

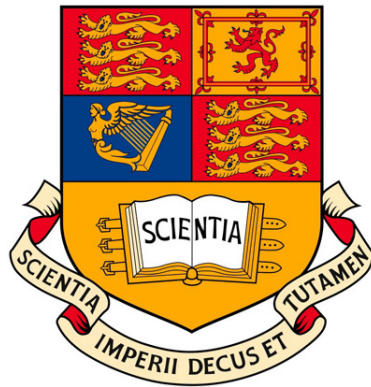
Classificaition of complex stresses in plants using machine learning

by

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Declarations:

Data provided by Dr Oliver Windram, Chris Adams and Abigail
Baines

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All mathematical models developed by Abigail Baines
All interpretations of results and analyses were developed by Abigail
Baines

Abstract

Determining the incidence and types of plant stresses has been of interest for many years, particularly due to their negative impact on agricultural production. Despite this, current methods are very qualitative, with many relying on manual detection which can be highly variable. With this in mind, our aim for this project was to see whether we could use machine learning, in particular Random Forests (RFs) and Convolutional Neural Networks (CNNs), to classify plant stresses from RGB and colour-pass filtered leaf images. Two plant leaf datasets were collected; an infection duration study conducted to assess the impact of *Botrytis cinerea* on *Arabidopsis thaliana* over a 72 hour period, and a complex stress study conducted on tomato leaves. Results showed support for four out of the five proposed hypotheses; hypothesis four (normalised images will improve model performance) was not supported by the data collected. We found strong support that machine learning can correctly identify both infection duration and complex/multi stresses in an image; both RFs and CNNs produced training accuracies of >85%. Finally, it was noted that the use of colour-pass filters improve model performance. Overall the results obtained from this study will be greatly beneficial to both the botanical and horticultural communities, and to the future of crop production.

Keywords: Digital Pathology, Random Forests, Python, Disease detection, Multi-spectral features, Machine Learning.

1 Introduction

Recently, the field of digital pathology has exploded; searching digital pathology on Web of Science returns over 3,000 hits, and 2013 onwards returns over 250 papers per annum. Despite this, plant pathology lags behind in using this technology; searching for "plant" within digital pathology only yields 18 results, in comparison to 455 for searching "human". This discrepancy is in part due to simpler disease detection methods, such as manual identification, being so heavily relied upon within the field of plant pathology up until fairly recently. (Lowe, Harrison and French 2017).

Plant Pathology

The study of plant pathology has always received much attention, due to its link with agricultural yield and crop productivity (Donatelli et al. 2017; Waller et al. 2005). Plant pathology is similar to other streams of pathology; susceptibility is the antithesis of resistance, with resistance being that a plant can overcome or exclude pathogenic effects/organisms. The cycle of resistance and susceptibility is powered by natural selection; plants become able to attack invading pathogens via their immune response, then pathogens evolve so that they can suppress this triggered immunity. This perpetual cycling results in a constant struggle to maintain resistance (Lapin and Van den Ackerveken 2013; Pel and Pieterse 2013; Zheng et al. 2012). Artificial selection in plants has routinely been used in agriculture, in order to introduce resistance to specific diseases into crops (Dennis et al. 2008).

Despite the existence of numerous control measures for many crop diseases (Wood 1996), those that still manage to impact upon crop production can be devastating (McCook 2006; Woodham-Smith 1962). This is further complicated by the fact that different pathogen races and variable environmental conditions can radically affect the degree of disease severity (Dangl and Jones 2006; Suzuki and Ron Mittler 2006).

It has been well documented that abiotic stress conditions also cause extensive losses to crop production (Ron Mittler 2006); nitrogen deficiencies, drought, and excess UV exposure are amongst several other factors influencing both plant immune system responses and plant growth (Karimi et al. 2006; Santos et al. 2018). Although these abiotic conditions receive much research attention,

the research conducted is often on individual stresses (Zhao et al. 2005; Santos et al. 2018). In the field, crops are routinely being subjected to various combinations of stresses, both biotic and abiotic (Ron Mittler 2006), meaning complex stress experiments are becoming essential research priorities.

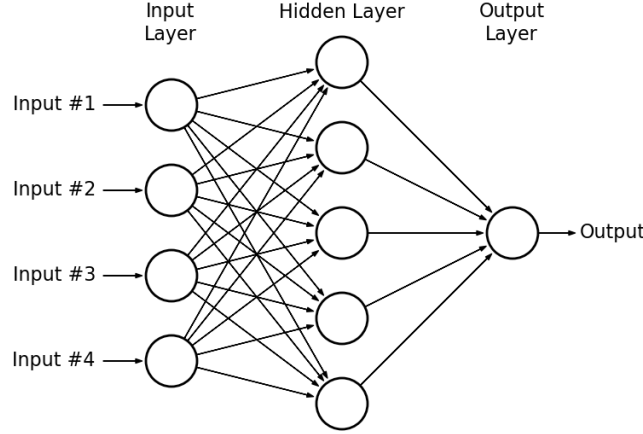
Due to the complex nature of stresses affecting plant growth, it can often be difficult to identify the cause of the stress; this is particularly apparent when multiple factors are influencing the phenotype expression. Recent studies have revealed that responses of plants exposed to multiple stresses are unique, and cannot be extrapolated from that of responses of plants exposed to the individual stresses (see: Rizhsky, Liang and R. Mittler 2002; Ron Mittler 2006). These findings make stress/ infection identification particularly complicated in the field; even when stress combination experiments are conducted, these primarily occur in the lab, with conditions that do not compare well to the field.

Past methods in detecting plant disease and other stress-inducing factors rely on experts simply observing the plant (V. Singh and Misra 2017); despite the rise in the use of machine learning for classification, many still rely on manual detection in this field. Manual detection, despite being easily applied in the field, is highly qualitative, and therefore is subject to interpretation. It is also noted that this method cannot quantify the level of disease severity, nor infection duration, accurately (Lowe, Harrison and French 2017). Manual identification also cannot correctly identify complex stresses or masking/ hidden factors, such as underlying root rot. Finally, manual detection limits the ability to compare data and results, due to the nature of the identification method.

