Project title: Early detection of stress in strawberry plants using hyperspectral image analysis

Project number: SF 144

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Report: Annual report, May 2016

Previous report: Annual report, April 2015

Key staff:

Location of project: University of Nottingham & NIAB EMR

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Date project commenced: 12 May 2014

Date project completed 30 April 2017
(or expected completion date):

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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.

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GROWER SUMMARY

Headline

- Progress is being made to develop the use of hyperspectral imaging to identify early symptoms of strawberry plant stress before it is visible to the human eye.

Background and expected deliverables

The aim of this research project is to automatically detect symptoms of stress (such as onset of disease or drought) of strawberry plants at an early stage, before symptoms are visible to the human eye. The intention is to detect indicators of stress in strawberry plant physiology using hyperspectral image analysis, a non-invasive technique. Finding early indicators of stress will aid crop management through timely intervention with appropriate treatments in order to reduce potential losses to the quantity and quality of the strawberry crop. To do this, novel image analysis methods will be developed to aid data measurement from the images, and a suitable analysis method will be identified to enable use of hyperspectral data as a predictor.

The images will be collected using hyperspectral cameras, and will include both 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). The plants will be imaged at NIAB East Malling Research (NIAB EMR) and The University of Nottingham.

Once the images have been captured, the strawberry plants need to be identified in the images using a technique known as 'segmentation'. This means labelling objects (leaves, in this case) in the image by finding similar properties such as colour, shape or texture. Once the leaves have been located in the images, the hyperspectral information can then be extracted and analysed over time.

Summary of the project and main conclusions

In the past year, work has been carried out through the collection of experimental datasets, and the development of image segmentation techniques. The data collected during the summer of 2015 involved two-spotted spider mite, powdery mildew and two drought sessions. Four varieties were imaged daily (spider mite, drought) or on alternating days (in the case of mildew) until visible signs appeared.

The challenge for the image analysis is selecting areas of the images from which to use the hyperspectral data, to maximise its quality. A novel segmentation approach based on a popular 'Level Set' technique is being developed to segment the leaves. Level sets are a

mathematical way of representing object boundaries in images. In combination with the level sets approach, a shape constraint has been introduced because the level sets alone could not reliably identify overlapping leaves. Also by using the shape constraint the aim is that angled leaves will not fit the shape criteria and not be selected for inclusion in the data analysis, as leaves facing away from the camera will likely affect final data quality. It is anticipated that this combination of approaches will yield high quality data for analysis via the reduction of noise in the dataset.

3D reconstruction has also been researched to find the depth of the leaves and possibly model their orientation; however this technique is still ongoing. The next 3-6 months will involve finishing the segmentation process to improve the accuracy and also to analyse the time series data with different predictive techniques (VI, SiVM, machine learning).

Financial benefits

- For this interim report it is not appropriate to undertake a cost/benefit analysis.

Action points for growers

- There are no action points for growers at this stage of the project.

SCIENCE SECTION

Introduction

The main focus of this studentship over the past twelve months was to collect datasets consisting of hyperspectral images from strawberry plants exposed to different biotic and abiotic stresses, up to the point of visible symptoms. These datasets were then used to develop segmentation techniques to locate the leaves.

Materials and methods

There were three planned experiments during a visit to NIAB EMR in May-July 2015. The experiments were set up to capture strawberry responses to the two-spotted spider mites (*Tetranychus urticae*), drought and powdery mildew (*Podosphaera aphanis*). These were selected because they are common problems that induce stress in strawberry plants. There are many diseases and pests of strawberry plants, but mildew and spider mites are very interesting for this work due to both the time they take to show symptoms and to the different physical effects that they have on the plant. Powdery mildew can take 14 days to see visible signs, providing a two week timeline for dataset accumulation. Drought can take several days before visible signs appear after water is withheld; limited watering will take longer. Spider mite infection time depends on the number of mites and eggs present on the plant; once critical numbers of mites are present, damage can occur very rapidly. Unlike thrips and aphids, which are visible by looking at the leaves, the mites are very hard to detect and see before visible deterioration in the plant is detected, hence the motivation for hyperspectral analysis.

Below is a description of the imaging process and datasets gathered in the last year. This is followed by an update on the subsequent analysis development.

Protocol for Imaging of strawberry plants using a hyperspectral camera

A protocol was developed at NIAB EMR to image strawberry plants with a hyperspectral imaging camera. This protocol ensures appropriate procedures and precautions are taken, including how to store the plants, how to inoculate or infect them and how to contain the disease/pest while imaging. The mildew and drought protocols are included in the document below while the spider mite protocol is in the Appendix.

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)

Data Collection and Recording

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)
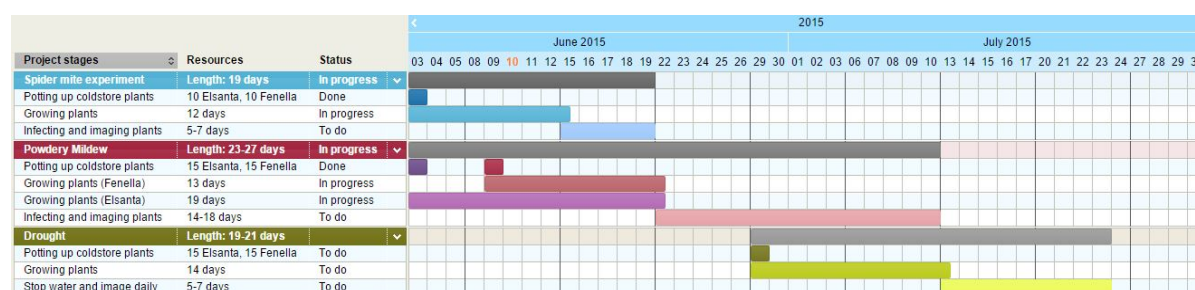
- General health

Figure 1. Gantt chart showing timeline for three of the four imaging sessions/experiments

Dataset 1 – Spider mite damage Experiment – 15/06/15 – 16/06/15

See protocol in Appendices

The data was collected until there were visible signs of damage on the plants. Signs of spider mite damage are white/light green speckles usually near the middle of the leaf, webbing on the leaf or underside and black dots or spots under the leaf, see figure 2.

