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Detection of the signs of flower senescence and pollination through image data paired with machine learning

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Abstract

Pollination deficits remain a key bottleneck in agriculture, with the spatial and temporal scales at which they occur being difficult to quantify due to time consuming methods. Being able to provide real time quantification of pollination is important in order to uncover where pollination deficits occur and allow for optimisation of pollination services. Here I investigate the ability of image data combined with machine learning to detect signs of senescence and pollination. Using time series photographs of wild strawberry *(Fragaria vesca)* flowers from a controlled pollination experiment, I investigate the ability of Random Forest (RF) and Convolutional Neural Networks (CNN) to predict flower age and treatment group (pollinated vs unpollinated) from image data. RF and CNN were both able to predict flower age on the scale of days with an accuracy of roughly 75%. Treatment group was more difficult to predict, with only CNN able to predict this better than random at around 64% accuracy. The ability of the machine learning methods to perform better than random suggest they are able to detect signs of senescence and pollination which are not visible to the eye, however further work is needed to improve the accuracy of prediction to a level usable for crop monitoring.

Declaration and Acknowledgements

I declare that the work in this thesis to be my own. The image data for this project is available upon request. The code required for this project can be found on github at: `https://github.com/elliottparnell/CMEEProject`

I'd like to thank Catherine Parry and my supervisor Rich Gill for all their advice and support with the project. I'd like to acknowledged the Imperial College Research Computing Service and thanks them for the access to the HPC that make computational projects like these feasible.

Elliott Parnell, 23rd August 2023

Keywords

Pollination, machine learning, floral senescence, remote sensing, strawberry, convolutional neural networks

1 Introduction

1.1 Importance of pollination in Agriculture

World hunger is once again on the rise [World Health Organization, 2019]. Food production is estimated to need to increase by 50% from 2010 in order to meet global needs in 2030 [Evans, 2009]. With increased competition for land, water and energy, agriculture needs to intensify in order to keep up with growing demand [Godfray et al., 2010, Sutton et al., 2013]. Technological innovations are needed to ensure this intensification occurs sustainably otherwise environmental damage caused by agriculture could be pushed to unsafe levels [Springmann et al., 2018]. With modern technology we are seeing optimisation within agriculture taking place. The implementation of artificial intelligence in farm management has lead to decreases in inputs of water, pesticides and herbicides, leading to more efficient farming and maintenance of healthy fertile soil [Talaviya et al., 2020]. Genetically modified crops and other new plant breeding techniques have produced cultivars with increased yields and other beneficial characteristic such as pest and herbicide resistances [Kumar et al., 2020, Qaim, 2020, Brookes, 2020]. While these innovations in farming are becoming increasingly common in agriculture, one area of agriculture that lags behind others in terms of optimisation is pollination.

Animal pollination (mainly by insects) is hugely important both for ecosystem function and maintaining genetic diversity amongst plants [Kevan and Viana, 2003]. It is estimated that 87.5% of flowering plant species are pollinated by animals globally [Ollerton et al., 2011]. Insect pollination is also vitally important in agriculture, with over three quarters of the top 115 most important food crops being dependent on insect pollination [Klein et al., 2007]. This includes most fruits, nuts and seeds alongside some important cash crops such as coffee, cocoa and oil-seed rape [Gallai et al., 2009]. Pollination can affect both yield and quality in the pollinator dependent food crops, with a complete absence of pollinators leading to around a 40% reduction in fruit yield [Klein et al., 2007, Gallai et al., 2009]. While in general, crop yields have increased thanks to technological advancements in agriculture, the rate of increase remains lowest in these pollinator dependent crops, suggesting pollination may be a key bottleneck in food production [Klein et al., 2007].

As agriculture has intensified, wild populations of pollinators have been in decline and have been unable to keep up with crop pollination demands, leading to pollination deficits [Dicks et al., 2021]. These declines have been linked with increases in landscape homogeneity, habitat fragmentation, climate change and pesticide use. While restoring wild habitats

and reducing pesticides has been shown to benefit wild pollinators [Kohler et al., 2008, Gabriel and Tscharntke, 2007], the most common approach in agriculture has been to supplement wild pollinator populations with managed bee colonies. While this approach in the short term may achieve increased yields, it may not be the most efficient. The most commonly used managed species, *Apis melifera* is a less effective pollinator per flower visit than wild bees, hence larger populations will be required in order to fulfill pollination deficits [Page et al., 2021]. Increasing managed bee numbers however can have negative impacts on fruit crops, with over pollination leading to a reduction in fruit quality [Garratt et al., 2021]. Furthermore, where and when these pollination deficits occur is not uniform and hence is unlikely to be solved by simply increasing number of pollinators. On a spatial scale, pollination services can vary over the space of just a few meters [Joshi et al., 2016], while on a temporal scale, flowering must remain synchronous with population growth through the growing season to provide pollination [Reddy et al., 2013]. Hence, being able to monitor where pollination deficits occur and optimise the pollination services provided by wild and managed pollinator communities is key for ensuring pollination deficits are reduced with the smallest level of input.

1.2 Current methods for measuring pollination deficits

Pollination deficits occur over a spatial and temporal scale. Pollinator levels will vary through a landscape, depending on land use. Proximity to natural environments has been demonstrated to enhance pollination visitation of crop plants [Joshi et al., 2016]. The positive effects provided by natural environments occur on small spatial scales of about 250m, so a better understanding of where pollination deficits occur within a crop would allow for optimum placement of re-wilded land [Kohler et al., 2008].

Pollination can vary over the space of just a few meters, yet mass flowering crops can span kilometers in agriculture. Technological advancements in harvesting machinery have allowed for yield variation to easily be assessed across a field. This is often used as a proxy for assessing pollination deficits [Bishop et al., 2022, Hünicken et al., 2021]. The issue with this is that while pollinator deficits have been linked to yield reductions through exclusion and association studies, yield remains a complex trait, dependent on interactions between a large number of environmental and plant based factors [Diepenbrock, 2000]. A further problem is that this method only allows for assessment of pollinator deficits post harvest, and does not allow for corrective management of that years crop.