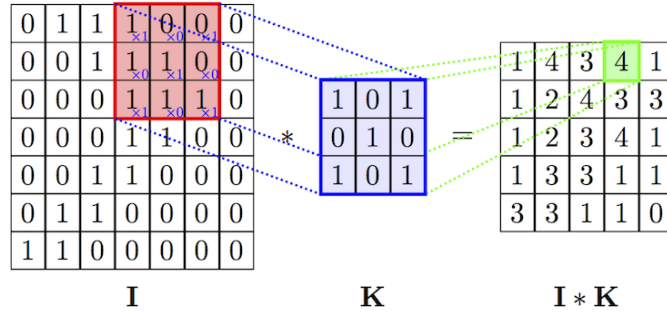
Machine Learning

Due to the limitations of manual detection, more recent studies have been using Machine Learning algorithms (Awate et al. 2015; Baranowski et al. 2015; Mohanty, Hughes and Salathe 2016; Lowe, Harrison and French 2017). Mohanty *et al* (2016) used a CNN to detect the presence/absence of 26 diseases, including leaf blight and tomato mosaic virus, in 14 crop species. The dataset used was open source (obtained from PlantVillage), and contained 54,306 images in total; the highest accuracy achieved was 99.35%, whilst a model trained on images under one condition and tested under another yielded results of only 31.4%. This discrepancy was primarily due to the factors affecting the image being taken, such as daylight, light exposure and type of camera being used; using more images and normalising images is supposed to minimise this discrepancy (Ciresan, Meier and Schmidhuber 2012; Krizhevsky, Sutskever and E. Hinton 2012). Another study, conducted by Zhu *et al* (2016), looked into early detection and classification of Tobacco Mosaic Virus; a range of machine learning algorithms were used, including Random Forests, Support Vector Machines and Back Propagation Neural Networks, with most models returning accuracy ratings of 85% or higher.

Neural Networks (NNs) have become of particularly important in the field of Plant Pathology, and Pathology in general, in more recent years (Awate et al. 2015; Sladojevic et al. 2016). A NNs basic structure consists of 3 types of layers, an input layer, one or many hidden layers and, an output layer, with each layer made up of 'neurons' (Figure 1a). Convolutional Neural Networks (CNNs), a subclass of NNs, have received much attention in the field of image recognition; in recent years, CNN's have out-ranked many other classification algorithms and achieved best performances on many major image recognition benchmarks (Krizhevsky, Sutskever and E. Hinton 2012; Simard, Steinkraus and Platt 2003). CNNs also consist of 'neurons'; these neurons primarily receive inputs in the form of 2/3D matrixes (e.g. single channel images, RGB images, image data), apply specific functions and biases, and return an output. CNNs contain layers called convolutional layers (these are within the aforementioned hidden layer(s)); within this layer a matrix dot function is applied using a matrix of size $h \times w$ (known as a convolution kernel/matrix) on an image or image data,



(a) Basic example of a Neural Network, with a simple input, hidden and output layer. The Input Layer contain the split image(s), which then progress onto the Hidden layers. The output layer is a single neuron containing all the predictions made by the hidden layers.



(b) Basic example of how a convolutional layer is created. A matrix dot function is applied to the layer (I) using a convolutional matrix (K); the resulting output layer is the matrix $I * K$.

Figure 1: Figures showing the basic layout of a Neural Network, (a), and a convolutional layer (b), found within the hidden layer of a network. All neurons within each layer connect to previous layers, as well as to future layers; as the layers 'learn', interaction strengths vary depending on how well each neuron classifies the data input into the model.

I (Figure 1b).

By overlaying the kernel on top of the image in all possible ways, the convolutional layer is able to extract valuable information from the image, including edges and shape (Zeiler and Fergus 2014). This process is aided by the non-linear activation functions applied to CNN layers, such as the ReLu function. These functions allow for true learning to take place, by allowing different input transformations to occur, which are learnt from the dataset itself (Punjani and Abbeel 2015).

Despite their excellent performance, CNNs require more resources in terms of computing power, as well as more time to train in comparison to other machine learning algorithms (e.g., K-nearest



(a) Dataset taken under RGB sensor (b) Dataset taken under RGB sensor and CalColor 90 pink colour-pass filter (c) Dataset taken under RGB sensor and CalColor 90 pink colour-pass filter

Figure 2: Samples of dataset taken under different filters. Both b and c highlight chlorosis occurring in the leaves more evidently than in a.

neighbours, Random Forests). This problem is further exacerbated because CNNs are difficult to design correctly, with many networks being created in the pursuit of the final model. Often, a balance must be struck between model factors (such as number of layers, layer type and parameters used) and the risk of over-fitting.

Another commonly used algorithm in Plant Pathology is a Random Forest model (Baranowski et al. 2015). Despite returning lower accuracies than CNNs (Baranowski et al. 2015), they are still used and relied upon as a classifier; Ilastik (Sommer et al. 2011), an ‘interactive learning and segmentation toolkit’, is developed in Python and utilises Random Forest Classifiers. It is also a primary tool in classification in both Human and Plant Pathology (see: Kleesiek et al. 2014; Haubold et al. 2016; Visschers, Dam and Peters 2018).

Image data

Despite the excellent track records of Machine Learning algorithms as image classifiers, the images used are still essential to creating a correctly classifying model. To ensure that the classifier does not overfit the data, images are often augmented prior to training (Simard, Steinkraus and Platt 2003; Ciresan, Meier, Masci et al. 2011; Ciresan, Meier and Schmidhuber 2012; Sladojevic et al. 2016); the images are often augmented in such a way as to retain the structure of the image (i.e. transformations, rotations) whilst also providing additional test data. Images can also be normalised in an attempt to further reduce over-fitting and to allow comparison between datasets.

The quality of the image is also essential when it comes to building a classifier; Mou *et al* (2017) note that hyperspectral imagery has started collecting considerable attention in the past few decades, in part due to its high spectral resolution. Both multispectral and hyperspectral image data have been used in CNNs, and have achieved great results (Harsanyi and Chang 1994; Delalieux et al. 2007; Baranowski et al. 2015; H. Zhu et al. 2016; Lowe, Harrison and French 2017; Mou, Ghamisi and X. X. Zhu 2017). Although these methods have attracted attention over the recent decade, these imaging techniques are still costly. Another method for extracting valuable information from data are band-pass (Interference filters) and colour-pass filters (high pass filters; figure 2); these work by filtering image wavelengths, and allowing only specific wavelengths to pass whilst others are blocked. Band-pass filters are non-coloured and can allow very narrow wavelengths through; colour-pass filters are coloured glass and work according to the principle of subtractive colour mixing (Sischka 2018). These filters allow for greater contrast within the image

(Figure 2), and are gaining in popularity in image analysis (Piron et al. 2008; Knoth et al. 2013).

Study - Organisms and Relevance/Outline

Several methods are currently being used to study disease classification; more advanced models (H. Zhu et al. 2016) are producing higher classification accuracies, and better quality images allow for additional information to be extracted (Baranowski et al. 2015; Lowe, Harrison and French 2017). This paper will utilise both advanced models (CNNs and RFs), and high quality images produced using colour-pass filters.

This study will be classifying the impact of complex stresses on tomato (*Solanum lycopersicum*) leaves; *Botrytis cinerea*, a common fungal infection, and both drought and nitrogen deficiency were used as the stresses in this image dataset. A further study will look at the effect of infection duration on *Arabidopsis thaliana*.