Figure 2. Examples of spider mites and spider mite damage to bean leaves. From left to right- light green speckles, black dots on the underside, spider mites, eggs on the leaf.

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This first dataset was a time series over a 24 hour period. Ten Fenella (five control and five infected) and ten Elsanta (five control and five infected) plants were used. Before they were infected, the plants were imaged for a control dataset, and then transported to a glasshouse containing spider mite infected plants. To contain the spider mites on each plant, the plants were placed on an overturned plant saucer and these were placed on a tray filled with water (Figure 3). Spider mites do not like the water and a moat kept the spider mites contained on the plants.

Figure 3. Experimental set-up for the spider mite infection experiment.
Left: spider mite set up. Right: Control plants.

Spider mite infection involved taking a leaf from a previously infected host plant and placing it at the base of the stem of the plant to be inoculated. Using this method enables the spider mites to crawl onto the leaves naturally rather than being placed on the leaf (Figure 4).

Figure 4. Left: Spider mite crawling up the stem. Right: the leaf placement for infection

The leaves used as inoculum had many of spider mites (50+), therefore by the next day there were already visible signs of infection (Appendix Figure A). The plants were imaged and then the data collection ended, as after visible signs are present no more useful information can be gained. The aim of this experiment was to have a slow progression for a time series dataset over several days; however, within 24 hours visible signs were already present.

Dataset 2 – Mildew infection experiment 22/06/15 – 08/07/15

Mildew can typically take 14 days from infection before visible signs appear. The signs of mildew are curling of the leaves, white spores on the underside of the curled leaves and small dark patches start to form on the underside. Figure 6, bottom right panel.

The plants used were 14 Fenella (7 control and 7 infected) and 11 Elsanta (5 control and 6 infected). The sixth plant in the Elsanta set was used for destructive sampling by removing leaves for imaging.

Due to the length of the experiment, the plan was to image every other day at the same time each day. This started with day 0 (before infection). The plants would be placed into controlled environment cabinets to try and control as many conditions as possible (Figure 5). After imaging, the plants would be placed back in the Mildew cabinet at random positions to prevent bias in the data. The two Mildew infected host plants are raised above the experimental plants for imaging to enable the mildew spores to naturally drop/float onto them to provide suitable inoculum for good infection (Figure 5).

Daytime temperature was set at 19°C and 17°C at night, with humidity set at 70%. The day/night cycle was 15hrs day/9hrs night. Plants were watered every other day.

Figure 5. Experimental cabinet setup. Left: mildew-infected plants. Right: control plants.

Technical problems during the data collecting of mildew -1

During day 9 adverse weather conditions affected the experiment. The temperature in Cabinet 6 rose from 19°C to 28°C within one hour, so the plants were moved into another cabinet. This also proved to be defective, which resulted in the humidity rising to 95% instead of 70%. After 24 hours Cabinet 6 reverted to 19°C and the plants were returned to it. The data

for the control plants during day 8-10 therefore may have been compromised due to the poorly-controlled conditions.

The rest of the data collecting went as planned and the imaging was completed on day 16.

Dataset 3 – Drought 1 Experiment – 13/07/15 – 23/07/15

Twenty one strawberry plants cv. 'Fenella', were placed into a heated glasshouse (Figure 6). The experiment contained 7 control plants, 7 drought plants and 7 over-watered plants. The plants were imaged daily until day 4 when the drought plants showed drooping/weakness by this point.

Figure 6. Layout for the drought-2 dataset.

**Dataset 4 – Drought 2 Experiment – 13/08/15 – 25/08/15**

At Nottingham, a second drought experiment took place using the cultivars 'Hawaii'(12), 'BSP-14'(12), 'Malling Centenary'(8) and 'Sonata'(6) during two weeks in August. The plants were evenly divided into control and drought plants and they were imaged daily for 5-7 days, depending on the plant variety and its sensitivity to drought. The data was collected until visible signs of stress appeared on all of the plants, therefore some plants were imaged for longer. Sonata was imaged from day 0 to day 6, there was also a day 9 collection where visible signs were present, and two plants (one control and one drought) were imaged on day 10. BSP-14 was imaged from day 0 to day 6. Malling Centenary was imaged from day 0 to day 4. Hawaii was imaged from day 0 to day 5.

The aim of this experiment was to develop the analytical methods using the comprehensive drought-1 and drought-2 datasets, with the intention to subsequently assess how well the approach generalised to the mildew-1 dataset.

Dataset 5 – 3D reconstruction from the drought dataset

The objective of this dataset is to see if extra spatial information helps with the plant leaf segmentation and also to determine the angle of the leaves because the leaves ideally need to be orthogonal to the camera. Usually when hyperspectral data is collected the object to be imaged is placed on a flat surface; however the plants are free-standing when they are imaged which means the leaves are at different orientations, and some need to be eliminated from the data before it is analysed since shadows and illumination variation can affect the spectral signatures (Ibrahim, 2014).

