Detection of the Tulip Breaking Virus (TBV) in tulip using spectral and vision sensors

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Abstract

Experiments are described for detection of Tulip Breaking Virus in tulip plants. Four optical techniques were investigated and compared with visual assessment by crop experts as well as with Elisa (Enzyme ImmunoAssay) analysis of the same plants. The optical sensor techniques used were: an RGB Color camera, a spectrophotometer ranging from 400-2400 nm, a spectral imaging camera covering a spectral range from 400-900 nm and a chlorophyll fluorescence imaging system which measures the photosynthetic activity. Linear discriminant classification was used to compare the results of these optical techniques with the Elisa score and the visual assessment. A good correlation was found when the results of the spectral camera and the results of the visual examinations of the crop expert were compared. These are very promising results which are translated in a follow up with as final goal an autonomous robot for detection and removal of diseased tulip plants in the open field.

Keywords: plant virus, image processing, hyper spectral imaging, spectroscopy, machine vision

Introduction

Tulip and other bulbous ornamental crops are plagued by viral diseases. One of the most important viruses in tulips is the potyvirus TBV (Tulip Breaking Virus). The virus symptoms can manifest itself in different ways, including striping of the leaves and abnormal flowers (Dekker *et al.,* 1993). The presence of virus causes a reduction of the quantity and especially the quality of the product and leads to sales and export restrictions. Current methods to keep the disease under control are twofold. Firstly, plants are sprayed with chemicals to control aphids, which spread the virus (Asjes and Blom-Barnhoorn, 2001). Secondly, crop experts go through the field and remove symptomatic plants as observed visually. The total costs of the viral problem in the Netherlands is estimated at over 9 M€ yearly.
A large problem with visual assessment of infected plants is, that the symptoms are often difficult to see and require a expert eye. The visibility of the symptoms is also largely influenced by the cultivar of the tulips, the weather conditions and can only be seen during a limited period of the growing season. This causes a high peak of labor of trained personnel, which is difficult to find. In order to reduce the amount of chemicals for aphids and the high labor pressure, various alternatives, were studied and it was concluded that presently the best possibility to control the disease was by means of imaging techniques to automatically find infected tulips. In this study, our final objective is to develop a robot system which automatically detects and removes diseased plants in the field. As a first step a feasibility study was carried out to test the detection performance of several optical sensors for virus symptoms in tulips under laboratory conditions.

Experimental setup

Three tulip varieties (Barcelona, Monte Carlo, Yokohama) with high TBV infection rates, as assessed in the former breeding season by Elisa, were used. The established infection rates for the bulb lots were 14% in Yokohama, 16% in Barcelona en 31% in Monte Carlo. Per cultivar between 400 and 800 plants per cultivar were planted in small plastic baskets and buried in the field.
Early in the growing season individual plants were visually assessed and marked when TBV symptoms were present. Afterwards, leafs of about 100 visually healthy and 100 visually infected plants were measured using four different vision sensors.
An Elisa (Enzyme ImmunoAssay) analysis using TBV-specific antisera and a validated protocol (Derks *et al.,* 1982) was carried out on the same leaves of the measured plants. These measurements were used as the reference analysis.

Optical techniques

Four different optical sensor techniques were assessed in this study: an RGB Color camera, a spectrophotometer ranging from 400-2,400 nm, a spectral camera ranging from 400-900 nm and the Multiple Imaging Plant Stress (MIPS) system (Jalink *et al.,* 2004; Polder *et al.,* 2007), which measures the photosynthetic activity using Chlorophyll fluorescence.

RGB Color camera
From each leaf a digital picture was taken under controlled light conditions. The camera used was a Nikon D70 with a Nikon 18-70 mm zoom lens in a closed cabinet equipped with high frequency fluorescent illumination (Osram Biolux daylight tubes). To correct for possible changes in the illumination, a Macbeth color chart was put in each image. This makes it possible to check whether there were color changes and to correct for them if needed. To identify each leaf, each image was coded by putting a 2D QR-barcode in the image. In Figure 1 a typical image is displayed.
After recording, the images were segmented and a number of shape parameters were calculated for each leaf. Table 1 gives a list of the calculated shape parameters.
Infected plants very often have a red/purple spot pattern on the leaves. These spots were quantified using color segmentation. Afterwards the total area of the spots, the total perimeter of the spots, and the number of separated spots were calculated (Figure 2).