Arabidopsis thaliana and Tomatoes were used as they are common study hosts in both host-pathogen and host-stress studies (Tank and Saraf 2010; A. Singh et al. 2016; Choi et al. 2017); both have also been used in studies involving *Botrytis cinerea* infection and resistance (Diaz, Have and Kan 2002; Choi et al. 2017).

Botrytis cinerea is a commonly studied disease with distinguishable characteristics (Awate et al. 2015); by using this fungal infection, we should obtain classifiable results, whilst also adding to the growing body of research in plant-host susceptibility and resistance. Finally, the abiotic stresses were chosen due to their notable effect on plant growth (drought: Santos et al. 2018, nitrogen: Zhao et al. 2005), and due to their ease of application.

Aims of Project

This study was undertaken to see whether machine learning can correctly classify instances of complex stress exposure in tomato leaves, as well as duration of infection of *Botrytis cinerea* on *Arabidopsis thaliana*.

Our study aims to address five questions: Can machine learning classify instances of complex stress (question 1) and infection duration (question 2), will including colour-pass filtered images affect the model outcomes (question 3), will models trained on normalised images produce different results (question 4), and, will CNNs differ in model outcomes in comparison to RFs (question 5)?

Based on previous studies looking into machine learning algorithms as plant disease classifiers (Awate et al. 2015; Baranowski et al. 2015; Sladojevic et al. 2016; V. Singh and Misra 2017; Ubbens and Stavness 2017), and on other image classification benchmarks that have been achieved using these algorithms (see: Krizhevsky, Sutskever and E. Hinton 2012), we believe both CNNs and RFs will be able to correctly classify (>85% training accuracy) both complex stresses (hypothesis 1) and infection duration (hypothesis 2) images. Including colour-pass filtered images should improve the classifiers accuracy (hypothesis 3); band-pass filters have already been used successfully in plant pathology (see: Piron et al. 2008) with colour-pass filters applying similar, but subtler, techniques. Models trained on the normalised dataset should have an improved accuracy rating in comparison to the original model accuracies (hypothesis 4); normalising the data should reduce the potential for over-fitting, and should also allow the model to be used on different datasets without requiring additional information. Due to the staggering performances of the top CNN models in many image classification challenges (for example, see: Krizhevsky, Sutskever and E. Hinton 2012), we also hypothesise that CNNs will produce better model outcomes in comparison to RFs (hypothesis 5).

2 Materials & Methods

Data and composition

The data used was collected during 2009 and 2017; the 2009 data was collected by Dr Oliver Windram, and the 2017 data was collected by myself and Chris Adams.

The 2009 data was collected to look at the effect of *Botrytis cineria* on *Arabidopsis thaliana*; the data was collected over a period of 72 hours, allowing us to look at the effect of duration of infection. The time periods for this dataset were 10hpi (hours post infection), 18hpi, 26hpi, 34hpi, 42hpi, 48hpi, 72hpi and non-infected/ control. Images in this dataset were taken under a RGB sensor only.

The 2017 data was collected to look at the effect of multiple stressors on tomato leaves; a combination of drought stress, Nitrogen deficiency, and *Botrytis cineria* were applied (see Table 1 each treatment type) and left for 42 hours, after which several images were taken, including a RGB image and a range of images under color-pass filters (appendix table 1).

Both datasets were then cropped to contain individual leaf images; once this was done each individual image was processed, as laid out in Figure 3, ready for image classification.

Colour-pass filters

Part of this papers aim was to ascertain whether colour-pass filters bring out more visual data than just RGB images alone; to test this, three multi-stress datasets were created, with increasing numbers of colour-pass filters used in each. The colour-pass filters were held over a normal RGB sensor camera (Figure 3).

The three datasets consisted of a RGB image dataset (dataset 1), a RGB, purple and magenta image dataset (dataset 2), and a dataset consisting of RGB images and all color-pass filters (dataset 3). The colour-pass filter colours used are noted in Table 1 in the Appendices.

The duration infection dataset did not contain additional filters; only one dataset (dataset 4) was created, containing RGB images.

Image Normalisation

In order to allow for easier comparison between datasets, Dataset 3 was normalised using the normalize function in OpenCV Python (Figure 3). Each image was normalised between absolute black (0) and absolute white (255); the

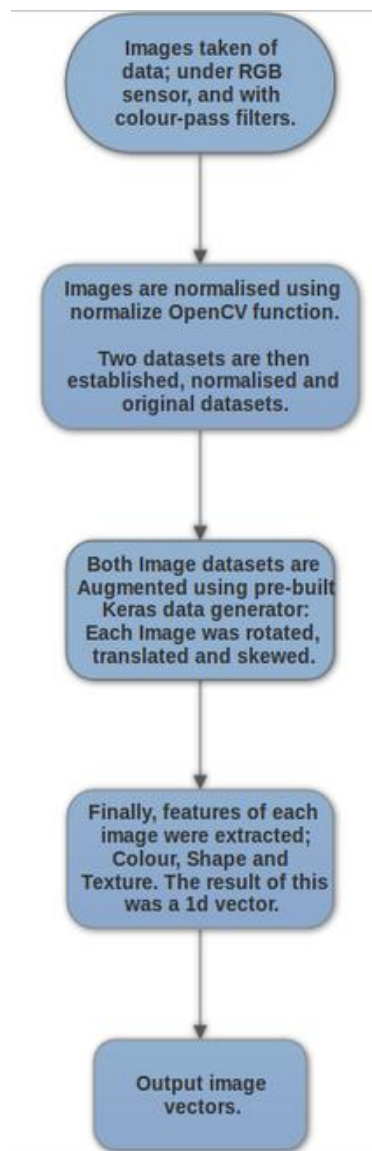


Figure 3: Outline of the workflow from raw images to final datasets.

Table 1: Treatment Types

		Drought			
		Present	Absent	Present	Absent
Nitrogen	Present	+++	++ -	+ - +	+ - -
	Absent	- + +	- + -	- - +	- - -
		Present		Absent	
		Infection			

original images were also kept to allow for results comparison. This dataset is referred to as Dataset 3-NORM.

Data Augmentation - Reducing over-fitting

One of the easiest and most common methods of minimising the risks of over-fitting on image data, is to artificially enlarge the dataset(s) (Figure 3: Simard, Steinkraus and Platt 2003; Krizhevsky, Sutskever and E. Hinton 2012; Sladojevic et al. 2016; Ubbens and Stavness 2017). Another benefit of this approach is that data generation/ augmentation has been noted to improve classifier performance (see: Yaeger, Lyon and Webb 1996, or Krizhevsky, Sutskever and E. Hinton 2012), by increasing the chance for the classifier to learn appropriate features of each treatment (Sladojevic et al. 2016). We augmented the dataset using a pre-built data generator in the Keras library (Chollet 2015); each image was randomly augmented 20 times using simple distortions such as translations, rotations, and skewing.

