Photo-optical, non-invasive detection of the fire blight pathogen *E. amylovora*

A. Hummrich[1]* and R.T. Voegele[1]

Abstract

Erwinia amylovora*, the causal agent of fire blight, causes enormous losses in pome fruit production, especially in apples and pears. The limited options for disease control make early detection of fire blight infections important in order to start specific and efficient counter measures in due time. Spectroscopic methods take advantage of non-invasiveness and speed of measurements. Measurements are repeatable, and a quick survey of large areas and remote sensing is possible. The method uses reflectance characteristics of plant tissue, which provides information about the health status of the plant. In this project, a fluorescence spectrometer was used to perform ratings on inoculated and healthy trees of different apple cultivars. First results indicate the possibility of differentiation between healthy and infected trees due to spectrometric data.*

Keywords: *E. amylovora*, remote sensing, fluorescence spectroscopy

Introduction

Fire blight is a major threat for pome fruit production, causing economical losses worldwide especially in apples and pears (Bonn and Van der Zwet 2000). Specific symptoms are the emergence of polysaccharide containing exudates, the burnt appearance of leaves and flowers, and the typical shepherd's crook, a bending of the shoot apex due to decreasing turgor pressure. Once the disease has occurred, there are very few countermeasures like the excision of diseased tissues or the removal of infected trees, even whole orchards. Besides these curative measures stands the protective use of antibiotics e.g. streptomycin or biological control using antagonists (Kunz *et al.* 2011). An early detection of fire blight infections, amongst other parameters, may be helpful for decision making in order to start specific and efficient countermeasures in due time. The advantages of spectroscopic methods are obvious: the measurements are non-invasive and therefore repeatable; a quick survey of large areas and remote sensing is possible. The method uses the reflectance characteristics of plant tissue, which provides information about the health status of the plant. Changes in plant reflectance spectra can be used to make statements about the presence of pathogens. In 1996, Luedeker *et al.* could use chlorophyll fluorescence to identify powdery mildew on apple leaves earlier than a visual rating. Experiments on scab-infected and healthy apple trees were successful putting into practice an infrared spectroscopy-based differentiation (Delalieux *et al.* 2007). In addition, attempts were undertaken using near infrared spectroscopy to detect fire blight in asymptomatic pear plants (Spinelli *et al.* 2006).

Material and methods

Apple plants from six different susceptible cultivars were used: *Adams Parmäne, Danziger Kantapfel, Gala, Öhringer Blutstreifling, Rewena,* and *Schneiderapfel*.

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For each cultivar, twelve apple shoots were used. One half of the shoots were artificially inoculated with a bacterial suspension of *E. amylovora* strain *Ea*385. Inoculation was performed by injection with a needle and syringe just beneath the youngest fully developed leaf. The other half was left untreated as control. The experiment was conducted in the greenhouse, with 12 h light and 27°C/15°C day/night temperature. Measurements were taken immediately before inoculation as well as 1, 2, 4, 7, 11, 16, 22 and 29 dpi. Leaves at five different positions on the shoot were measured three times at each point; additionally photographs were taken to document symptom development. The sensor used was the fluorescence spectrometer Multiplex® (Force A, Orsay, France). As an active sensor it is independent from ambient light because of its internal light source, allowing measurements regardless from external light conditions. The sensor registers twelve signals resulting from four excitations (UV, blue, green and red light) and three emissions (blue, red and far-red). Out of this signals several ratios are calculated, which give information about different plant constituents/components (Multiplex® User's guide 2008). The main two categories are fluorescence excitation ratios of far-red chlorophyll emission (FRF) and fluorescence emission ratios (e.g. FRF/RF and BGF/FRF).