An alternate method is performing pollinator targeted monitoring. This can be done through

floral observations and lethal trapping [Carvell et al., 2016]. Visitation does not necessarily mean pollination, however this can be accounted for by modelling approaches that calculate the necessary number of visitations for species to pollinate the flower [Garibaldi et al., 2017]. Floral observations are time consuming and lethal trapping is destructive to pollinator populations, hence farmers often ignore pollinator monitoring [Carvell et al., 2016]. To provide a good measure of spatial pollination deficits, monitoring must be repeated across the landscape, increasing the time input required. Furthermore, the datasets produced can be biased depending on factors such as methodology, landscape and the pollinator community present [Hutchinson et al., 2022].

It is clear that in order to optimise pollination in agriculture, we need a more robust and efficient way of calculating pollination deficits over a spatial scale. A possible solution could be to directly assess pollination state in the crop plants themselves.

1.3 Using the senescence pathway as an indicator for pollination

Pollination is the first step in triggering a cascade of changes in a plants life cycle. Pollination leads to early flower senescence, which depending on plant life strategy, can lead on to whole plant senescence when enough flowers have been pollinated. The visible changes in senescence such as petal drop and fruit development are the result of a change in gene expression and metabolism [van Doorn and Woltering, 2008, Jones, 2013]. Tapping in to these changes in metabolites could be a possible way to categorise flowers as pollinated or not. Traditional methods of this would be to use molecular based lateral flow kits, however this would only give you a result for a single flower, and hence struggles with scalability.

An alternate approach would be to identify changes using image based technology. Imaging approaches have the benefit of being extremely repeatable and recent advancements in image analysis algorithms have allowed us to better identify changes in image data. The changes in gene-expression and metabolism that accompany pollination may carry phenotypic changes in spectral reflectance that are not visible to the naked eye [van der Kooi et al., 2019]. Previous studies have identified regions of the spectrum that change in the early stages of plant stress [Junttila et al., 2022]. These changes were linked with changes in leaf thickness, mesophyll density and pigment composition [Zhao et al., 2016, Rumpf et al., 2010, Mahlein et al., 2013]. With pollination leading to senescence, we would expect that large level cellular changes involved in programmed cell death such as cell membrane degradation and the breakdown of the vacuolar in epidermal cells would cause changes in spectral reflectance [Tripathi and Tuteja, 2007]. Spectral reflectance can be measured using spe-

cial probes, however these are expensive and require calibration. Alternatively, spectral reflectance can be estimated from photographic data [Stigell et al., 2007]. If changes in spectral reflectance can be detected post pollination through photographic data this could provide a cost effective root for the development of remote crop monitoring technologies to provide instant answers regarding pollination status of the crop.

1.4 My machine learning tool

I aim to investigate some of the routes in which machine learning could be used to analyse photographic data in order to detect senescence and pollination. Through investigating the merit of machine learning algorithms at predicting flower age, I aim to investigate whether indicators of senescence can be detected through image data. I also aim to investigate if there are differences in the changes of spectral reflectance estimated from the image data for pollinated flowers compared to unpollinated flowers, which could then be scaled up and used in remote sensing to provide an accurate, real time pollination status of flowers.

2 Methods

2.1 Plant rearing, pollination treatment and time series imaging

Wild strawberry (*Fragia vesca*) were grown from seed on Levington F2+S medium in a controlled environment room. The controlled environment room was set to a 16 hour day at 22°C and an 8 hour night at 18°C. Artificial grow lights provided a light intensity of $120\,\mu\text{mol}\,\text{m}^{-2}$, and humidity was maintained at 60% throughout. Seedlings were transplanted from seed trays into 9cm pots after three weeks. Plants were watered twice a week, and fertilised once a week with a high phosphate fertiliser designed for strawberry plants.

As flowers emerged they were labelled with coloured collars to allow for time series photographs to be taken over the flowers lifespan. Time series photos were taken with a unique QR code label in frame, this contained information on the flower and time of photo (see appendix 1 for labelling system). In order to minimise variation in lighting conditions, all photos were taken in the same place, with flowers placed against a black felt background to minimise emissivity. A canon DLSR with a macro-lens was used in manual mode, with settings kept the same (ISO:250, Shutterspeed:1/50, focal length:0.4m). Images were saved as raw files on the camera to avoid compression.

Each morning, I removed the anthers of emergent flowers before anthesis could take place. Flowers were then photographed at 10:30 to give time point zero. Plants were split into two

treatments groups (pollinated and unpollinated) with 8 plants in each group. After time point zero, pollinated group flowers were hand pollinated by pollen provided by two donor plants. These were kept under the same conditions in a separate control environment room to avoid inadvertent pollination. The unpollinated group were not pollinated to act as a control for the experiment. Subsequent time series photos were taken in two hour intervals throughout the day at 12:30, 14:30, 16:30 and 18:30. I continued to photograph the flowers over consecutive days until the petals dropped.

After petal drop, flowers were left to develop into fruit. After the experiment concluded, the fruit development of each flower was assessed by eye to see if pollination had occurred. This allowed for the success of emasculation to be assessed, and also allowed for the removal of any anomalous flowers.

2.2 Image pre-processing

Images were pre-proceesed to extract the relevant information needed and prepare images for analysis, an overview of this is shown in figure 1a. This was done using python [Van Rossum and Drake, 2009] and a full breakdown of packages used can be found in appendix 2. A QR reader was used to extract the label info from the image [Hill and Whitty, 2021].