Feature extraction

Prior to training, features of each image dataset (Figure 3: shape, colour, texture) were extracted; this was done as preliminary training was returning poor classification results when trained on the original image dataset. This method of feature extraction is also done to reduce the computational workload, and to further minimise over-fitting. After the features were extracted, each image's data consisted of a 532 x 1 array.

Test images for each dataset were kept separate from the training datasets; these images consisted of data from the original experiment and from other experiments with the same treatment types.

The final datasets consisted of 3,600 duration infection image vectors; 450 for each stage of infection, and 7,952 multi-stress image vectors; 994 for each treatment. These datasets were split by 80:20 for training/validation of the models. There were also 42 multi-stress test images, and 209 duration infection test images not input into any models; these were used to test the final models for a final accuracy/prediction score.

Data modelling

Image classification problems have utilised various machine learning algorithms in the past, ranging from Linear Regression to Deep CNNs (Naseem, Togneri and Bennamoun 2010; Krizhevsky, Sutskever and E. Hinton 2012; Tripathi and Maktedar 2016). The two main classification algorithms investigated in this project are Random Forest classifiers, and Convolutional Neural Networks.

Both algorithms have been successfully applied for image classification tasks (Krizhevsky, Sutskever and E. Hinton 2012; Malof et al. 2016), and are currently being utilised in the field of plant pathology (Awate et al. 2015; A. Singh et al. 2016).

Machine Learning

To get the best classifier comparison, several classifiers were trained on the image data, including Random Forests, K-Nearest-Neighbours and Support Vector Machines. An ensemble approach was also conducted, consisting of Decision trees, Random Forests and KNNs. Random Forests produced the best results, and so further testing was using RF alone.

Each RF classifier had the same random state, and consisted of 100 trees unless otherwise stated. All RF classifiers then underwent a ten fold cross validation, with accuracy of each classification used as the scoring method.

The CNN used in this study (Appendix Figure 3) consisted of many layers including several convolutional layers, a dropout layer and, a dense layer. A Relu activation function was used in all convolutional and dense layers; this activation function was used to model nonlinearity in the data, as it can be computed faster than more traditional functions, such as sigmoid or hyperbolic tangent functions. The dropout layer had a 40% dropout function, and the learning rate was 15% unless otherwise stated. The Tensorflow framework (Abadi et al. 2016) within Python was used to create this CNN.

Pixel Intensities

In order to further validate the predications the classifiers made, and to ensure that there are valid differences between each treatment, pixel intensities were gathered.

To do this, the background was first masked, so that only the leaves were visible (Figure 4). After this each leaf was labeled in relation to the treatment, and pixel intensities of each treatment leaf were gathered using Python 3.7 (Rossum and Drake 2001). These Pixel Intensities were then compared using an ANOVA.

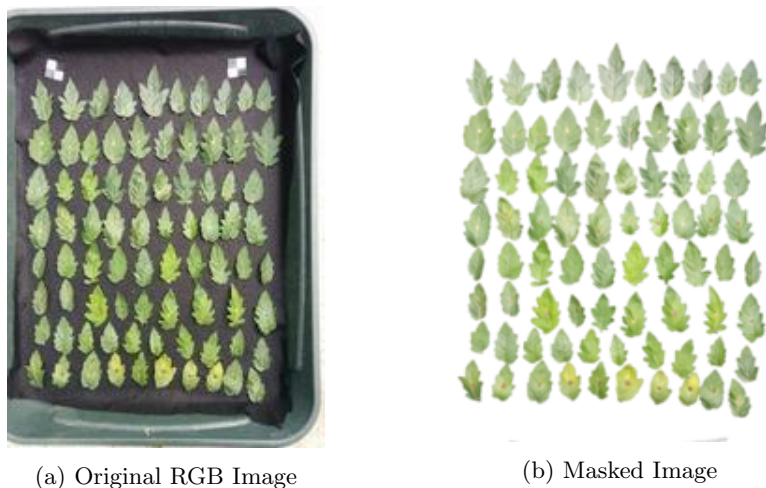


Figure 4: Original RGB Image before and after applying mask

Model comparison

To compare between classifiers, separate test datasets, consisting of 209 duration images, and 74 treatment images were used to obtain predictions. These predictions consisted of a predicted class and a percentage of certainty, which were also used in comparison analysis.

Statistical analysis

All statistical analyses were carried out in R version 3.2.3 (Team 2013). ANOVAS (Analysis of Variance) were used to explore variance between treatments within both datasets.

3 Results

All model results were noted, and models were adjusted as we saw fit; the number of trees in the Random Forest model were adjusted, as were the CNN models learning rates (Table 2). These were changed to assess the impacts on the model accuracies.

All CNN models were stopped between 3000 and 5000 training iterations; accuracies had exceeded 90% by this time, and model loss (the number of inaccurate predictions) was <0.20 (Figure 5). Allowing the model to run for excess iterations did not improve the training accuracy nor did it drastically improve the loss; stopping the models allowed for over-fitting to be minimised whilst also allowing model losses to remain low.

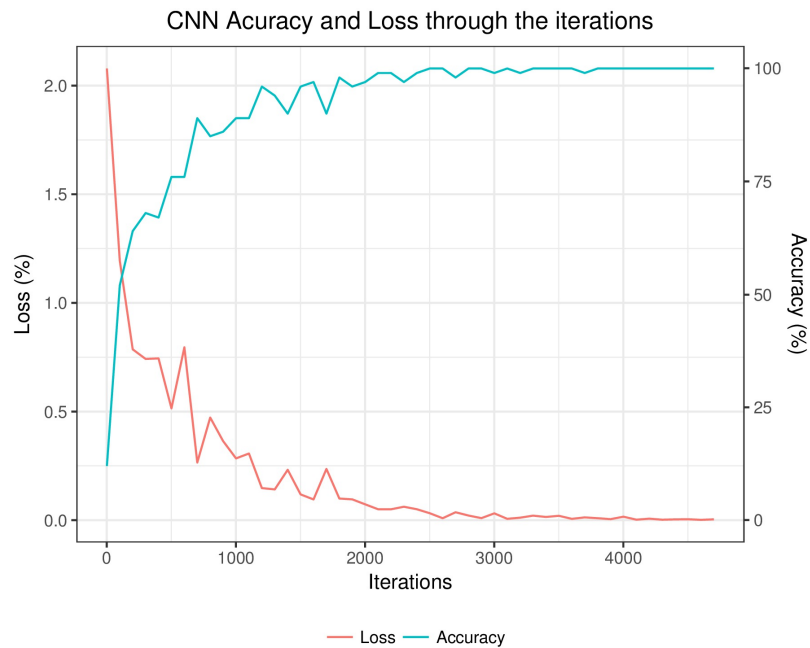


Figure 5: CNN accuracy and loss over 4800 iterations for infection duration classification.