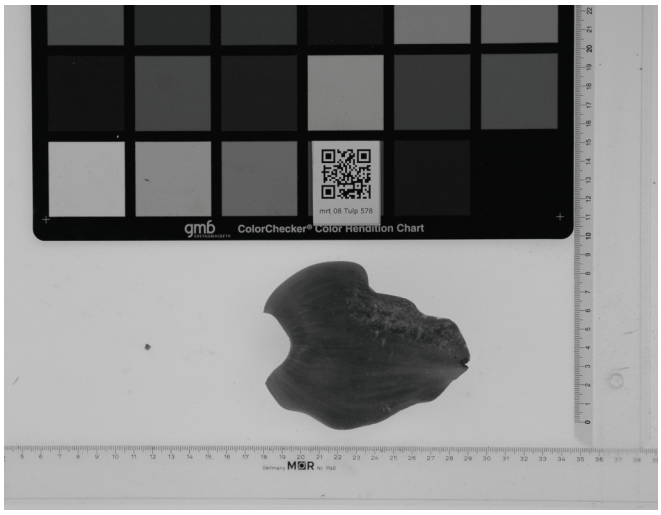

Figure 1. RGB image of tulip leaf, Macbeth color standard and barcode.

Table 1. Calculated shape parameters.

Name	Description
Dimensions	dimensions (length, width, depth) of the object
Mean	mean object intensity
StdDev	standard deviation of object intensity
Size	number of object pixels
Perimeter	perimeter length of the object
Inertia	moments of inertia of binary object
Mu	elements of the inertia tensor
CCBendingEnergy	bending energy of object perimeter (chain-code method)
P2A	circularity of the object (perimeter^2/area)
PodczeckShapes	Podczeck shape descriptors

Figure 2. Image of infected tulip leaf (left), segmented color pattern (middle) and contour pixels of color pattern (right).

Spectrophotometer

Using a spectrophotometer the reflection spectrum of two spots was measured on each leaf. The spectrometer used was a FieldSpec Pro FR spectroradiometer from Analytical Spectral Devices (ASD). The total range was (350-2,500 nm), with a resolution of 3 nm in the visible range and 10 nm in the infrared. A leaf clip was used to measure the reflection spectrum of a circular part of the leaf with a diameter of 2 cm in a standardized way.

Since the spectrophotometer consists of three different sensors, sometimes a small mismatch between the adjacent spectral regions was present. Each spectrum is corrected for this mismatch before further processing using standard protocols. The spectrophotometer outputs 2,151 data points per spectrum. This is far too much for classification and also much more than the physical resolution of the sensors. Therefore the data were reduced to 40 points, using Savitzky–Golay smoothing and sub sampling (Savitzky and Golay, 1964).

The analysis of the spectrophotometer data was needed to answer two questions: can we distinguish between healthy and diseased plants using only spectral information and secondly how informative are the data in the various parts of the spectrum, especially in the (near) infrared region. In order to answer these questions the difference between the mean spectrum of the healthy and diseased plants was investigated. Also the total spectral region was subdivided in small spectra of 100 nm, with an overlap of 90 nm. The height of the classification error of these subsets is an indication of the importance of the subsequent spectral region.

Spectral camera

Whereas the color camera only has a red, green and blue value per pixel, the spectral camera gives the complete reflection spectrum from 430-900 nm with a resolution of 4.5 nm. The spectral imaging system is build around an imaging spectrograph from Spectral Imaging Ltd (Specim). A detailed description of the system has been published elsewhere (Polder *et al.,* 2003). Figure 3 depicts an example of spectral image data, showing images at three different wavelengths as well as the reflectance spectrum of one pixel.