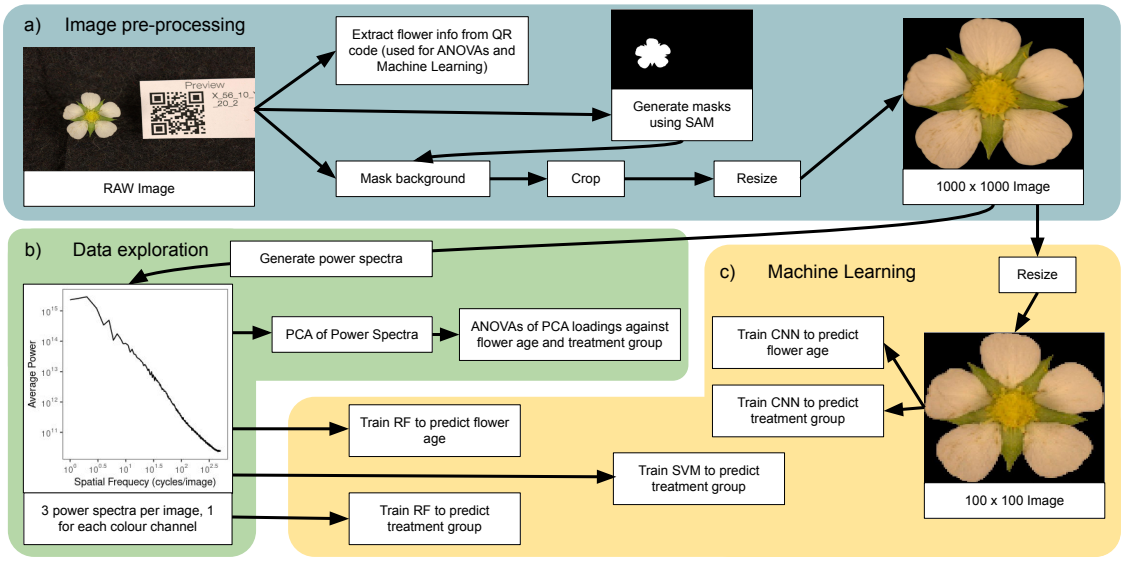

Figure 1: An overview on my data analysis pipeline. a) Pre-processing: extracting the data contained in the label and remove any background noise in the image via mask generation using SegmentAnythingModel (SAM). b) Data exploration: Representing image data as power spectra, principle component analysis (PCA), and testing of PCA loadings for differences with regards to flower age and treatment. c) Machine learning: Prediction of flower age and treatment from image data and power spectra using convolutional neural network (CNN), random forest (RF) and support vector machines (SVM)

To further minimise background emissivity, masks were generated to segment the floral pixels from the background pixels, which could then be set to a value of zero. To do this the SegmentAnythingModel (SAM) from Meta was used [Kirillov et al., 2023]. This model is a pre-trained segmentation model able to generate accurate masks based on prompts. I supplied the model with the pixel co-ordinates of 9 boxes that the flower in each of my images should be contained in. SAM then produce a segmentation mask that best fitted the item contained in the box. Each mask generated also had a confidence score attached with it which is calculated based on how stable the mask is when the image has thresholding applied to it [Kirillov et al., 2023]. Of the 9 masks generated from the boxes, only the mask with the highest confidence score was saved. This mask was then used to set the background pixel values to zero for all three colour channels of the image. Images were then cropped to a tight fitting square around the floral mask and resized to 1000 x 1000 pixels.

2.3 Data exploration

To explore the data, each colour channel of the image had a power spectra generated for it, an example of which is seen in figure 1b. Power spectra represent the distribution of power into the frequency domains that make up that signal. For images this will assess the variance of the features in the image at different resolutions [Van der Schaaf and van Hateren, 1996, Vandenbroucke, 2019]. In order to do this the image is transformed from spatial domain into a frequency domain through a Fast Fourier Transform (FFT), with the distribution of pixel values at different resolutions (cycles per image) now represented as Fourier components in sine and cosine waves. By taking the amplitudes of these Fourier components and the frequency it represents, we can calculate a mean for Fourier amplitudes at the same frequency giving us the power at that resolution [Van der Schaaf and van Hateren, 1996, Vandenbroucke, 2019]. Power spectra were generated in python [Van Rossum and Drake, 2009], with a full breakdown of packages used found in appendix 2.

Dimensionality reduction and further data exploration of the 3 power spectra per image was done in R [R Core Team, 2021]. I performed principle component analysis to summarise the variance in the power spectra on fewer axis. The axes that explained 98% of the variance of the dataset were selected and the positional loadings of each image on the axis was tested with a two way mixed ANOVA to test whether the axis contain any information about flower age or treatment (pollinated vs unpollinated). Normality of the loadings was checked prior to the ANOVA by visual inspection of QQ plots. Between group variance was also checked to be homogeneous through a Levene test. Treatment (pollinated or

unpollinated) was the between-subject factor variable, while flower age was the within subject-factor variable. The Greenhouse-Geisser correction was used as the data did not meet the assumption of sphericity.

2.4 Machine learning

Following on from confirmation that the image data and power spectra contained information regarding to flower age and treatment, I used machine learning techniques to predict flower age and treatment, an overview of this is seen in figure 1c. I used three different algorthims; Convolutional Neural Networks (CNN), Random Forest (RF) and Support Vector Machines (SVM). Data was split into a training group with 60% of the images and a test group with the other 40%.

Training of the CNNs was carried out in python using the keras package [Chollet et al., 2015]. CNNs use image data as an input, and in order to increase model speed I resized images to 100x100 pixels. I trained two models: one to predict flower age and the other to predict treatment group. For the model architecture of these see appendix 3.

I trained a RF regressor in R (full package list in appendix 2) to predict flower age using the power spectra data of each flower. A RF classifier and a SVM classifier were also trained in R to predict treatment group [Liaw and Wiener, 2002, David Meyer, 2023, R Core Team, 2021].

3 Results

3.1 Image data collection

Over a 25 day period the strawberries produced 191 flowers that had a minimum of 5 time points before petal drop. I removed unpollinated group flowers that formed fruit from the analysis, as well as pollinated group flowers that didn't form fruit. I further removed any flowers that did not have two complete days worth of time point post opening, leaving 158 unique flowers.