Results

Data were tested for normality of distribution to decide which classification method should be used. Testing was done using the Shapiro-Wilk test in R (R Development Core Team 2011) for each of the datasets (324 sets for each cultivar: two treatments – inoculated and control, nine dates - 0 to 29 dpi, and 18 variables tested individually). Out of these datasets, only 21% (*Adams Parmäne*, Fig. 1) respectively 25% (*Rewena*, Fig. 3) were normally distributed with a p-value greater than 0,05 in Shapiro-Wilk test. The four other cultivars showed similar results (data not shown). Since most of the data were not normally distributed, a tree based modeling was done to decide, which of the variables were most useful in differentiating between healthy and infected plants. Decision trees consist of nodes, connected by branches and ending in leaf nodes. At each decision node the measured attributes are tested, resulting in a branch that leads either to another node or to a terminating leaf node (Larose 2005). From these trees it is possible to generate decision rules to decide between healthy and infected populations based upon new measurements. The technique used in this work could accurately classify the measured attributes based on recursive partitioning (Breiman *et al.* 1984). The decision trees were created using "rpart" in R (R Development Core Team 2011).

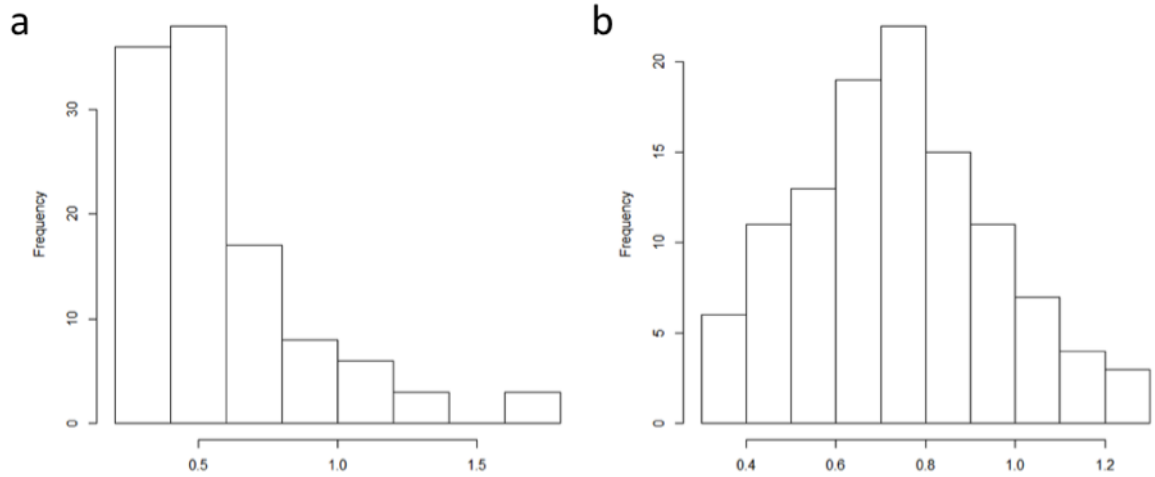

Fig. 1: Histograms representing a non normal (a) and a normal distribution (b) of the variables BRR_FRF (p-value=8,355E-7) and NBI_R (p-value=0,1119) on infected *Adams Parmäne* plants at 4 dpi. BRR_FRF: Blue-to-Red Fluorescence Ratio under UV excitation; NBI_R: Nitrogen Balance Index, red and UV excitation.