By collecting two images side by side, information such as a distance from the camera can be derived, and a 3D model can be produced through stereo imaging in a similar way to how humans perceive depth due to having two offset eyes. During data collection, some plants were imaged twice, but moved 30 cm to the right for the second image. An alternative approach is take different views of the plant, not just move the camera. Figure 7 shows an example where five images from different angles were selected (two are displayed) to create the 3D reconstruction using VisualSFM (Changchang Wu, 2011).
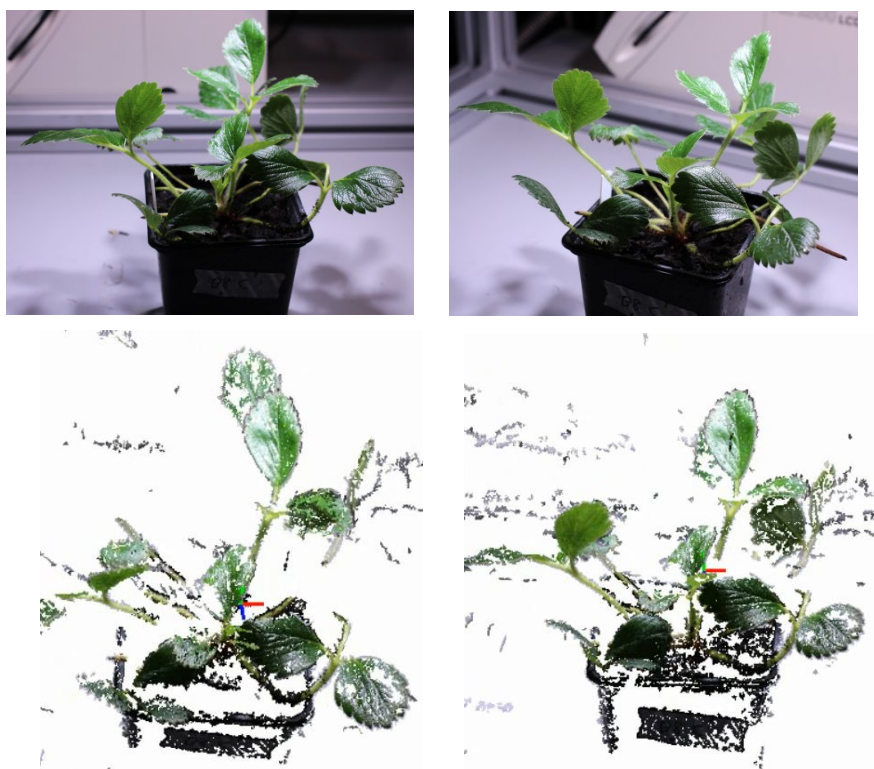
Figure 7. Above: images of BSP-14 plant from different angles.
Below: reconstructed plant at different angles (using VisualSFM).
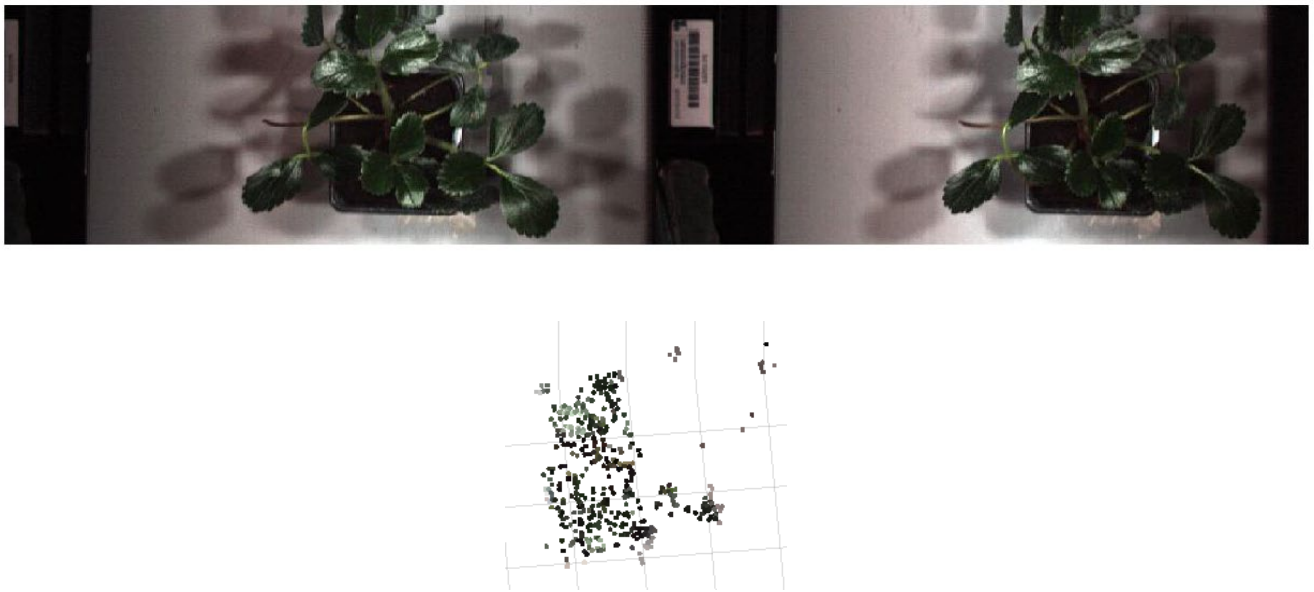
Figure 8. Above: stereo pair of RGB images taken from the hyperspectral data. Below: Point cloud created using SFM in Matlab.