3.2 Principle component analysis

Dimensionality of the power spectra data was reduced by principle component analysis (PCA). This revealed that 98% of the variance was contained in the first 15 components, with component one explaining 86% of the variance as shown in figure 1.

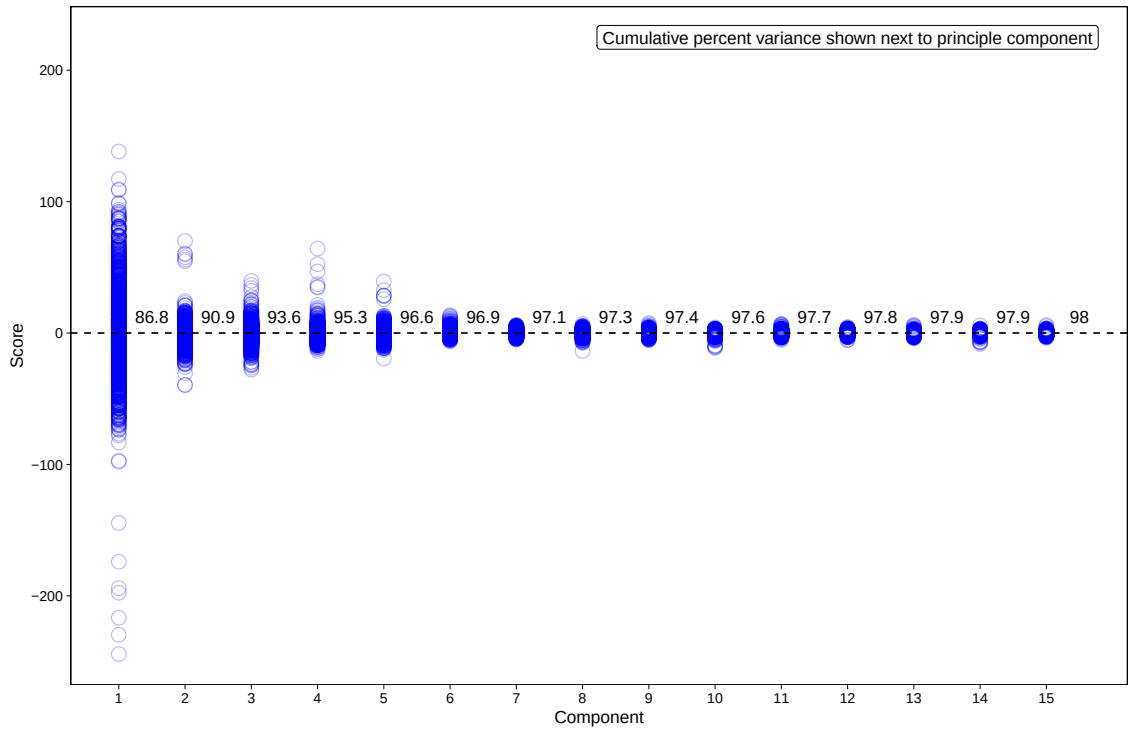

Figure 2: Percentage of total variance captured in the first 15 principle component axes. Score represent the position of each image on the axis.

With dimensionality reduced, a two-way mixed ANOVA was used to see if the principle components contained any information relating to flower age or treatment group. The ANOVA showed there to be no significant interaction between flower age and treatment group (see appendix 4). Loadings were significantly different with regards to flower age for PC1-7, PC9 and PC13. Treatment group was only significantly different on PC2.

A post-hoc one way ANOVA was applied to the principle component loadings that showed significance for treatment or flower age. The ANOVA tested for significance between treatments when subsetted by flower age (see appendix 5). Late stage flowers (hours 24, 26, 30 and 32) were more frequently significantly different, showing the later stage flowers were significantly different between treatments.

3.3 Predicting flower age from power spectra

The predicted values for the test data by the random forest regression model are shown in figure 3. The linear model shows that the ability of the model to predict is better than random, with the linear regression line being close to y = x . However, an R-squared value of 0.4126 suggests the reliability of the models predictions are low. The box plots of the model predictions show that the random forest model is unable to distinguish between

time-points on the same day, however is able to distinguish between days.

