lighting
- Frame
- Board
- Software – HSI
- PC
- Plants
- Box (to carry the plants in)

Recording data

During imaging write down

-plant variety, number, date, time and time after infection

-Position of the plant during imaging

- Speed, start and stop position, lighting, exposure rate and frame rate

- Any visible marks/signs (including before infection to discount it)

- General health