The point cloud (Figure 8) is part of 3D reconstruction showing a set of points in a 3D space, represented by a pixel location on the x, y and z axes. Points are derived from comparing and extracting features from the pair of images. Not all of the pixels are in the point cloud because they could not all be matched and located from the pair of images. If more images are available, more information could be extracted - for example Figure 7 uses 5 images from different angles around the plant to construct the image.

Image Analysis Techniques - Developing techniques for image segmentation

The technique of non-negative matrix factorisation was introduced in this project's 2015 report. This process was found to work well for separating the plant from the background, and to distinguish between correctly-oriented and overturned leaves, but was unable to locate individual leaves because the pixels within the leaves share a very similar spectral signature. The technique also used the spectral signatures alone as a way to segment the materials, yet this technique does not make use of geometric image information such as the boundary of the leaves. Isolating individual leaves is necessary because to record accurate data they need to be as flat as possible and orthogonal to the camera, so the objective is to detect the best available leaves for imaging, in terms of how flat they are, and in which direction they are oriented.

There are many approaches that can be used for segmenting objects, from low level techniques such as edge detectors to more complex techniques. This research project will

investigate two complementary approaches, level sets to find the individual leaves and 3D reconstruction for depth information.

Level sets

Level set methods (LSM) are a framework for numerical analysis of surfaces and shapes which enable numerical computations involving curves and surfaces. Segmentation is an important technique used in image processing to identify the objects in the image.

There are different implementations of level sets. One version is the CV (Chanvese) model by Chan and Vese, Equation 1:

$$E(c1, c2, \emptyset) = \int (u - c1)^2 \, H(\emptyset) + (u - c2)^2 (1 - H(\emptyset)) dx \qquad (1)$$

Where c1 is the area inside and c2 is the area outside, $\emptyset$ is the level set function and H(x) is the Heaviside function where $x \geq 0 \ = 1$ and $x < 0 \ = 0$. Equation 1 is minimised to segment the image into two regions. (Chan & Zhu, 2005). Chanvese was used here because this model is adapted to stop at weak edges and also evolve over noisy data. The individual leaves have weak edges compared to the edges of the plant and background. Also hyperspectral data is typically noisy.

Figure 9 shows an example level set segmentation, and the resulting signed distance function. The panel on the right shows the final level set, where the orange section is positive, the green/blue is negative and the section between the orange and green is the final contour of the star object, which is at the zero-point.
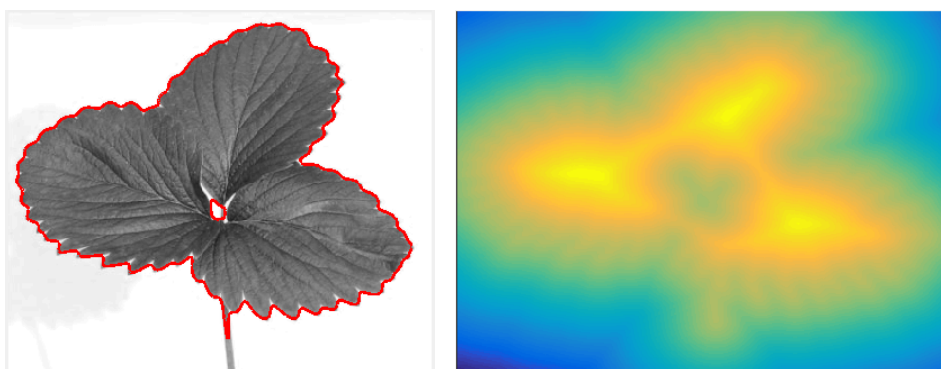

Figure 9 – Left: Leaf image. Right: The Signed distance function graph, plotted as a heat map; yellow/orange represents positive regions, green/blue negative

Results

Dataset 1 – Spider mite

The spider mite data collection was cut short. Also there were thrips on the leaf and a few more plants after checking the other 9 plants. This is a problem because thrips prey on spider mites (Maas, J. L. 1998). (Appendix Figure B).

The image collection schedule is shown in Table 1. Elsanta had more visible signs than Fenella.

Table 1: Hyperspectral data collection for the spider mite experiment.

	Plants	Time	Images	Location	dates
Spider mite	10 Elsanta 10 Fenella	24 hours, imaged daily	40 (20 x 2 days)	EMR - glasshouse	15-16th June

Dataset 2 – Mildew

Data collection for the mildew infection lasted 16 days. One of the 13 infected plants that were imaged over the 16 day period is below to show the changes during this period (Figure 10). Fenella 34 has a slow progression of leaf curling before finally seeing the spores on the undersides that have curled. All 13 plants showed signs of stress by day 16.

Figure 10: Upper left: Day 0, centre: Day 4, right: Day 8, Lower left: Day 10, right: Day 14,

The data collected consisted of 25 images every two days (image per plant) plus the white balance. Each image in RAW format is ~1.2GB.

Table 2: Hyperspectral data collection for the mildew experiment.

	Plants	Time	Images	Location	Dates
Mildew	13 Elsanta 12 Fenella	16 days – imaged every two days	225 (25 x 9 days)	EMR - Cabinets	22nd June – 8th July

Dataset 3 – Drought-1

The soil was very damp due to over watering so the experiment was delayed for a few days and Sunday 19th became day 0. The data collected consisted of 21 images per day over a five day period.