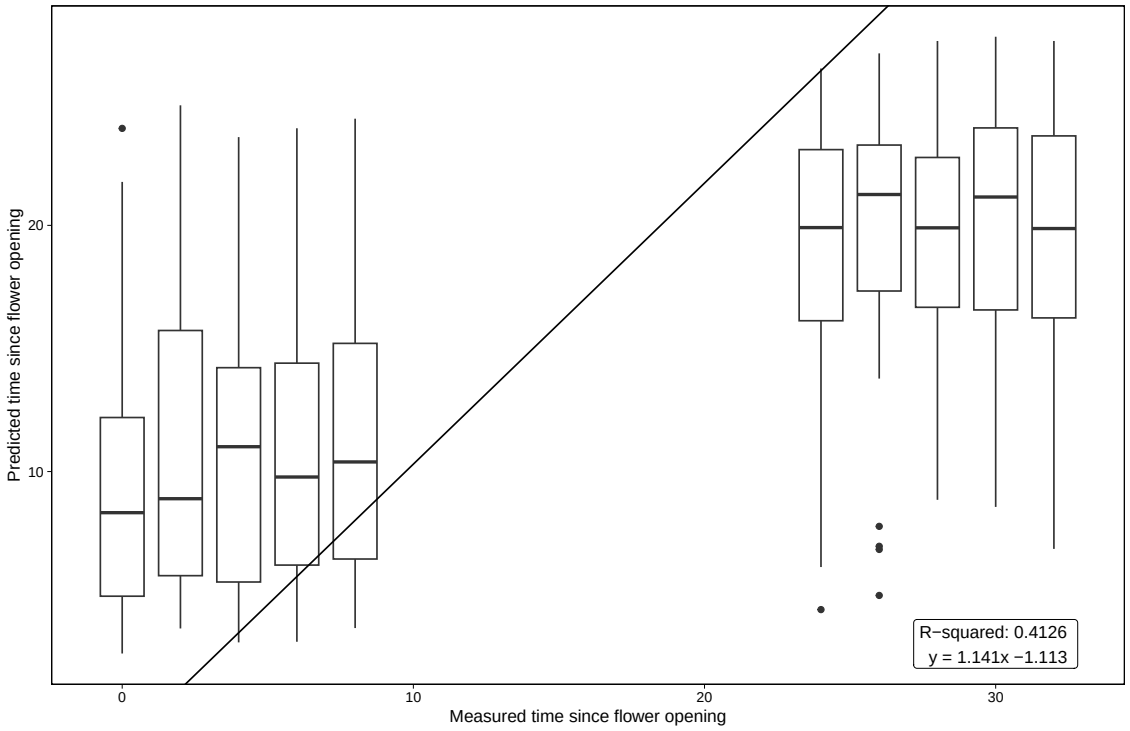

Figure 3: Correlation of flower age predicted by random forest regression against actual flower age

Following on from this observation, I trained a random forest classification model with the same training:validation split to classify whether the flower is in its first day or second day. This random forest classifier was 74% accurate at predicting day 1 and 78% accurate at predicting day 2 as shown in table 1.

Table 1: Accuracy of RF classifier at predicting flower age in days from power spectra (632 datapoints per day grouping).

		Actual	
		Day 1	Day 2
RF Predicted	Day 1	74%	22%
	Day 2	26%	78%

3.4 Predicting treatment from power spectra

The ability of RF and SVM to predict treatment group (pollinated vs unpollinated) from power spectra is shown in figure 4. Using all the time points, RF did not predict flower age much better than random chance (figure 4a), with 31.9% accuracy for unpollinated flowers and a 57.1% accuracy for pollinated flowers. SVM using all time points showed slightly better accuracy (figure 4b), but still not much better than random chance, with

46.1% accuracy for unpollinated and 66.0% accuracy for pollinated. Using only late stage flowers did not significantly improve the predictive power of RF or SVM. RF had a 54.5% accuracy for unpollinated and 56.2% for pollinated (figure 4c). SVM had an accuracy of 41.6% for unpollinated and 66.0% for pollinated (figure 4d).

a)

		Actual	
		Unpollinated	Pollinated
RF Predicted	Unpollinated	31.9%	42.9%
	Pollinated	68.1%	57.1%

b)

		Actual	
		Unpollinated	Pollinated
SVM Predicted	Unpollinated	46.1%	34.0%
	Pollinated	53.9%	66.0%

c)

		Actual	
		Unpollinated	Pollinated
RF Predicted	Unpollinated	54.5%	43.8%
	Pollinated	45.5%	56.2%

d)

		Actual	
		Unpollinated	Pollinated
SVM Predicted	Unpollinated	41.6%	34.0%
	Pollinated	58.4%	66.0%

Key
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%

Figure 4: Accuracy of predicting treatment group (pollinated vs unpollinated) from power spectra. a) and b) use data from all time points (0 to 32), while c) and d) only use later stage flowers (time points 24 to 32). a) uses a RF classifier with 182 unpollinated, 350 pollinated. b) uses a SVM classifier with a radial kernel and uses 308 unpollinated, 324 pollinated. c) uses a RF classifier with 154 unpollinated, 162 pollinated. d) uses a SVM classifier with a radial kernel and uses 154 unpollinated, 162 pollinated.

3.5 Predicting flower age with CNN

Predicted values from the CNN regression compared to actual value are shown in figure 5. The linear model has a regression line close to y = x, suggesting this model has predictive power. The reliability of this model is low as shown by the R-squared of 0.4169. The box plots of the model predictions suggest this model may be able to distinguish flower age at a two hour time scale, particularly during the first 8 hours. The box plots after 24 hours show more variability in prediction, showing the model may not be able to predict flower age at a two hour scale here. Box plots in the first 24 hours have little overlap with those in the second 24 hours, suggesting this model will be able to reasonably successfully predict flower age at a scale of days.