The same shape features as with the RGB images were calculated. The red/purple spots were quantified by dividing the sum of the images from 560-590 nm by the sum of the images from 740-780 nm.

Chlorophyll fluorescence imaging system

The chlorophyll fluorescence imaging system measures the photosynthetic reaction on stress factors. The output of this system is an image where each pixel value gives the Photosynthetic Efficiency (PE) between 0 and 1. The system is developed at our institute by Jalink, (2004). The images were analyzed by calculating the mean and standard deviation for each leaf. Furthermore thresholds were applied with small differences in PE, e.g. 0.4-0.5, 0.5-0.6 etc. The size of the objects after these thresholds was used in the classification. Figure 4 gives an example.

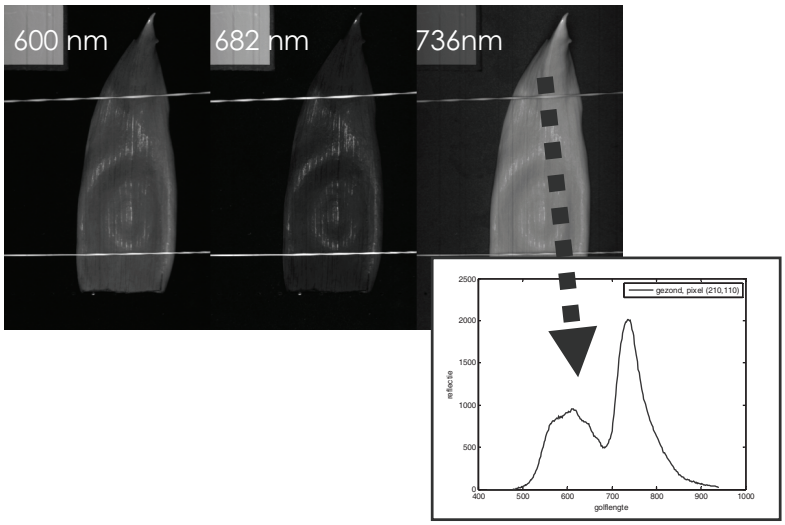

Figure 3. Part of the spectral image of a tulip leaf. Only three images are shown. In reality 257 images at wavelengths from 430-900 nm are available. The spectrum shows the reflection of all wavelengths at the position of the selected pixel.

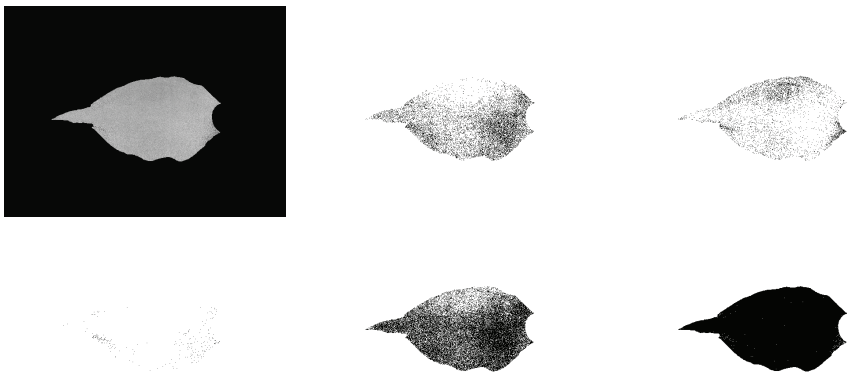

Figure 4. Photosynthetic efficiency image (upper left) and segmented images at different threshold values.

Data analysis

Each optical technique described above gives a list of 10-40 features. These features were used in the data analysis. Linear discriminant analysis (LDA) using leave one out cross validation was used to predict whether a plant is healthy or diseased. LDA is a so called supervised technique. A model is trained using the features of known healthy or diseased leaves. As ground truth (ultimate reference) the Elisa measurements were used. The training algorithm calculates the optimal separation function. In leave one out cross validation all the samples except one are used for training and the remaining sample is used for validation. This is repeated such that each observation in the sample is used once as the validation data.