Table 3: Hyperspectral data collection for the Drought-1 experiment.

	Plants	Time	Images	Location	dates
Drought-1	21 Fenella	5 days – imaged daily	105 (21 x 5 days)	EMR - glasshouse	13th – 23rd July (Imaged 19th-23rd)

Dataset 4 – Drought-2

There were four varieties imaged two varieties at a time over a 13 day period and the plants took 5-7 days to display early signs.

Table 4: Hyperspectral data collection for the Drought-2 experiment.

	Plants	Time	Images	Location	dates
Drought-2	6 Sonata 12 BSP-14 8 Malling Centenary 12 Hawaii-4	7 days 7 days 5 days 6 days	123	Nottingham Sutton Bonington	13th – 25th August

Manual analysis of Sonata plants from the Drought-2 dataset

To provide a ground truth analysis, a simple manual approach to data extraction was explored. Graphs were produced from plant images in the Drought-2 experiment to see if there were any noticeable differences between the drought and control plants. Six Sonata plants were imaged, three were control plants and three were drought-induced plants. The image analysis software package 'Fiji' (Schneider et al, 2012) was used to manually select sections of leaves from three plants and taking the average over three days for the control and drought plants. Figure 11 shows the control plants' and drought plants' spectral signatures, Figure 12 shows day zero control and drought plants' spectral signature, Figure 13 shows day three, Figure 14 shows day five and Figure 15 shows day 9.
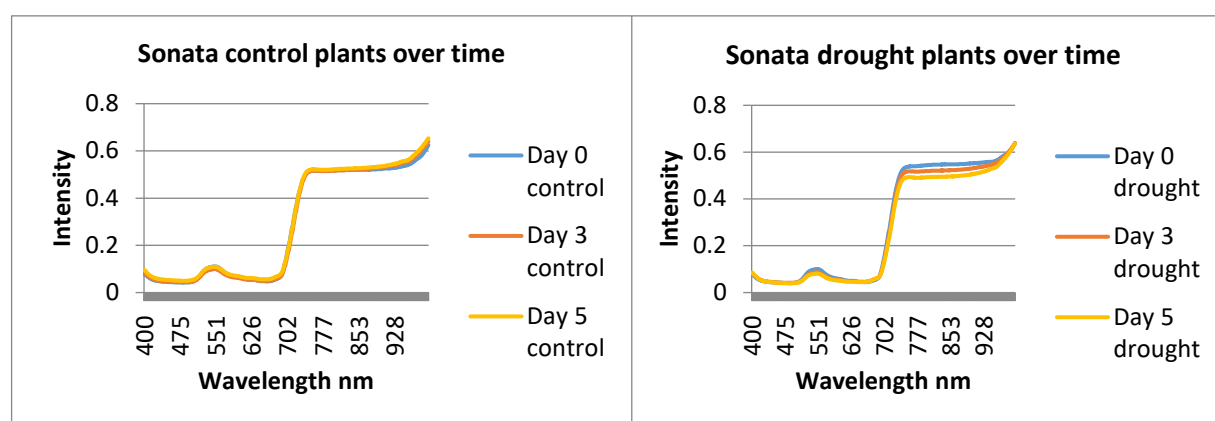

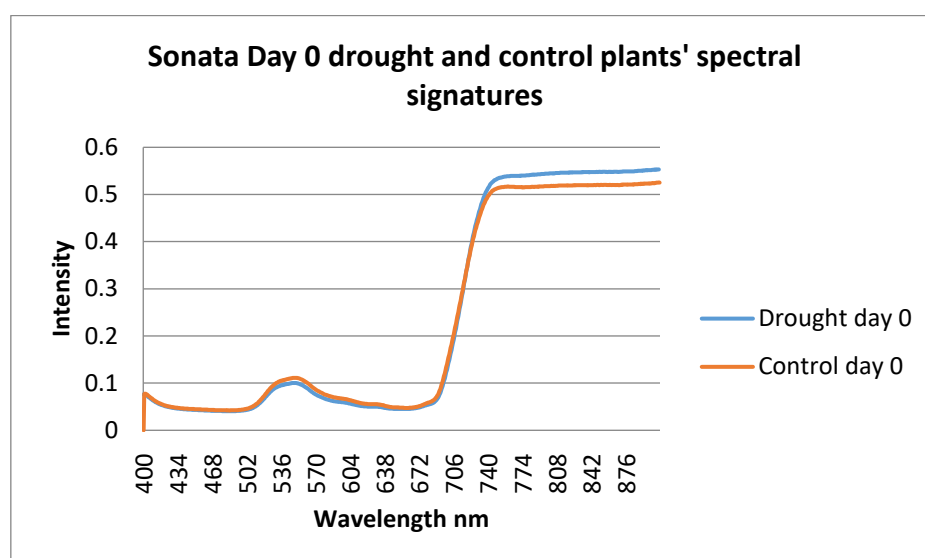*Figure 11: Left: Control plant spectral signature from day 0, 3 and 5. Right: Drought plants.*