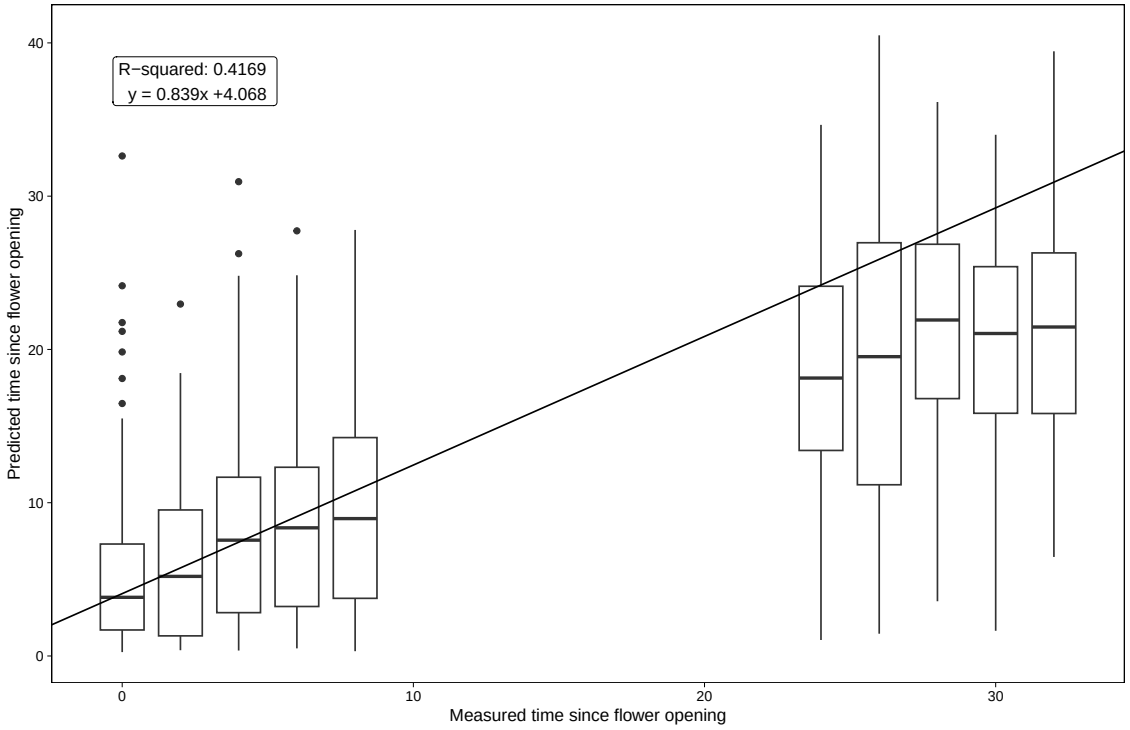

Figure 5: Correlation of flower age predicted by CNN against actual flower age

3.6 Predicting treatment with CNN

CNN was able to predict treatment group better than random chance as shown in table 2. There is still a large amount of error in this prediction, however the CNN is able to predict unpollinated treatment correctly 61% of the time and the pollinated treatment 68% of the time. This suggests there may be a small difference between the treatment groups that the CNN is only able to detect sometimes.

Table 2: Accuracy of CNN classifier at predicting treatment (pollinated vs unpollinated) using image data as input

		Actual	
		Unpollinated	Pollinated
CNN Predicted	Unpollinated	61%	32%
	Pollinated	39%	68%

4 Discussion

Machine learning paired with RGB image data could be a viable option for detecting signs of floral senescence and pollination. While none of the methods at this stage provide reliable prediction of flower age or pollination status, some of the algorithms were able to predict better than random chance, suggesting they were able to detect indicators for flower age

and pollination status to some extent. Random forest was able to predict flower age on a scale of days with an accuracy of around 75%. A CNN classifier was able to predict treatment group (pollinated vs unpollinated) around 64% of the time.

Although neither machine learning method was able to predict flower age with a low margin of error, both RF and CNN were able to predict flower age significantly better than random chance, suggesting they were able to detect signs of senescence. Both models were able to correctly predict the age of the flowers in days with reasonable accuracy, however they struggled to predict it down to the two hour increments the photos were taken at. A possible reason for this is the inclusion of both treatment groups together in the test and training data. This variable was not included in the data fed to the machine learning model, adding extra variation that could obscure trends within the data. Pollination leads to early senescence and hence this treatment group is likely to exhibit signs of senescence at a faster rate. This creates variation within the images taken at each time point. The 12 hour time gap between days then provides a long enough gap so that in both treatment groups, the indicators of senescence are further along the process than all the time points from day one, allowing for more accurate prediction between days. In order to test this hypothesis, the model would need to be retrained whilst either sub-setting the data for treatment group or including this as a variable in the data fed to the machine learning model. An improvement in accuracy would suggest this hypothesis to be correct.

The ability of machine learning to predict floral age is promising for early indication of flower senescence, as we are unable to see any visible signs of senescence by the end of 32 hours, as shown by figure 6. It is possible that the models are able to detect changes in reflectance due to the breakdown of pigments in the flower that accompany senescence's. This is an early step in flower senescence, pigment is a heavy energetic investment for plants, so it must be broken down and the nutrients re-mobilised before petal fall [van Doorn and Woltering, 2008]. While it is known that this pigment breakdown occurs in senescing petals, what happens to the re-mobilised pigments remains unclear [Rani and Singh, 2014]. These changes in pigment levels are undetectable to human eyes, however it has been shown that insect pollinators are sensitive to changes in pigment level that would accompany senescence [Papiorek et al., 2013]. It has been hypothesised that the changes to pigment level from pollination triggered early senescence could act as a signifier to insects to avoid pollinated flowers, however this may just a by product of floral senescence rather than an active strategy [Weiss and Lamont, 1997].