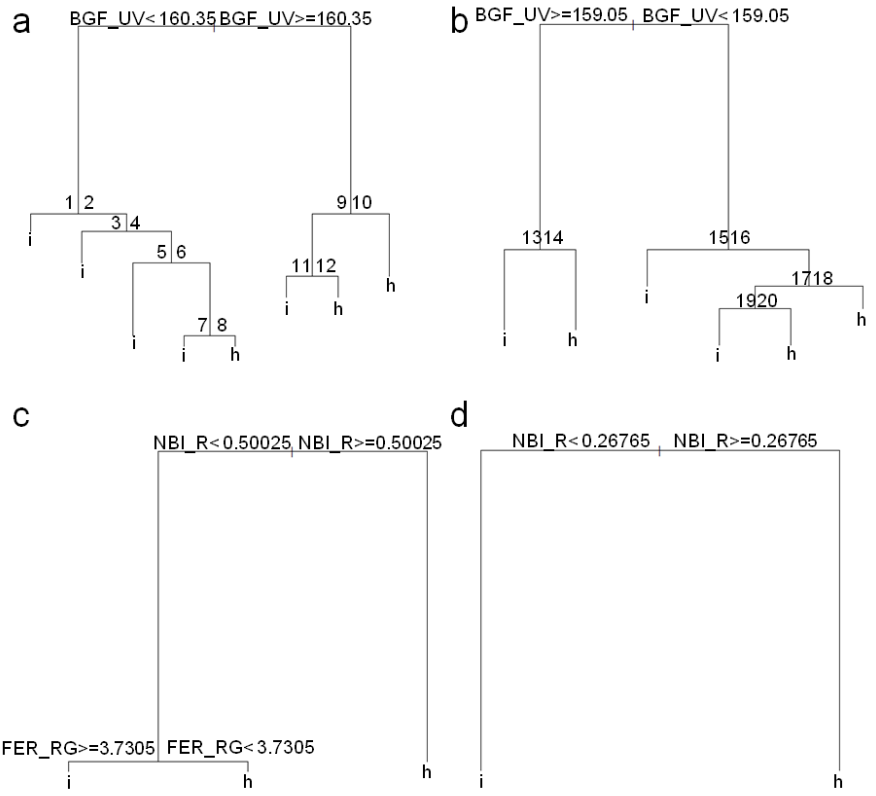

Fig. 2: Decision trees derived from measurements on healthy and infected *Adams Parmäne* plants. a: 1 dpi, b: 7 dpi, c: 16 dpi, d: 29 dpi; i: infected leaves, h: healthy leaves; 1: RF_UV>=120.4; 2: RF_UV< 120.4; 3: BGF_UV< 155.9; 4: BGF_UV>=155.9; 5: BGF_UV>=159.15; 6: BGF_UV< 159.15; 7: NBI_G>=2.7775; 8: NBI_G< 2.7775; 9: BGF_UV>=165.05; 11: BGF_UV< 167.75; 12: BGF_UV>=167.75; 10: BGF_UV< 165.05; 13: NBI_G< 4.229; 14: NBI_G>=4.229; 15: RF_UV< 51.55; 16: RF_UV>=51.55; 17: BGF_UV>=157.75; 19: BGF_G>=412.5; 20: BGF_G< 412.5; 18: BGF_UV< 157.75. Abbreviations for the attributes are explained in the Multiplex® User's guide (2008).