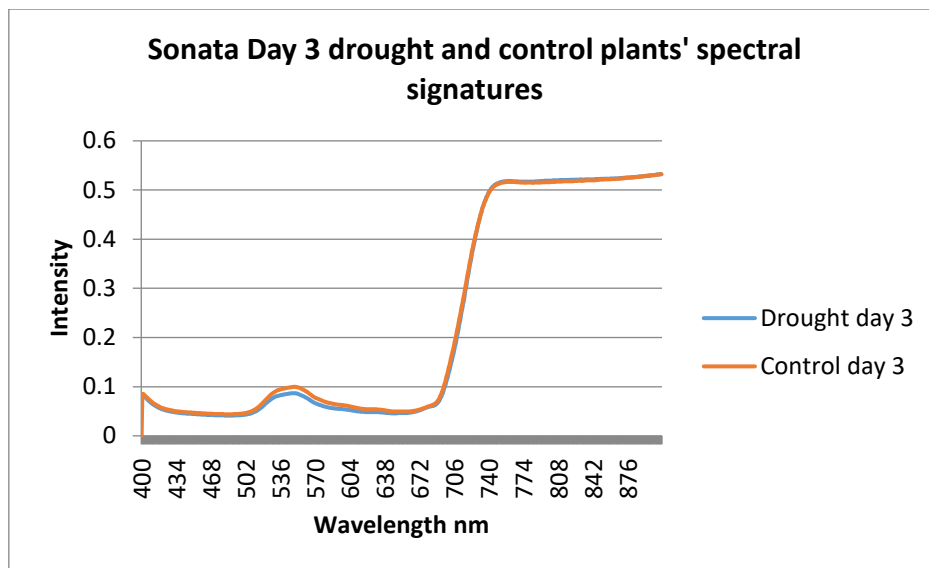*Figure 12: Day zero spectral signatures of drought and control plants.*

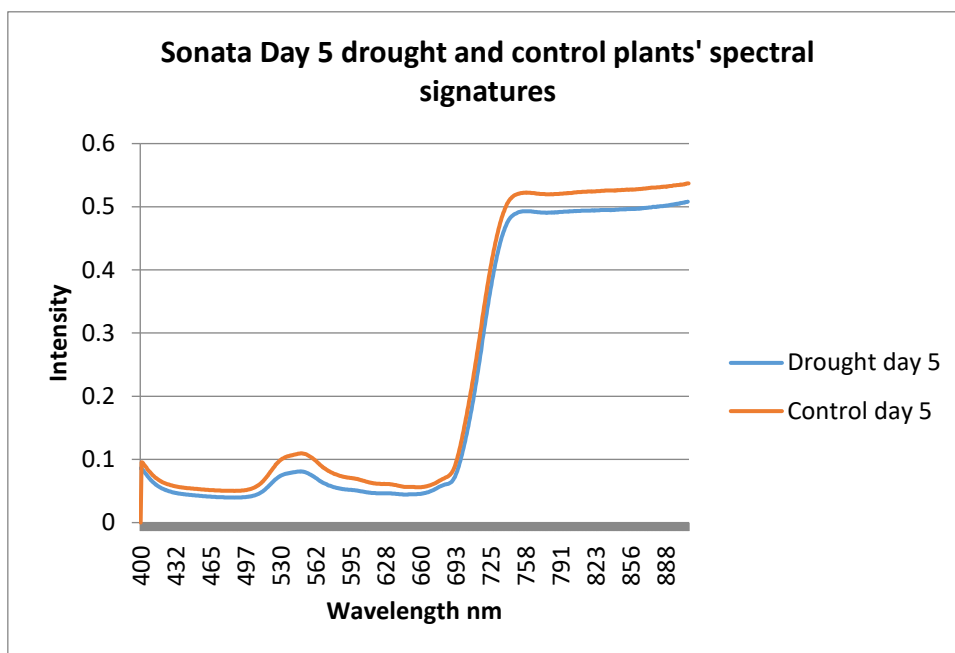*Figure 13: Day three spectral signatures of drought and control plants.*

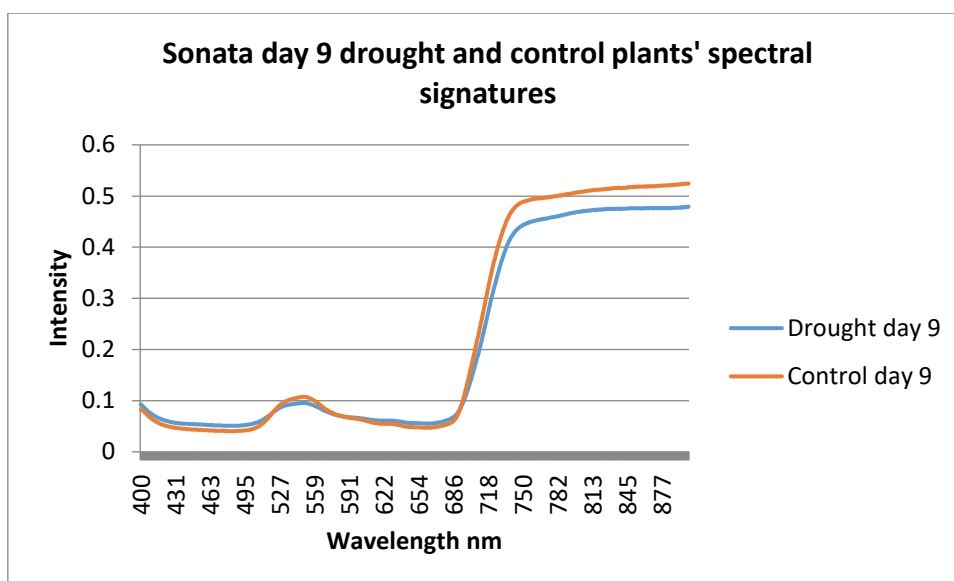*Figure 14: Day five spectral signatures of drought and control plants.*

Figure 15: Day nine spectral signatures of drought and control plants.