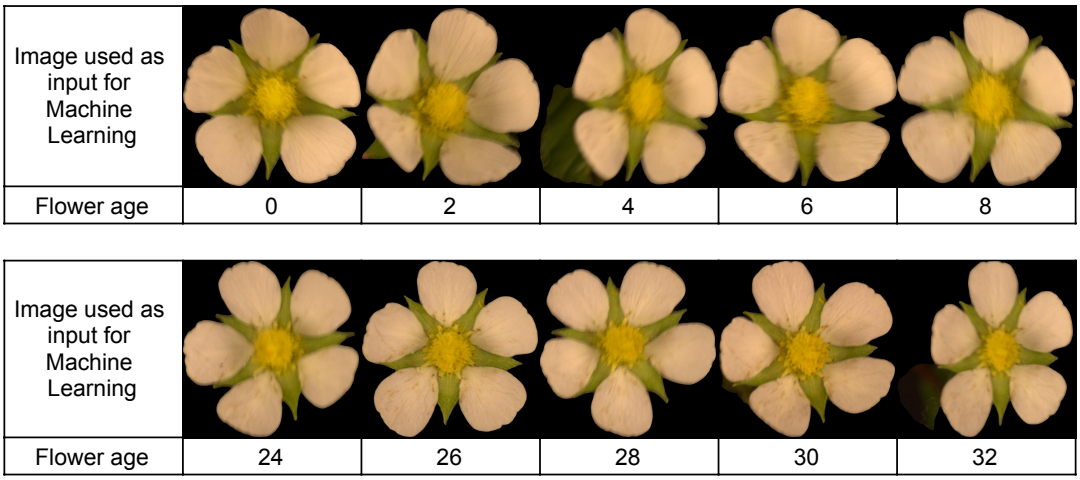

Figure 6: Time series images of a typical flower from my experiment

Treatment group of pollinated vs unpollinated flowers was more difficult to predict than flower age. In the data exploratory phase, the absence of an interaction between treatment group and flower age, combined with the post-hoc ANOVA only finding significance in late stage flowers, suggested that a signal for pollination that would separate the treatment groups may be hard to detect from the power spectra. This was confirmed, with neither random forest or support vector machines able to provide significantly better predictions of treatment group than random chance. CNN provided better results with prediction accuracy being better than random chance, indicating it was able to pick up a signal to separate the two treatment groups. It is important to verify that this signal is due to the pollination treatment. In the training and test data for this machine learning the split did not group flowers together based on unique id, meaning the same flower at different time points could end up in both the training and the test data. Hence the algorithm may have learnt to identify treatment group based on learning the unique characteristics of the flowers in that group instead of identifying an underlying signal present in all flowers in the treatment group. A way to test this hypothesis would be to retrain the model, ensuring the groups are split by unique flower id, or the collect a new set of time series photos to validate the model with.

Assuming that the models continue to perform better than random given the adaptions stated above, I believe image data and machine learning could provide a feasible option for monitoring flower senescence and pollination in crops. Of the machine learning methods used, I believe CNN to be the most likely to provide further accurate predictions of flower age and pollination status. CNN was the only algorithm used that was able to predict

treatment group to some degree, and while the results for predicting flower age were similar to random forest, the means of the groups at early stage did centre around the actual value, indicating CNN may be able to predict flower age on the scale of hours. One benefit of CNN is that it is able to use the raw image data, taking into account the two dimensional relationship between pixel values [O'Shea and Nash, 2015]. For random forest, image data must be transformed to power spectra, this leads to the loss of some spatial information because the Fourier transform used only investigates the correlation between pixels on the horizontal axis and then the vertical axis before averaging those results together [Vandenbroucke, 2019, Van der Schaaf and van Hateren, 1996]. CNN has already had successful results in crop monitoring, with successful results in disease detection [Radha and Swathika, 2021].

Further training with more image data may improve the predictive power of the CNN, alternatively image augmentation methods could be used. Image augmentation works by modifying your training images through techniques such as rotations and re-scaling to create new images. This combats over fitting, but also helps improve the predictive power of models that involve computer vision such as CNNs [Kostrikov et al., 2020]. Alternatively, removal of noise from the image data to amplify indicators of pollination and senescence would help improve the accuracy of the CNN. This could be done by identifying the areas of the light spectrum where the changes in reflectance occur, and then designing filters for the camera lens that only let in those targeted areas [Valero et al., 2007]. This provides wavelength selectivity that is a benefit of hyperspectral imaging, whilst still using standard RGB cameras making it much more practical for scaling up into a full crop monitoring system at later date.

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5 Appendices

5.1 Appendix 1 - QR labelling system

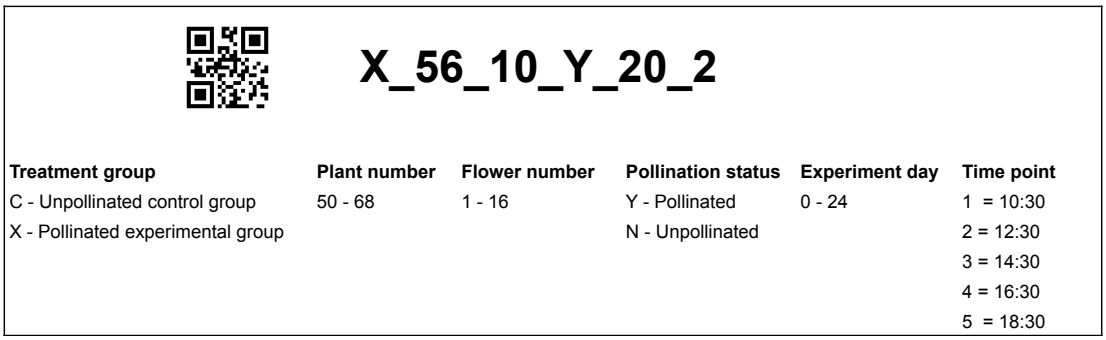

Figure 7: System used to create QR code labels that contained all the information required for analysis.

5.2 Appendix 2 - Table of packages used

Table 3: Full list of packages and their uses in analysis.

Language	Package name	Use	Authors
Python (standard library)	sys	Programme structure	[Van Rossum and Drake, 2009]
	os	Input and output	
	glob	File searching	
	shutil	File copying	
	tkinter	User interface	
	pickle	Storage of data	
	time	Time functions	
	re	Regular expression	
	csv	reading of csv files	
Python (other)	keras	CNN machine learning	[Chollet et al., 2015]
	rawpy	processing of raw images	[Riechert et al., 2014]
	S.A.M.	Masking background	[Kirillov et al., 2023]
	torch	CPU management	[Collobert et al., 2011]
	numpy	Arrays and Fourier transform	[Harris et al., 2020]
	pandas	Dataframes	[McKinney et al., 2010]
	matplotlib	plot visulisation	[Hunter, 2007]
	opencv	Handling of images	[Bradski, 2000]
	PIL	Handling of images	[Umesh, 2012]
	pyzbar	QR code reading	[Hudson, 2022]
	sklearn	Machine learning tools	[Pedregosa et al., 2011]
	SciPy	Statistics	[Virtanen et al., 2020]
	pyreader	R interface	[Fajardo, 2023]
R	reticulate	Python interface	[Ushey et al., 2023]
	tidyverse	Dataframe manipulation and plotting	[Wickham et al., 2019]
	rstatix	Two way mixed ANOVA	[Kassambara, 2023]
	caTools	Data split for ML	[Dietze, 2021]
	caret	Data split for ML	[Kuhn and Max, 2008]
	randomForest	Random Forest	[Liaw and Wiener, 2002]
	e1071	Support Vector Machine	[David Meyer, 2023]
	cvms	Confusion matrix	[Ludvig Renbo Olsen, 2023]
	Metrics	Statistics	[Ben Hamner, 2018]

5.3 Appendix 3 - CNN model architecture

Table 4: Model architecture for both CNNs. All nodes use ReLu activation function except the final dense node. Final node in the flower age predicting CNN uses a linear activation on the final node, while the CNN for predicting treatment group uses a sigmoid activation function.