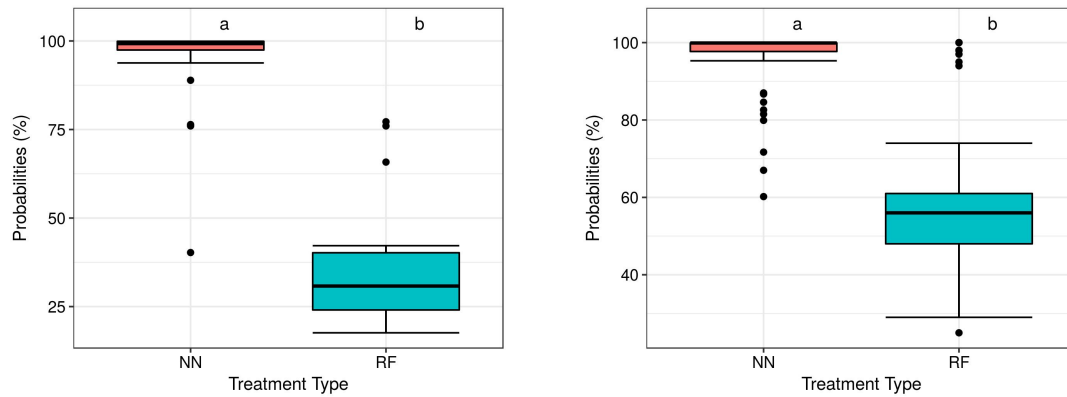
Table 2: Outputs of all models trained on all datasets; training and testing accuracies were compared between models to assess model fit.

<i>Model Type</i>	<i>Training Images</i>	<i>Training Accuracy (%)</i>	<i>Test Accuracy (%)</i>
<i>CNN</i> <i>Learning rate 0.2</i>	Dataset 1	99.1	31
	Dataset 2	99	48
	Dataset 3-NORM	92.2	55
	Dataset 3	93.9	57
	Dataset 4	98.6	94
<i>CNN</i> <i>Learning rate 0.15</i>	Dataset 3	88.3	62
<i>Random Forest</i> <i>100 trees</i>	Dataset 1	99.8	42
	Dataset 2	99.4	45
	Dataset 3-NORM	95.9	57
	Dataset 3	96	62
	Dataset 4	97.9	94
<i>Random Forest</i> <i>500 trees</i>	Dataset 2	99.4	48
	Dataset 3	96.3	60

Multi-stress treatments

Results from the multi-stress classification show both algorithms correctly classify all datasets (1-3), with accuracy increasing with additional colour-pass filters. However, accuracy decreased when using the normalised dataset (3-NORM).

Both RFs and CNNs returned high (>88%) training accuracies for all datasets; training accuracies decreased as datasets contained more colour-pass filter images (Table 2). Despite this, testing/ validation accuracies increased as more colour-pass images were included. Models trained on the normalised dataset (Dataset 3-NORM) returned lower accuracies in comparison to Dataset 3 (Table 2).



(a) Results for treatment models trained on dataset 3. CNNs were significantly more certain ($p < 0.0001$) in comparison to RFs. (b) Results for duration models trained on dataset 4. CNNs were significantly more certain ($p < 0.0001$) in comparison to RFs.

Figure 6: True Positive test accuracy results for RF and CNN models trained on multi-stress dataset 3 and duration dataset 4.

Due to both CNN and RF models returning similar accuracy ratings, the percentage certainties of correct predictions were compared (Figure 6a). The outcome of this comparison was that the CNN models predictions were significantly more certain than that of the RFs (ANOVA: $F_{1,50} = 218.1$, $p < 0.0001$).

Duration Infection

Results from the duration infection classification show both algorithms can correctly classify dataset 4.

Both RF and CNNs managed to return a $>97\%$ training accuracy and a 94% testing accuracy (Table 1); when true positive prediction certainties were compared (Figure 6b), CNNs were found to be significantly more certain in comparison to the RF model (ANOVA: $F_{1,120} = 216.3$, $p < 0.0001$).

Pixel Intensities

Pixel intensities for both multi-stress and infection duration treatments show that all treatments are statistically different from one another, allowing for further support of the models classification results.

When pixel intensities were compared between treatments, both multi-stress and duration infection treatments returned significant results (ANOVA multi-stress: $F_{7,4646161} = 38676$, $p < 0.0001$; ANOVA duration infection: $F_{2,1628925} = 32894$, $p < 0.0001$); Figures 7 and 8 show that all treatment types were significantly different from one another. Further comparison shows that channels significantly differ both within and between treatments (ANOVA duration infection: Appendix

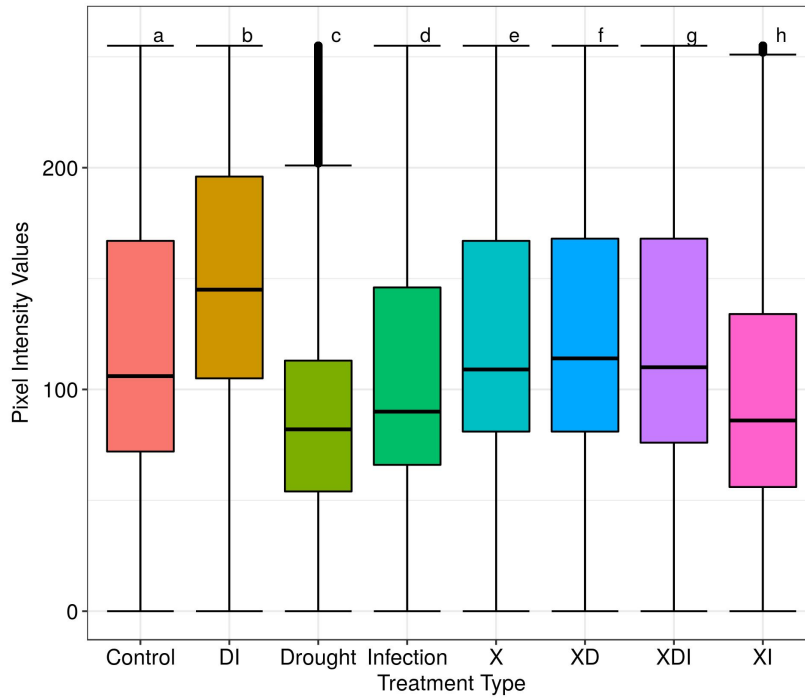


Figure 7: Pixel Intensities of the multi-stress dataset (RGB images only). All treatments were proven to be statistically different from one another.