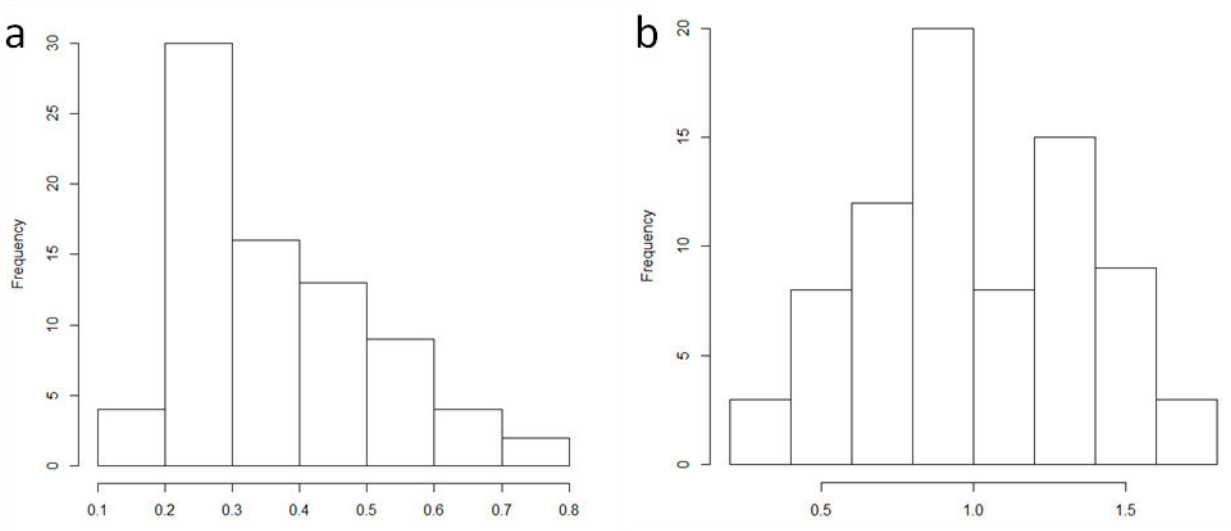

Fig. 3: Histograms representing a non normal (a) and a normal distribution (b) of the variables BRR_FRF (p-value=1,99E-4) and NBI_R (p-value=0,1269) on infected *Rewena* plants at 4 dpi. BRR_FRF: Blue-to-Red Fluorescence Ratio under UV excitation; NBI_R: Nitrogen Balance Index, red and UV excitation.

