DETECTION OF LATENT POTATO LATE BLIGHT BY HYPERSPECTRAL IMAGING.

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ABSTRACT

Phytophthora infestans causes late-blight in potatoes. Without control *P. infestans* causes severe damage to the foliage and tubers, leading to yield loss. A way to suppress the disease without chemical control would be to remove the primary inoculumm sources originating from infected seed tubers or oospores in the soil. These latently diseased plants, if detected before symptom expression and sporulation, could be removed. To do so early detection is required. A pot-experiment to detect latent late blight using hyperspectral imaging was conducted. Several inoculation rates and both spray inoculation and point inoculation were used. The spectral signature of the soon to die tissue was learned which enabled early detection of latent potato late blight, well before it was detectable by a trained human eye.

1. INTRODUCTION

Phytophthora infestans causes late-blight in potatoes. Without control *P. infestans* causes severe damage to the foliage and tubers, leading to yield loss. To suppress the disease without chemical control removal of the infected plants is an option. Therefore, it is necessary to detect infected plants before symptom expression and sporulation of *P infestans* occurs. Primary inoculum sources for potato late blight are latent infected seed potatoes and soil borne inoculum in the form of oospores. Secondary inoculum comes from infected volunteer potatoes and neighbouring potato crops. In the beginning of the season primary sources are the main factor, while in the course of the season secondary inoculum sources take over [8]. Alternative hosts for *P. infestans* are of no importance in the Netherlands. Generally, potato late blight is controlled by applying fungicides and growing cultivars which are less susceptible to *P. infestans*. To interrupt the disease cycle it would be interesting if we could take out the primary inoculum sources. Typically the first potato plants to become infected in a potato crop are the ones originating from infected seed potatoes. If we are able to detect and remove these potato plants before symptoms expression no transmission of inoculum to neighbouring plants would occur. Consider a rough estimation that maximum 1 in 1000 seed tubers might be infected. Shattock [5] showed a transmission rate from infected seed tuber to infected potato plants between 1.5% and 5% depending on the isolate used. Ware potatoes are often planted at a density 40000 tubers per hectare, which would mean that potentially 2 potato plants on a hectare need to be detected. Hands on experience suggest that the disease incidence and transmission rate from infected tubers to plants is even lower.

To detect those few infected plants techniques should be low cost and non-destructive. All kind of autonomous platforms have been developed to enable disease detection in the field [2]. Detection of virus infected potato plants by hyperspectral technology and artificial intelligence was successfully developed under laboratory conditions. The research of Polder et al [3] proved that disease symptoms caused by PVY virus could be detected with machine vision techniques using hyperspectral cameras with a precision which was almost equal to the accuracy of an experienced crop expert under field conditions. Similar techniques are being developed in the cultivation of flower bulbs, especially for tulips [4]. For potatoes an extension is foreseen for detecting bacterial blight with hyperspectral cameras. For tomatoes *P. infestans* infections of tomato plants have been predicted using a spectral range from 750 to 1350 nm. Adding a technique for early detection of potato late blight would be complementary to virus and bacteria detection in potatoes.

For this work we conducted a greenhouse experiment to detect latent late blight using hyperspectral imaging. In 2019 some preliminary work was performed. Using a camera having a spectral range of 400-1000 nm, images of 90 plants that were spray inoculated with 3 different densities, were taken on the 4 days after inoculation. There did not seem to occur a decoloration effect on the inoculated plants, even though most of the inoculated plants died within a week. Therefore, another experiment was set up in which on parts of the plants the location of the inoculation was known: so not only spray inoculated plants were imaged but also a series of plants that were point inoculated. The disease spread clearly from this point allowing to find the spectral signature of the tissue that was going to express symptoms within the following 24 hours and die. These signatures have been tested on non-, low- and

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high-inoculated plants. Allowing to detect the disease a day before it is visible by a trained human eye. Given that in a greenhouse the life cycle is usually shorter than in the field these are promising results for future developments.