Figure 15 shows spectral signatures where first visible signs appeared. The drought plant is slightly lower in the green and near infrared sections whilst the red wavelength section (596-681nm) is now higher than that of the control.
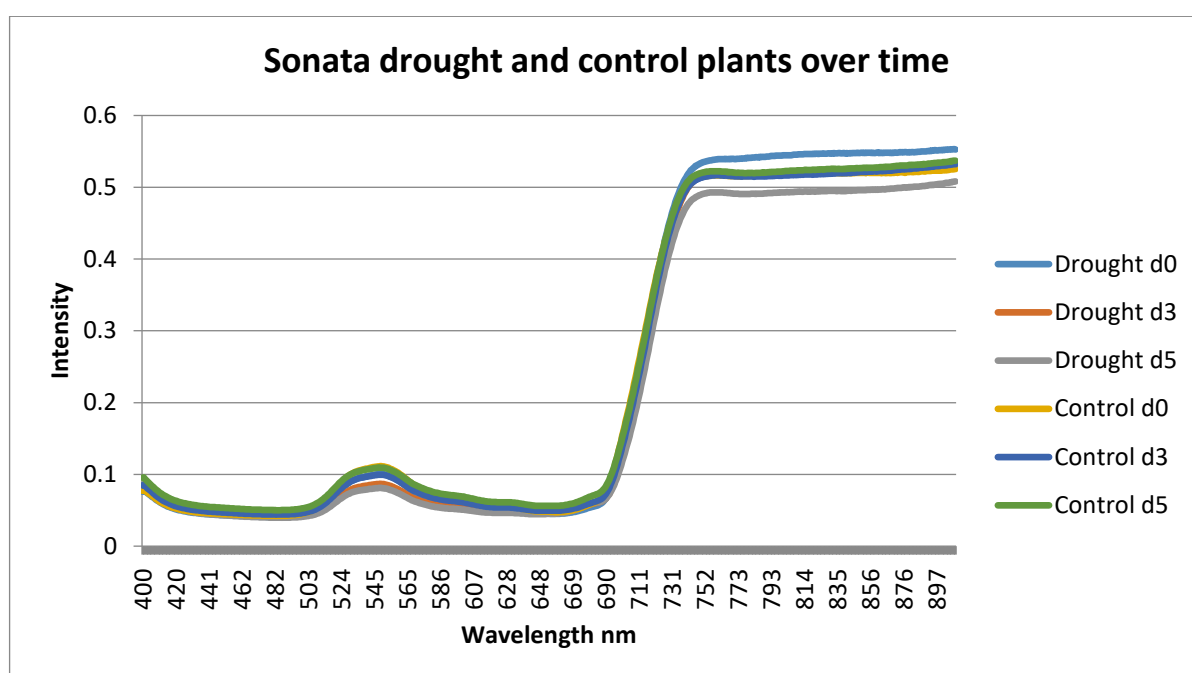

Figure 16: Control and drought plant spectral signatures - days 0, 3 and 5.

It is difficult to draw clear conclusions from the graphs alone. Figure 11 indicates a potential difference in signature for the droughted plants, but not the controls. This suggests the hyperspectral data is demonstrating a response to stress, but establishing the significance

requires further analysis. Figures 12-14 indicate that there is a change over time as the spectral signature of the droughted plants slowly changes in the green (~545nm) and the near infrared (>750nm) parts of the spectrum, and that these changes appear before any visible signs are apparent. Figure 15 shows the spectral signatures of the plants at the point when visible signs appeared - droughted plants are lower in the green and near infrared section, and higher in the in the red wavelength section (596-681nm). Figure 16 shows a composition of all the spectral signatures from the drought plants and control plants.

Further analysis is needed before any conclusions can be determined. This will involve analysing all of the plants over time by using new approaches currently under development which can analyse the whole dataset much more quickly than manual segmentation and analysis whilst offering greater accuracy. These techniques will include calculating vegetation indices and employing machine learning techniques such as Support Vector Machines (SVM), but these will need to be carefully calibrated and depend on completion of the segmentation technique described below.

Level set image segmentation results

The level sets were evaluated to determine how well leaves are segmented; the first example is a simple leaf and the aim is to find the individual leaflets. Figure 17 displays the Chanvese level set where the red line is the evolving contour.
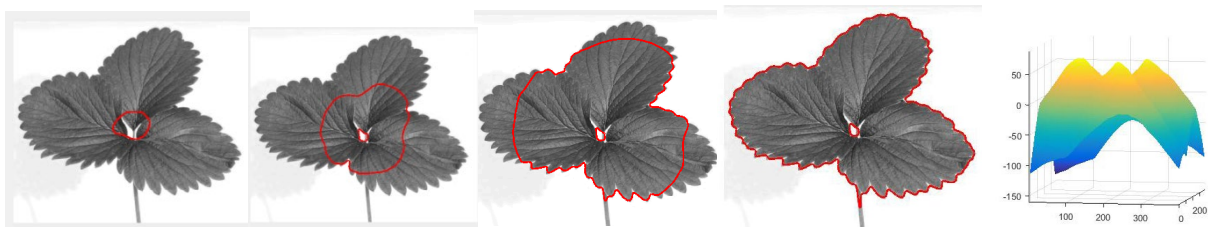

Figure 17: A leaf where the contour from the level set is shown in red.
From left to right, the initial contour in red, then the evolving contour after 20 iterations,
40 iterations and the final level set.