Results

Visual assessment

Table 2 gives the score of the visual assessment by the crop expert compared to the Elisa score, the latter considered as the ground truth. Columns two and three give the number of plants which were scored as healthy and diseased by both the Elisa test and the crop expert. Columns four and five gives the number of plants which were diseased and healthy according to the Elisa test, but were scored as healthy and diseased respectively by the expert. The last column gives the total percentage error.

Color camera

Analysis of the RGB values of the Macbeth color standard showed minimal differences between images. We concluded that the illumination was consistent and correction between images was not needed. Classification is done on the shape parameters, the red/purple spot parameters and a combination of the features. The results of the combination was the best of the three and is shown in Table 3.

Table 2. Visual assessment score compared to Elisa analysis (H=Healthy, I=Infected).

Variety	Elisa and expert: H	Elisa and expert: I	Elisa: I Expert: H	Elisa: H Expert: I	Total error
Barcelona	89	86	15	10	13%
Monte Carlo	100	22	22	25	28%
Yokohama	103	88	16	4	9%

Table 3. Color camera score compared to Elisa analysis.

Variety	Elisa and Camera: H	Elisa and Camera: I	Elisa: I Camera: H	Elisa: H Camera: I	Total error
Barcelona	83	72	29	16	22%
Monte Carlo	93	30	14	32	29%
Yokohama	101	84	19	5	12%

Spectrophotometer

The difference between the mean spectrum of the healthy and infected plants was the largest in the region between 500 and 700 nm. The error of the classification of the 100 nm subsets was smallest in the same region, as showed for variety Yokohama (Figure 5). Table 4 shows the classification result for the full range spectra.

Spectral camera

The same procedure as with the color images was applied on the spectral images using only shape parameters, spot parameters and a combination of the features. Here also the combination gives the best result, as is shown in Table 5.

Table 4. Spectrophotometer score compared to Elisa analysis.

Variety	Elisa and Camera: H	Elisa and Camera: I	Elisa: I Camera: H	Elisa: H Camera: I	Total error
Barcelona	147	137	65	51	29%
Monte Carlo	202	63	25	48	24%
Yokohama	189	172	36	25	14%

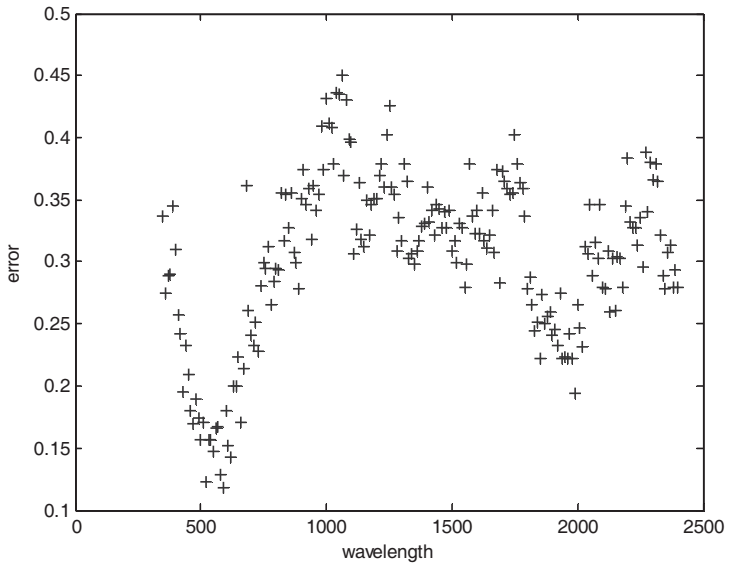

Figure 5. Classification error for subsets of 100 nm (variety Yokohama).

Table 5. Spectral camera score compared to Elisa analysis.

Variety	Elisa and Camera: H	Elisa and Camera: I	Elisa: I Camera: H	Elisa: H Camera: I	Total error
Barcelona	94	71	30	5	17%
Monte Carlo	89	31	13	36	29%
Yokohama	102	88	15	4	9%

Chlorophyll fluorescence imaging system
The results for the Chlorophyll fluorescence imaging system is shown in Table 6.