2. METHOD

2.1. Plant preparation

The cultivated potato plants (cv. Bintje) were grown in pots. The pots with a content of 5 litres were filled with potting soil and the potato tubers were placed at a depth of 10 cm. In total 80 potato plants were raised. From emergence until inoculation the plants were placed in the greenhouse at Lelystad. A *P. infestans* isolate belonging to the EU-13-A2 (Blue-13) clonal lineage was used. This isolate was chosen because it belongs to one of the most important genetic groups of *P. infestans* in Europe. The isolate was stored in liquid nitrogen at Wageningen University & Research business unit BioInteractions & Plant Health until use. From the isolate a plate culture was made. The inoculum suspension was made by rinsing a one week old culture of *P. infestans* with water. The inoculum density was set at approximately 10,000 zoosporangia/ml and 3,000 zoosporangia /ml. Inoculation was carried out by 20 spraying potato plants with approximately 10 ml of inoculum per plant of either spore suspension. Additionally, potato leaves of a fourth batch of 20 plants were inoculated with a droplet of 100 µm of the zoosporangia suspensions of 10,000 sp/ml on the leaf at 10 different spots. On each of the plants one leaf was inoculated. Inoculation was carried out on air dry plants. The experiment was inoculated on 31 March 2020, hence 1, 2 and 3 April correspond to the 1st, 2nd and 3rd day after inoculation. This way 4 groups of 20 plants each were created: UTC sprayed with water only, LOW (3000 spores/ml), HIGH (10000 spores /ml) and POINT (10000 spores /ml). After inoculation the plants were placed in a climate room at 18°C and high relative humidity during 12 hours to allow for infection. On 1 April the plants were transported from the climate chamber to a greenhouse in Wageningen. Disease observations were carried out four times. The percentage necrotic foliage per plant was estimated, 1, 2, 3 and 6 days after inoculation. The standard area under the disease progress curve (StAUDPC) was calculated. The trial was layed out as a randomized block design with 20 replicates. Analysis of variance was carried out using Genstat 19th ed. Disease severity data were log10(X+1) transformed to meet the requirement for a normal distribution and back transformed.

2.2. Image capturing

The images were taken in a dark tent in the glasshouse using an Imec Snapscan-vnir camera , with a spectral range of 467 to 900 nm. Illumination was provided by 6 15 Watt halogen light bulbs that were mounted next to the camera. The camera was placed about 30 cm above the plant facing down. At the beginning of the day, after heating up of the halogen lights and when the camera had been switched off the white balance was captured with white spectralon. The plants were ordered in blocks, each containing the four inoculation densities in a random order. The plants were ordered in 20 blocks, each containing the four inoculation densities in a random order. Every day the plants were captured from block 1 to block 20, starting around 8am and finishing measurements around 2pm.

2.3. Data analysis

Of all the blocks, 14 randomly selected were used for training and 6 were used for testing. From the images pieces of these leaves were taken on the same part of each leaf. In figure 2 A, an illustration of such a piece is presented. The data-processing pipeline comprised several steps, as is described below. A schematic overview of the steps taken is in figure 1.

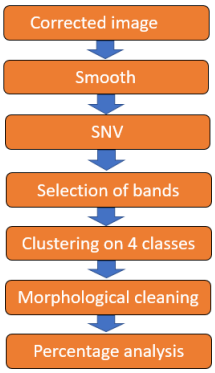

Fig. 1. The processing flow of the data analysis

The camera suffered from some noise, therefore, in a first step the signal was smoothed using a Savitzky-Golay filter of window length 7 and a polynomial order 3. The complex geometry of the plants cause illumination effects, which cause spectral variability that are unrelated to the plants reaction towards the *P. infestans* inoculation. In [6, 1] it is shown that standard normalized variate (SNV) is a good way to compensate for these illumination effects. The SNV is defined as:

$$\mathrm{SNV}(X) = \frac{X - \mathrm{mean}(X)}{\mathrm{std}(X)}, \qquad (1)$$

where X is a vector that the describes a pixel of the smoothed corrected image, $\mathrm{mean}(X)$ and $\mathrm{std}(X)$ are the mean and standard deviation of that vector, respectively.