Model: "sequential"		
Layer(type)	Output shape	Number of parameters
conv2d(Conv2D)	(None, 100, 100, 32)	896
max pooling2d(MaxPooling2D)	(None, 50, 50, 32)	0
conv2d 1(Conv2D)	(None, 50, 50, 32)	9248
max pooling2d 1(MaxPooling2D)	(None, 25, 25, 32)	0
conv2d 2(Conv2D)	(None, 25, 25, 64)	18496
max pooling2d 2(MaxPooling2D)	(None, 12, 12, 64)	0
flatten(Flatten)	(None, 9216)	0
dense (Dense)	(None, 64)	589888
dropout(Dropout)	(None, 64)	0
dense 1(Dense)	(None, 1)	65
Total parameters : 618,593 Trainable parameters : 618,593 Non-trainable parameters : 0		

5.4 Appendix 4 - Two-way mixed ANOVA

Table 5: Two-way mixed ANOVA of the first 15 principle component loading's to determine whether treatment (pollinated or unpollinated controls) or flower age were significant. Treatment is the between-subject factor variable. Flower age is the within-subject factor variable. Columns are effect, degrees of freedom numerator (DFn), degrees of freedom denominator (DFn), F value (F), probability (p), $p < 0.5$ and greenhouse-geisser adjustment (ges).

	Effect	DFn	DFd	F	p	p<.05	ges
PC1	treatment	1	156	0.691	0.407		0.002
	flower_age	7.08	1104.43	44.043	1.34E-55	*	0.156
	treatment:flower_age	7.08	1104.43	1.469	0.174		0.006
PC2	treatment	1	156	10.417	0.002	*	0.013
	flower_age	7.62	1188.44	13.146	1.41E-17	*	0.063
	treatment:flower_age	7.62	1188.44	1.105	0.357		0.006
PC3	treatment	1	156	0.14	0.709		0.000368
	flower_age	6.9	1075.8	2.754	0.008	*	0.01
	treatment:flower_age	6.9	1075.8	1.459	0.179		0.005
PC4	treatment	1	156	0.338	0.562		0.000714
	flower_age	6.26	976.18	4.691	0.0000773	*	0.02
	treatment:flower_age	6.26	976.18	0.775	0.595		0.003
PC5	treatment	1	156	0.7	0.404		0.001
	flower_age	7.21	1124.45	4.499	0.0000476	*	0.02
	treatment:flower_age	7.21	1124.45	1.754	0.091		0.008
PC6	treatment	1	156	1.8	0.182		0.003
	flower_age	7.53	1174.68	5.5	0.00000147	*	0.026
	treatment:flower_age	7.53	1174.68	0.72	0.666		0.003
PC7	treatment	1	156	0.94	0.334		0.002
	flower_age	7.31	1139.76	12.413	6.16E-16	*	0.047
	treatment:flower_age	7.31	1139.76	1.236	0.278		0.005
PC8	treatment	1	156	0.636	0.426		0.001
	flower_age	7.31	1140.35	0.971	0.453		0.004
	treatment:flower_age	7.31	1140.35	0.561	0.795		0.003
PC9	treatment	1	156	0.69	0.407		0.001
	flower_age	6.77	1055.55	2.623	0.012	*	0.012
	treatment:flower_age	6.77	1055.55	0.838	0.553		0.004
PC10	treatment	1	156	0.298	0.586		0.000369
	flower_age	7.24	1128.94	0.468	0.864		0.002
	treatment:flower_age	7.24	1128.94	0.417	0.897		0.002
PC11	treatment	1	156	0.065	0.799		0.000141
	flower_age	7.15	1114.63	1.362	0.216		0.006
	treatment:flower_age	7.15	1114.63	1.006	0.426		0.004
PC12	treatment	1	156	0.093	0.761		0.000129
	flower_age	7.32	1142.13	1.34	0.225		0.007
	treatment:flower_age	7.32	1142.13	0.26	0.972		0.001
PC13	treatment	1	156	3.131	0.079		0.006
	flower_age	7.77	1211.76	3.368	0.000911	*	0.015
	treatment:flower_age	7.77	1211.76	0.193	0.991		0.000877
PC14	treatment	1	156	0.003	0.959		0.00000261
	flower_age	8	1248.76	1.8	0.073		0.01
	treatment:flower_age	8	1248.76	0.459	0.885		0.002
PC15	treatment	1	156	0.024	0.877		0.0000446
	flower_age	7.1	1108.19	0.313	0.95		0.001
	treatment:flower_age	7.1	1108.19	0.952	0.466		0.004

5.5 Appendix 5 - Post hoc one way ANOVA

Table 6: Post-hoc one-way ANOVA for treatment subsetted by flower age for the loading's of PC1-15 which showed significant effect for treatment or flower age. Columns are flower age, effect, degrees of freedom numerator (DFn), degrees of freedom denominator (DFn), F value (F), probability (p), p < 0.5, greenhouse-geisser adjustment (ges) and adjusted p value (p.adj). Only significant results showed

	flower_age	Effect	DFn	DFd	F	p	p<.05	ges	p.adj
PC2	2	treatment	1	156	4.854	0.029	*	0.03	0.29
	30	treatment	1	156	7.792	0.006	*	0.048	0.06
	32	treatment	1	156	5.64	0.019	*	0.035	0.19
PC3	26	treatment	1	156	5.089	0.025	*	0.032	0.25
PC5	0	treatment	1	156	4.422	0.037	*	0.028	0.37
PC7	24	treatment	1	156	4.325	0.039	*	0.027	0.39