One weakness associated with the use of level sets is that it finds any connecting part of the image instead of individual leaves. This is visible in Figure 17 as the contour fails to segment individual leaflets and instead, finds the contour around the whole leaf. There are different versions of level sets which constrains the contour to a specific area in order to find the nearest edges/contours. However the leaf boundary is hard to see with the human eye therefore additional steps are needed. When the image information alone cannot find the leaf,

then incorporating shape information is a possible solution. This involves creating a shape model that resembles a strawberry leaf and incorporating it into the level set.

$$E(c1, c2, \emptyset, \emptyset o) = E(c1, c2, \emptyset) + \alpha\, shape(\emptyset, \emptyset o) \tag{2}$$

$$shape(\emptyset) = \int (\emptyset - \emptyset o\;)^2\, dx \tag{3}$$

Where $\emptyset o$ is the shape and it is a signed distance function. Equation 2 is the updated level set.

As well as image information, the level set will also have a leaf shape model (Figure 18), as developed by Cremers, Sochen and Schnorr (Cremers et al, 2003).

Figure 18: Mean shape from a set of strawberry leaves.
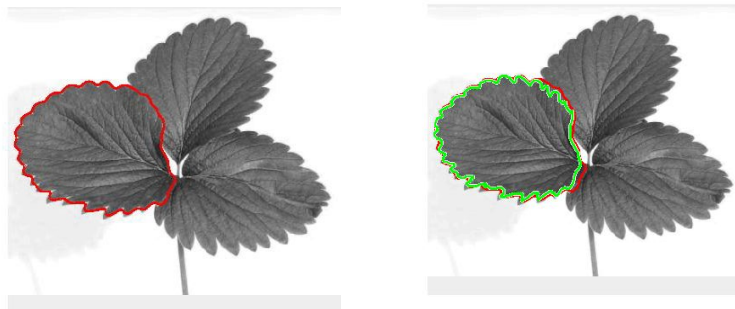
Figure 19: Left: Contour of the individual leaf after shape model is included in the level set. Right: The green line represents the shape model and the red line is the final contour.

The accuracy between the ground truth data and the contour is 94.84%, using the Jaccard similarity index which measures the difference between the ground truth and segmented object. The two binary images (object is 1 and background is 0) are compared using A∩B / A∪B.

The level set method is still being implemented and evaluated, and further development is needed for using this method as the model needs to be constantly translated and rotated to identify all the leaves.

Discussion

The past year has involved collecting image datasets and developing approaches to segment the data to find the leaves. 3D reconstruction has been explored to appraise the plant depth, and implementation of level sets to segment leaves. The level sets need more work to include translation and rotation, but initial results are promising.

The data was manually segmented using cv. Sonata to provide initial benchmark results; the aim is for the whole dataset to be re-analysed using the new segmentation approaches and machine learning for faster and more accurate results.

If time permits, further image datasets will be collected, including additional 3D data, but it is likely that sufficient datasets now exist to develop the computational approaches.

Conclusions

Over the past year five datasets have been collected for the following treatments: Spider mite, Powdery mildew, Drought 1, Drought 2 and Drought 2 stereo pair data. Segmentation techniques have been developed to distinguish plants from their background. This has proved to be relatively simple, but the current objective is to identify individual leaves – this is more challenging because the leaves are oriented in different directions. The task is to select the leaves orthogonal to the camera and this is still ongoing.

Segmentation is important to select the best areas to be analysed. Shadows and variations in illumination can change the spectral signatures and if the plants display small changes during the time period then these could be overshadowed by noise. Segmentation can also can improve accuracy, objectivity and speed when dealing with large image files and consequently large datasets.

Knowledge and Technology Transfer

- Study visit to EMR May-July 2015 to collect data and transfer strawberry plants for a repeat drought experiment in Nottingham.

- EMR/HDC Soft Fruit Day, 22 July 2015 – Discussing the project at a stand.

- HDC 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.
- Literature review paper being finalised which is about the detection and classification of the early onset of plant disease and stress using hyperspectral image analysis techniques.

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Appendix

Figure A: Spider mite damage. Left: one day after infestation; Right: 7 days after infestation.

Figure B: Thrips found and removed from the plants

Protocol for Strawberry Plants - adapted for the Spidermite-1 experiment

Plant Material Preparation

Dormant strawberry plants were purchased from propagators and kept in a cold store at 2°C until required. The dormant 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, plants were potted into FP9 pots using standard compost and grown for 2-3 weeks until they have 2-3 leaves.

At this stage, a thorough inspection of the leaves was undertaken and any marks or 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 to spider mites. The area was cleaned before and after the experiment using 70% ethanol.

Spider mite preparation

Use spider mite infested 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 infested.

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.

Infestation process

Place an infested leaf section on the bottom of the stem of each plant. This will allow an even distribution and also a more natural infestation compared to placing the infested 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 between 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 record:

- 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.

Protocol – Strawberry Plants - adapted for drought experiment

Plant Material Preparation

Dormant strawberry plants were purchased from propagators and kept in a cold store at 2°C until required. The dormant 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 plants were potted into FP9 pots using standard compost and grown for 2-3 weeks until they have 2-3 leaves.

At this stage, a thorough inspection of the leaves was undertaken and any marks or 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 between 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
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Equipment:

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

Recording data

During imaging record:

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

-Position of the plant during imaging

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

- General health