The point inoculated plants developed clear diseased signatures, with the disease spreading over the plant. Therefore, pieces of the leaves that would show symptoms in the next 24 hours could clearly be marked. Also, part of point inoculated leaves that still visually looked as uninfected, was sampled

under the name *ok*. The procedure is illustrated and explained in figure 2.

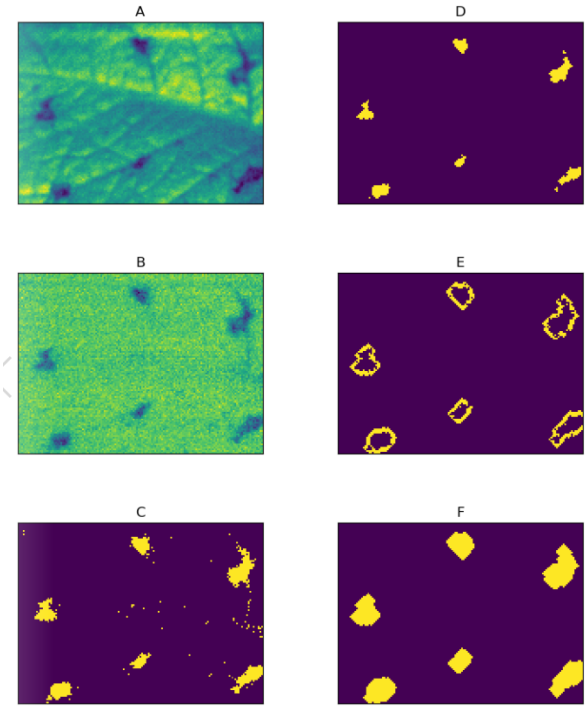

Fig. 2. In A) the piece of the leaf at band 83 that corresponds to 738.314nm, in B) the SNV of the original image in band 83, in C) the threshold of the SNV, in D) the erosion of B these yellow pixels are classified as infected, F) the dilation of E) the purple pixels here are classified as *ok* and E) the difference between D) and F), these pixels are classified as *ring* pixels.

In this way pixels of the four classes were sampled, with between the brackets the number of pixels from the training and test plant respectively: *infected* (2036,2157) *ring*(5432,3284), *ok* (171284,74689) and *utc* (247987,95549). Where *utc* comprised pixels of the plants that were not inoculated. All these pixels were collected from the images taken on the 2nd day after inoculation.

From these pixeldata the relevant bands were selected, by taking the mean values of the *ring* and *ok* pixels and computed the difference. Those bands for which the difference was larger than 90% of the maximal difference were selected. In that way 14 bands were selected corresponding to 735.571, 748.512, 751.165, 755.835, 758.651, 762.101, 764.902, 765.767, 768.447, 889.988, 891.896, 895.393, 897.454, 900.239 nm. See figure 3.

Restricting the pixels to those bands a clustering algorithm was trained to classify the pixels in the four classes. As clustering algorithm a random forest was used. To get balanced training and test sets and to avoid over-fitting not all collected pixels were used, but 5432 training pixels for each class except *infected* selected at random and all *infected*. For

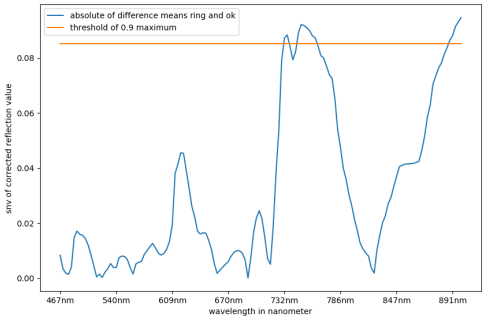

Fig. 3. Selecting the relevant bands were the difference occurs between the *ring* and the *ok* data.

testing the number of pixels was 3284.