Combination of techniques
Several combinations were investigated with the data from the four methods. The most interesting combination was that the result of color camera and spectral camera is taken if they predict the same and the result of the spectrophotometer is taken, if color camera and spectral camera predict differently. In this case the error for variety Monte Carlo drops significantly, as can be seen in Table 7. Finally the optical techniques were compared to the manual assessment. Table 8 gives the result for the spectrophotometer, the color – and spectral camera.

Table 6. Chlorophyll fluorescence imaging system score compared to Elisa analysis.

Variety	Elisa and Camera: H	Elisa and Camera: I	Elisa: I Camera: H	Elisa: H Camera: I	Total error
Barcelona	70	57	44	29	36
Monte Carlo	68	24	20	57	46
Yokohama	73	73	31	34	31

Table 7. Score where if color camera and spectral camera predict different, the result of the spectrophotometer is taken.

Variety	Elisa and Camera: H	Elisa and Camera: I	Elisa: I Camera: H	Elisa: H Camera: I	Total error
Barcelona	88	74	27	11	19%
Monte Carlo	100	31	13	25	22%
Yokohama	102	87	16	4	10%

Table 8. Comparison between manual assessment and optical techniques.

Variety	Camera system	Number of errors	Number of errors expert	Error in same plants	Percentage correspondence
Barcelona	Spectrophotometer	47	25	10	40%
Monte Carlo	Spectrophotometer	50	47	24	51%
Yokohama	Spectrophotometer	37	20	6	30%
Barcelona	Color camera	45	25	13	52%
Monte Carlo	Color camera	46	47	18	38%
Yokohama	Color camera	24	20	7	35%
Barcelona	Spectral camera	35	25	9	36%
Monte Carlo	Spectral camera	49	47	16	34%
Yokohama	Spectral camera	19	20	7	35%

Discussion and conclusions

The control of plant viral diseases in ornamental crops as for instance tulips by using plant protection agents is more and more restricted. Integrated crop protection management systems are still in progress; certain chemicals are restricted, and not only in the Netherlands.
For the control of TBV the vectors (aphids) can be controlled by the use of mineral oil with insecticides (pyrethroides) (Asjes and Blom-Barnhoorn, 2001). However, due to new regulations chemical-free methods are needed to control the incidence of virus-infected tulips.
As standard the results of the Elisa measurements were taken, as this serological method has been proved to be a reliable, sensitive and reproducible method. Also detection of TBV using RT-PCR might be an additional control to further confirmation of the presence of virus, also other viruses as TBV (Dekker *et al.,* 1993). This will be needed in future, as certain other viruses than TBV might be present in tulips and noticed by the vision techniques, giving rise to false-negative results.
It was found that the error of visual assessment of symptoms differed between 9 and 28% for the different varieties (Table 2). The best result for the optical methods was for the spectral camera (Table 5). This result is only slightly worse than the visual assessment by the expert.
The overall results of variety Monte Carlo were bad, which was due to hail and other problems which causes severe damage to the leaves during cultivation of these plants. Also the number of infected plants for this variety was too low for a proper statistical analysis.
The analysis of the spectrophotometer data shows that the most important features (wavelengths) are in the visual range (below 1000 nm). This implies that for practical implementation no expensive infra-red sensors are needed, which improves the economical feasibility of the system. Results for the chlorophyll fluorescence imaging system indicates that this method is not suitable for detection of TBV. A possible explanation for this is that this system measures overall plant stress and can not distinguish between different stress sources such as virus symptoms.
The fact that the spectral camera performs similar as the crop expert is very promising and gives reason for follow up with as final goal an autonomous robot for detection and removal of diseased tulip plants. Although an RGB color camera is easy to implement and it performed reasonably well in the laboratory test, we opt for the spectral camera for the field test of 2009. Reason for this is that field conditions are much more difficult to control and therefore we like to get the highest signal to noise ratio from the camera system.

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