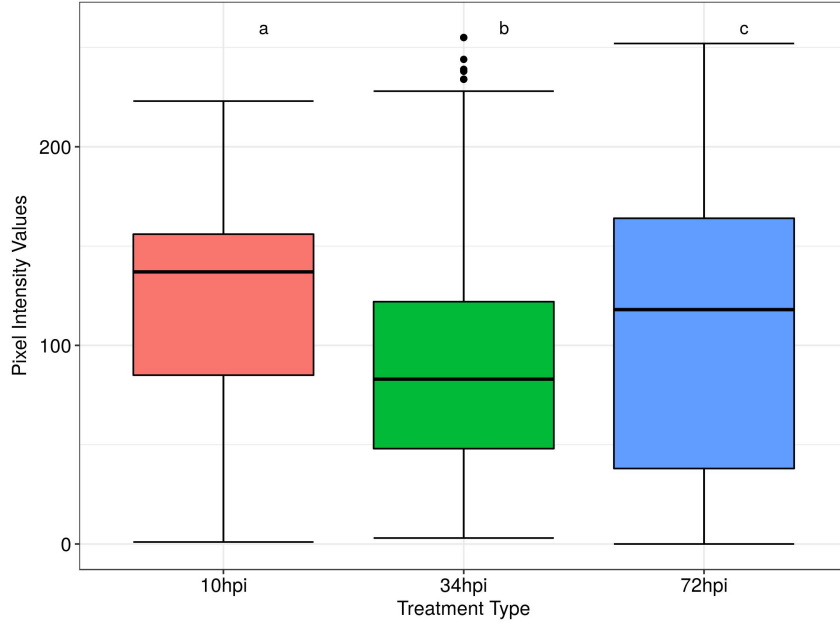


Figure 8: Pixel Intensities for part of the infection duration dataset (dataset 4). All treatments were proven to be statistically different from one another.

Figure 1, $F_{8,1628919} = 3437540$, $p < 0.0001$; ANOVA multi-stress: Appendix Figure 2, $F_{23,4646145} = 17195$, $p < 0.0001$)

4 Discussion

All classification models achieved $>88\%$ training accuracy, testing accuracy ranged from 31% to 94%. Four out of the proposed hypotheses were supported by the data collected; hypothesis 4 (Normalised images will improve model accuracies) was not supported by the data obtained. Furthermore, a website containing some of these models was also built to allow others to test their own images using the models and to allow people to use these techniques in future research.

Multi-stress treatments

The results obtained from the multi-stress classifiers support hypothesis 1; both machine learning algorithms will be able to correctly classify the images with a training accuracy of $>85\%$. Several studies have looked into classifying simple treatment images (see: Baranowski et al. 2015; Mohanty, Hughes and Salathe 2016; Sladojevic et al. 2016; V. Singh and Misra 2017) with classification accuracies ranging from 31.4% to 99.35%; despite this, there are no studies clearly documenting multi-stress classification experiments. Due to this, we believe that our results will be extremely significant both in the field and in future experiments. Knowing the impact that both biotic and abiotic factors have on plant growth, particularly when combined, will become extremely useful in disease identification as well as future treatment measures.

Validation accuracies for models trained on datasets 1-3 were obtained from a mixed test set; several images were collected from various datasets. This may partly explain the lower testing accuracy (Table 1); despite this, the results obtained are indicative of a good overall model, with

the potential to be used in future treatment studies. A Website was created **maybe a link/name?** containing the models created, allowing individuals to classify their own images. We aim to develop this into a fully functioning application to be used in future studies, whilst also allowing other users to add improvements and further train the models on new datasets.

Despite the obvious benefits of CNNs in comparison to RFs, classification results were almost identical (Table 1); the prediction certainties of true positive test results (Figure 5a) show that CNNs produce a significantly higher percentage certainty in comparison to RFs. This then allows support for hypothesis 5; CNNs are better classifiers in comparison to RFs. This has already been noted in several studies, with Baranowski *et al* (2015) noting a classification rate of 55% for a RF model, and 80% for a Back-Propagation NN. With many ways to interpret a models output, it can often be difficult to settle on one statistic; it is believed that by producing several model comparison statistics, a more general view of the model can be obtained. Calculating percentage certainties when model outputs appear similar allows for the user to be more certain in the case of an unknown test image, whilst also allowing for another means of comparison when comparing known images.

The use of colour-pass filters increased the models accuracy (Table 1); hypothesis 3 was thereby supported by this study. Colour-pass filters block out their colour specific wavelengths, and have been used before in an ecological classification survey (Knoth et al. 2013). By using them in classification studies, the object in question can come into focus whilst also allowing certain aspects of the object to become more apparent, as shown in Figure 2. Due to the price and accessibility of colour-pass filters in comparison to Hyperspectral imaging cameras, as well as the results obtained from this study, we recommend that colour-pass filters be included in more classification studies to allow for greater data to be brought out of a dataset. Due to the ease of applying a colour-pass filter to a dataset, this method will also allow for the wider botanical and agricultural community to apply these techniques in future research.

Training and testing on a normalised dataset resulted in models of a lower accuracy than identical models trained on non-normalised images; normalised images appeared to have a negative effect on classification rates, thus resulting in the rejection of hypothesis 4. It is possible that the process of normalising an image distorts the feature space of the image, thus making it harder for a model to classify. It is also possible that the method used to normalise the images was not sufficient; future studies should look into a wider array of normalising approaches, so as to ensure sufficient evidence has been obtained before making any compelling statements.

Duration Infection

The results of both models trained on the duration infection dataset support hypothesis 2; RFs and CNNs will be able to correctly classify the images with a training accuracy of >85%. Another study, conducted by Zhu *et al* 2016, also looked into classifying infection duration images; classification accuracies of up to 95% were established. Their study noted that is it hyperspectral imaging that has the potential to aid presymptomatic disease detection; our results indicate that even simple RGB images have the ability to advance detection. Being able to classify between healthy, 10hpi and 72hpi leaves will greatly improve presymptomatic treatment; applying this technique in the field will allow for more precise pesticide and insecticide application, which is beneficial to both the community and the environment.

As in multi-treatment classification, the results for both models were almost identical (Table 1), whilst the prediction certainties (Figure 5b) show that CNNs produce a significantly higher percentage certainty in comparison to RFs. This then allows for further support for hypothesis 5.

Pixel Intensities

Pixel intensities for both datasets show that each treatment was significantly different to all other treatments within that dataset (Figures 7 and 8); this allows for further support of the classifiers built, by acknowledging that they are classifying on relatively discrete and distinct treatments.