The next step was to use the model to classify the pixels in the images. The images created as such were slightly noisy so we removed the noise by morphological closing of the pixels that were classified as *utc* or *ok*. In figure 4 the results of the clustering before and after noise reduction is illustrated, on pot 78 which was a plant in the test set.

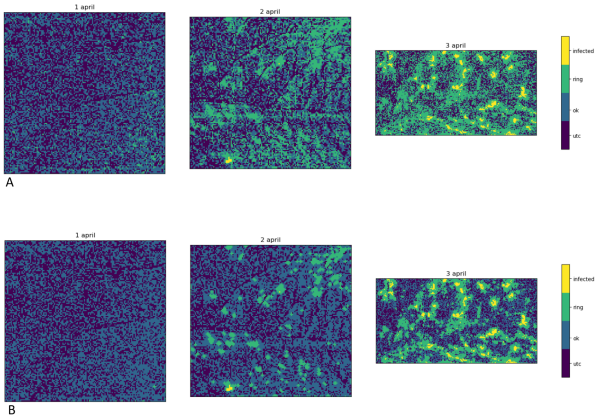

Fig. 4. Pot 78 with high inoculation dose, and phy1dpi = 0.0, phy2dpi = 0.0, phy3dpi = 25, A before noise reduction and B after noise reduction.

Finally a statistical analysis between the percentages of pixels falling in the classes *utc* and *ok* was performed. Those two classes were bundled as the classifier showed a lot of confusion between the two type of pixels. For the plants that were not inoculated these percentages were almost always above 99%, while the percentages of the other groups of plants showed more variance. Therefor, to test and quantify the difference, a Welch test was used [7]. For this testing we used all plants, that is, both the plants used for training the classifier and the plants that were used for testing.

3. RESULTS

The inoculated potato plants were readily infected by *P. infestans*. In fact disease incidence was 100 % for the inoculated plants, whereas the untreated control showed no symptom expression. Disease severity based on the StAUDPC differed significantly between groups, see table 1.

treatment	*StAUDPC*	Disease severity (%)			
		1 dpi	*2 dpi*	*3 dpi*	*6 dpi*
utc	$0.0a$	$0.0a$	$0.0a$	$0.0a$	$0.0a$
high	$32.5c$	$0.0a$	$0.6b$	$24.3c$	$94.5c$
low	$25.3b$	$0.0a$	$0.5b$	$10.5b$	$83.3b$
point	$50.7d$	$0.1b$	$11.9c$	$68.2d$	$99.9d$
F. Prob.	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 1. The disease severity. Dpi stands days past inoculation. Values followed by the same characters are not significantly different (P = 0.05)

At day 1 dpi symptom expression was none fore utc, high and low and minimal for point inoculation. Both disease severity and disease incidence increased in the following days.

The confusion matrix of the random forest algorithm trained on the pixels in the classes *utc*, *ok*, *ring*, *infected* is given in table 2.

The *infected* pixels are clearly separable from the other pixels. However, the *utc* and *ok* are confused almost as if they are randomly distributed between the two classes. From the *ring* pixels 78% are correctly assigned, which shows that these indeed differ from the other kind of pixels. In particular because in the selection method of *ring* pixels and *ok* some randomness was involved; it is not exactly possible to see which pixels correspond to tissue that is going to show *P. infestans* symptoms and subsequently die in the coming 24 hours. Therefore, some confusion between *ring* and *ok* was expected.

For the testing of the percentages pixels that were classified as *utc* or *ok* a Welch test was performed table 3. Similar to the more well known t-test it produces a P-value, where a low P-value (< -0.01) corresponds to rejection of the hypothesis that there is no difference between means of the two tested groups. See figure 5 for a box-plot illustrating the differences between the classes at the first, second and third day after inoculation.

		Predicted			
		utc	*ok*	*ring*	*infected*
Actual	*utc*	1813	969	121	30
	ok	1218	1648	531	3
	ring	253	667	2555	210
	infected	0	0	77	1914

Table 2. The confusion matrix of the random forest.

p-value	low 1 april	high 1 april
utc1 april	0.5552	0.3669

p-value	low 2 april	high 2 april
utc 2 april	0.0011	0.0073

p-value	low 3 april	high 3 april
utc 3 april	0.0001	$2.1 * 10^{-6}$

p-value	1 april versus 2 april	2 april versus 3 april
utc	0.3693	0.3221
low	0.2081	0.0019
high	0.0073	$6.5 * 10^{-5}$

Table 3. The values between the different groups and days. The values are computed using a Welch test.

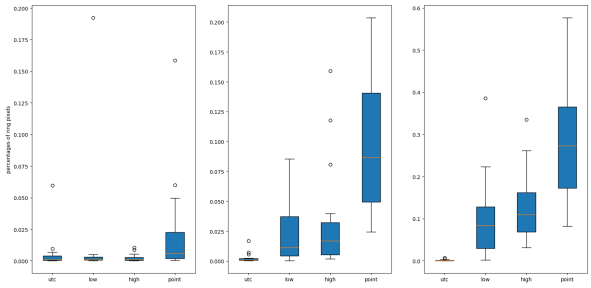

Fig. 5. Box plots of the percentages of pixels that are classified as ring, on the first, second and third day after inoculation.

4. CONCLUSIONS

This study shows that it is possible to detect latent late blight using close range hyper spectral imaging techniques. The spectra in which the difference is detectable are given above. Detection is possible two days after inoculation and one day before it is detectable by a trained human eye. One day after inoculation detection is not possible.

5. DISCUSSION

The objective of this study was to detect potato late blight before symptom expression in the potato plant. The potato plants were successfully inoculated, as a matter of fact all inoculated potato plants showed symptoms at the end of the experiment, whereas the water treated plants showed no symptoms. The rate of symptom expression was depended on inoculation method and inoculum density. Drop inoculation led to quicker symptom expression and higher disease severity than inoculation with a spore suspension by misting. The high spore density led to a higher disease severity than inoculation with a low spore density. The StAUDPC describes the potato late blight epidemic in 1 digit and it shows that the late blight epidemic was significantly more severe after point inoculation than inoculation with a spore suspension.

In addition the late blight epidemic was also significantly depended on the inoculum density. As detection technique in this study hyperspectral imaging was chosen. It is a relatively new, non-invasive, potentially cheap technique. Already several studies have shown that it can be used in the field [3, 4]. As it gathers spectra impossible to detect by a human, early signals could be expected, and were indeed found. In this experiment we have shown that we can detect potato late blight 24 hours before symptom expression under greenhouse conditions using a hyper spectral camera. In this experiment the first symptoms were found by a trained human eye two days after inoculation. Under field conditions symptom expression is typically slower and takes 3 to 4 days depending on average temperature in the field, which gives us potentially more time to detect latent diseased potato plants. The question remains whether we pick up a signal of the plant to the infection of the pathogen or the pathogen itself. And if the plant responds to the infection whether this response is systemically or local around the infection point. In this study we started to address infection of the leaves after inoculation. The reason for that is that our first goal was to detect a response. Obviously under field conditions we have to deal with natural infection originating from infected seed tubers, oospores or zoosporangia blown in from elsewhere. The first expression from latent tuber infection and oospores as inoculum sources is usually stem blight and occasionally leaf blight. It remains a question whether the potato plant responds and if so whether this signal is local or systemical. In future experiments we aim to detect potato late blight in potatoes before symptom expression after inoculation of seed tubers.

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