Appendix figures 1 and 2 show a more detailed view of the pixel intensities for each treatment. Two clear patterns are observed in Appendix Figure 1; the blue channel in the infection duration study significantly decreases as time increase, whilst the green and red channel significantly increase at 72hpi. This is likely an indication of ongoing infection, with the overall leaf colour changing from a green in 10hpi to a yellow/brown in 72hpi. Between 34 and 72hpi it is likely that chlorosis began, thus resulting in an increase in intensity in the red and green channels in the 72hpi treatment.

5 Conclusions

There has been a noteworthy increase in recent years in the use of digital pathology techniques. Plant disease detection and management are important activities for both agriculture and horticulture; by utilising digital techniques to identify diseases, it allows for quicker interventions on the infected individual(s).

This study shows that it is possible for relatively simple machine learning methods to identify both complex stress treatments, and duration of infection. These techniques will allow farmers, growers and horticultural enthusiasts to begin earlier treatment, thus minimising crop loss and reduced crop quality.

This study also sheds light on the impact that both colour-pass filters and image normalising techniques have on digital classification. These findings should benefit future research and agricultural practises alike.

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6 Appendix

Table 1: Colour-pass filters used in the multi-stress study

<i>Roscolux Filter Number</i>	<i>Colour Name</i>
#3407	Roscosun
#4590	CalColor 90 Yellow
#12	Straw
#318	Mayan Sun
#4690	CalColor 90 Red
#4890	CalColor 90 Pink
#4760	CalColor 60 Magenta
#4790	CalColor 90 Magenta
#39	Skeleton Exotic Sangria
#41	Salmon
#47	Light Rose Purple
#4930	CalColor 30 Lavendar
#59	Indigo
#3220	Double Blue
#65	Daylight Blue
#2005	Storaro Cyan
#77	Green Blue
#378	Alice Blue
#80	Primary Blue
#4490	CalColor 90 Green
#93	Blue Green

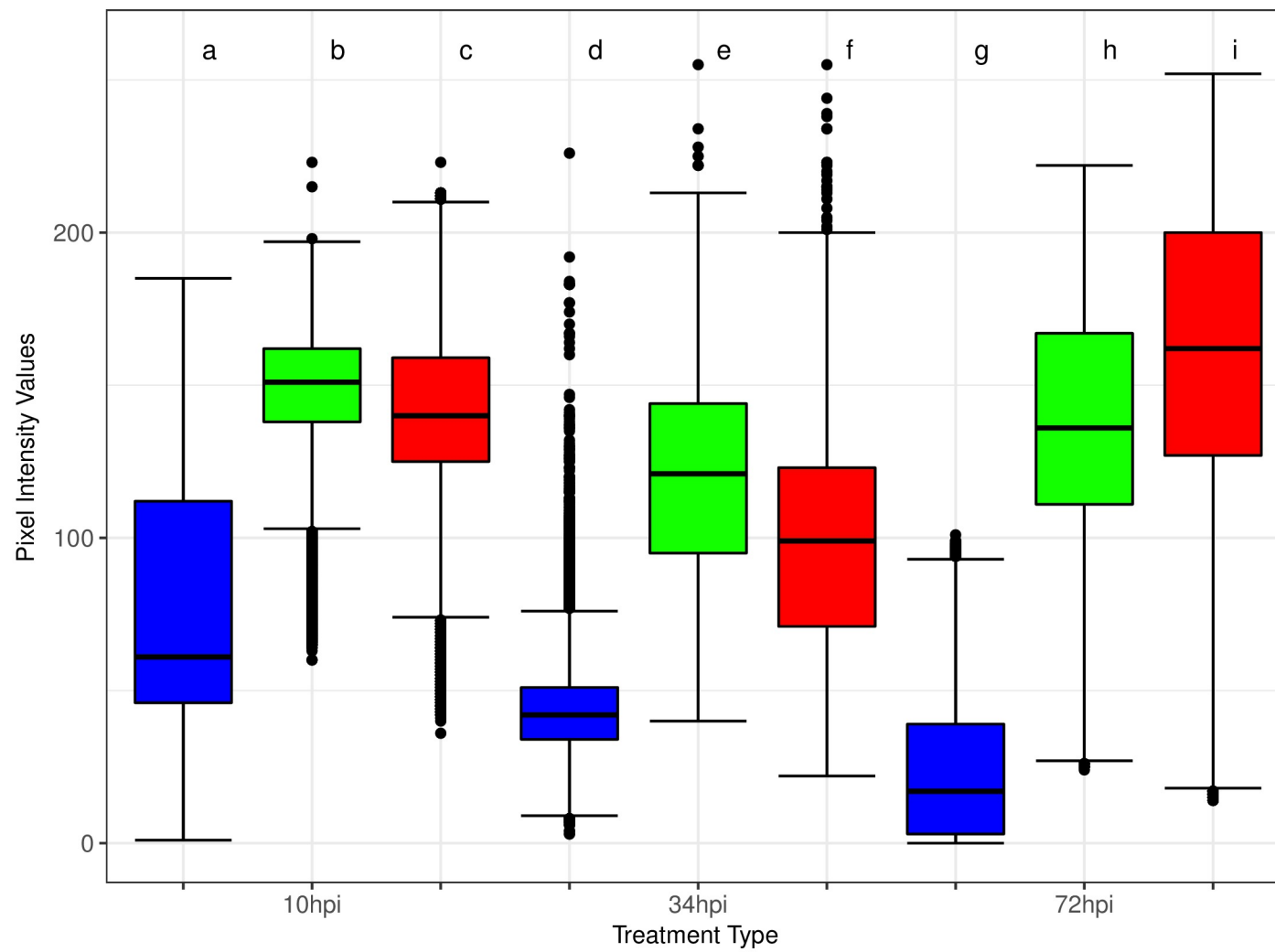


Figure 1: True Positive test accuracy results for RF and CNN infection duration classifiers.

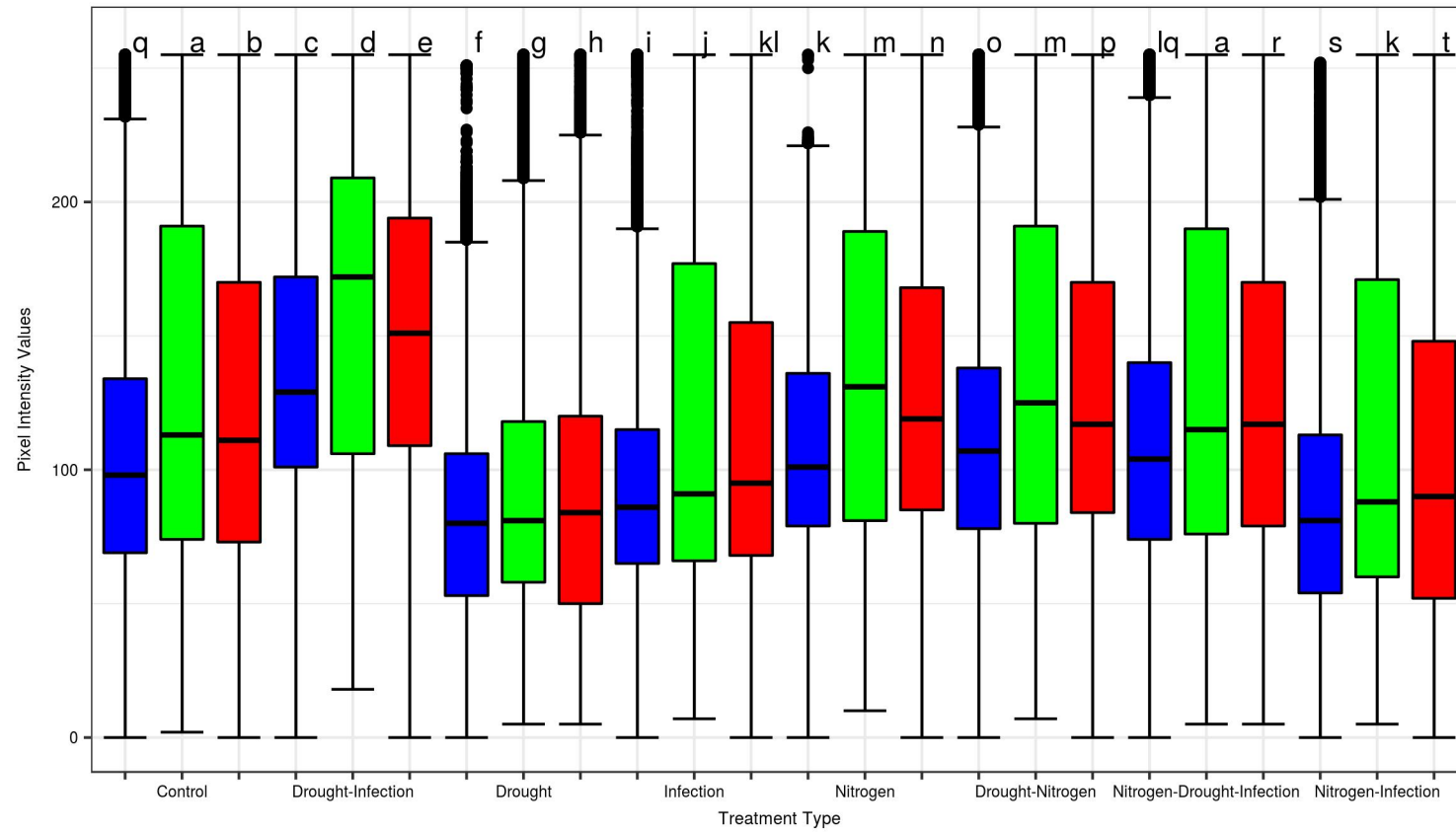


Figure 2: True Positive test accuracy results for RF and CNN treatment classifiers.

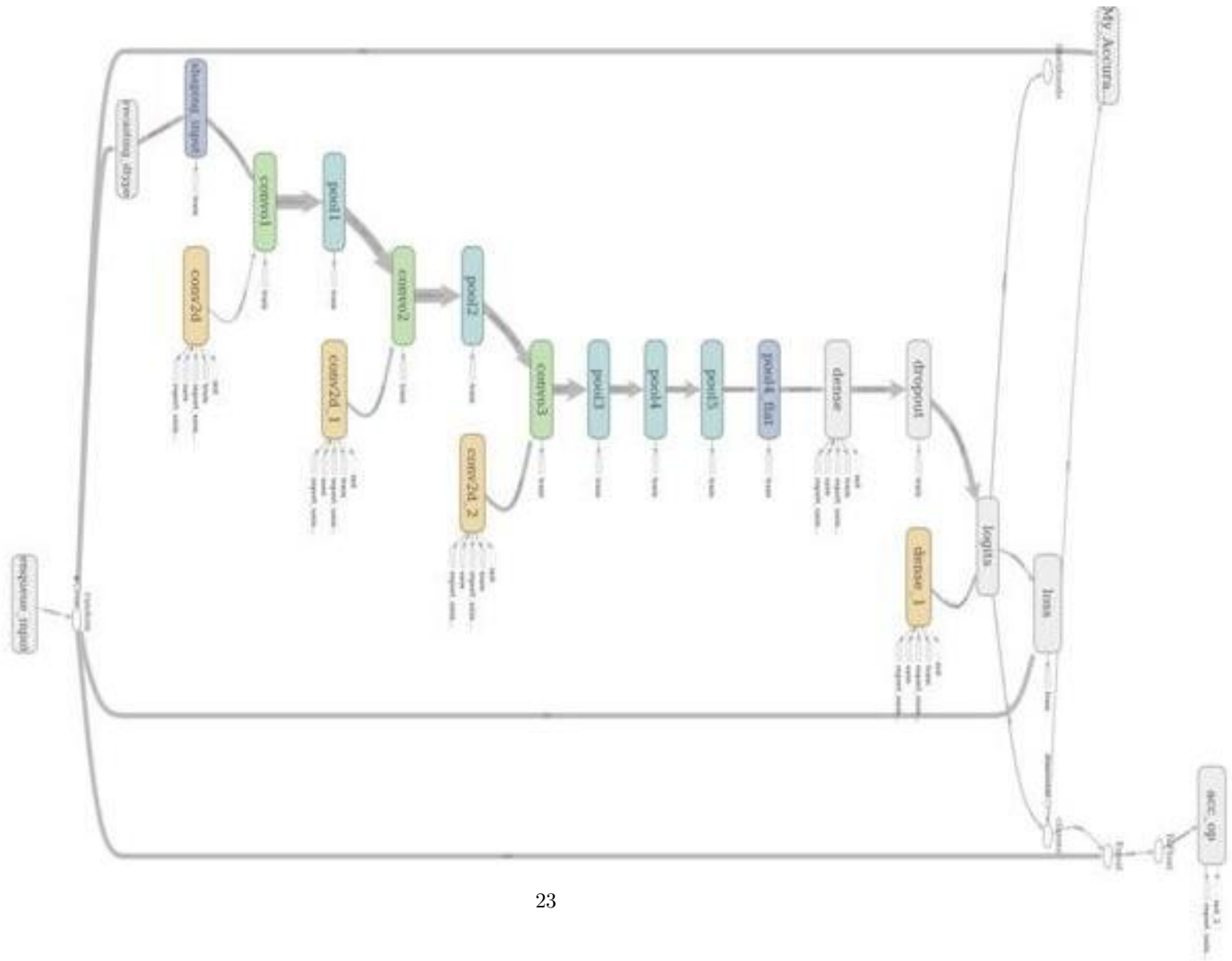


Figure 3: Layout of the CNN used.