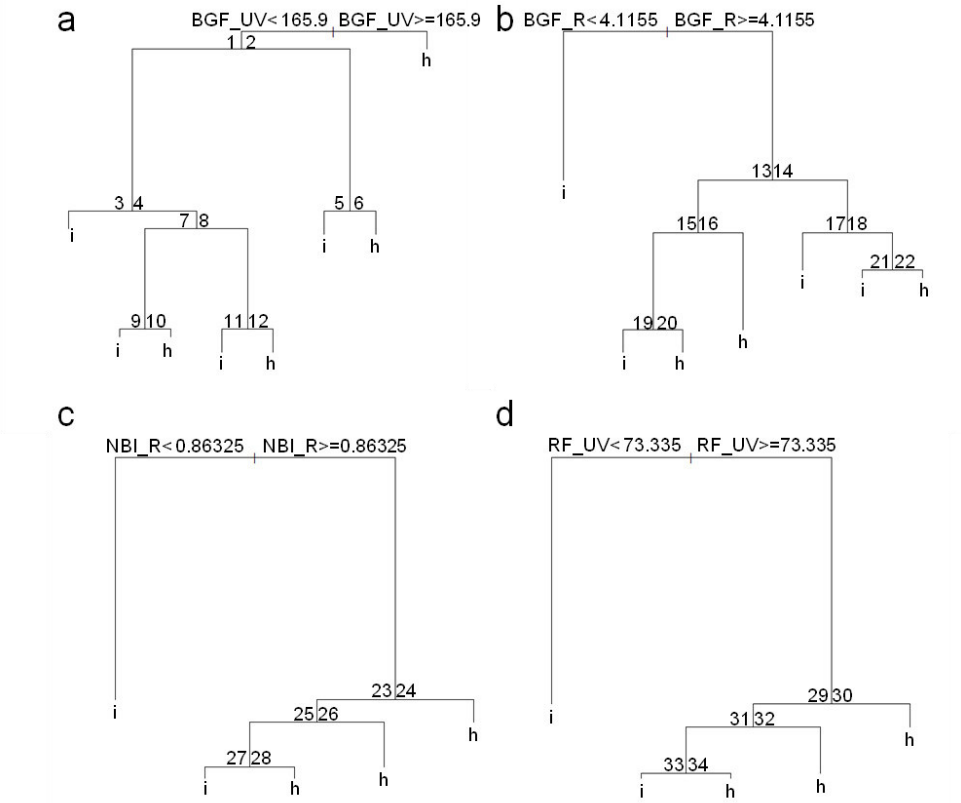

Fig. 4: Decision trees derived from measurements on healthy and infected *Rewena* plants. a: 1 dpi, b: 7 dpi, c: 16 dpi, d: 29 dpi; i: infected leaves, h: healthy leaves; 1: BGF_G>=383.65; 2: BGF_G< 383.65 51; 3: RF_R< 448.3; 4: RF_R>=448.3; 5: NBI_G< 4.145; 6: NBI_G>=4.145; 7: SFR_G< 6.1815; 8: SFR_G>=6.1815; 9: SFR_R>=4.4185; 10: SFR_R< 4.4185; 11: BGF_UV>=159.55; 12: BGF_UV< 159.55; 13: NBI_R< 1.0555; 14: NBI_R>=1.0555; 15: SFR_R>=4.9815; 16: SFR_R< 4.9815; 17: BGF_UV>=169.15; 18: BGF_UV< 169.15; 19: FRF_G< 808.2; 20: FRF_G>=808.2; 21: RF_UV>=210; 22: RF_UV< 210; 23: BGF_UV>=164.05; 24: BGF_UV< 164.05; 25 FRF_UV>=488.3; 26: FRF_UV< 488.3; 27: RF_UV< 150.7; 28: RF_UV>=150.7; 29: BGF_UV>=161.45; 30: BGF_UV< 161.45; 31: SFR_G< 8.744; 32: SFR_G>=8.744; 33: BGF_G< 405.2; 34: BGF_G>=405.2. Abbreviations for the attributes are explained in the Multiplex® User's guide (2008).

Decision trees were used to derive a set of conditions that permit accurate classification of infected and healthy trees based on the measured attributes. Fig. 2 and 4 show the results from the measurements at 1, 7, 16 and 29 dpi. At 7 dpi all *Adams Parmäne* trees showed visible symptoms, *Rewena* trees at 16 dpi. Taking Fig. 2d as example, the tree can be interpreted as follows: if the value of NBI_R is smaller than 0,26765 one would take the left branch and the corresponding measurements would be classified as infected. In more complex trees (Fig. 2a, b and c) one has to decide on more nodes which direction to take until the leaf node is reached. While at 1 dpi – regarding the measurements on *Adams Parmäne* - many nodes are needed to separate the populations, the number of nodes gets smaller until at 29 dpi only one attribute is needed to completely separate infected from healthy plants. With the less susceptible cultivar *Rewena*, the number of nodes at 1 and 7 dpi (Fig. 4a and b) is comparable to the ones of *Adams Parmäne* but at 16 and 29 dpi (Fig. 4c and d) the decision trees don't enable a proper differentiation like the ones derived from the susceptible cultivar. Similar results were obtained from the other cultivars: The classification of susceptible cultivars is more distinct than of less susceptible cultivars. Additionally, error rates of the classification accuracy for each tree were obtained in calculating the prediction column and confusion matrix. The corresponding error rates in table 1 indicate a better distinction for infected and healthy *Adams Parmäne* plants regarding further developed infections, too. Error rates are higher at the beginning and decrease with progression of infection; the distinction between healthy and infected populations gets clearer. The error rates for *Rewena* don't show this decline up to 29 dpi.

Table 1: Error rates of the classification accuracy.

dpi	1	2	4	7	11	16	22	29
error rate [%]								
Adams P.	15,97	13,89	9,14	12,78	7,22	5,00	4,39	0
Rewena	12,5	8,33	10,93	14,44	8,33	12,22	3,95	6,78

Discussion

This experiment gives insight into the potential of fluorescence spectroscopy to differentiate between fire blight infected and healthy apple trees. Even though a secure classification is only possible after visible symptoms are present, the photo-optical detection of fire blight infections could be useful in reducing the effort of manual ratings of orchards. Results suggest that decision trees are able to distinguish apple plants infected with fire blight from healthy plants of the susceptible cultivar *Adams Parmäne*. These results are of considerable interest for pome fruit production, where an early detection based on photo-optical input could lead to earlier and better counter measures and thus to secure and increased production. For the less susceptible cultivar *Rewena* this distinction is not that well-defined. Other statistical methods should be taken into account. In further studies, other parameters like secondary plant metabolites such as flavonoids that might be generating the changes in reflectance characteristics of infected plant tissue and the quantitative detection of *E. amylovora* using Real Time PCR could be combined with the results of spectroscopic measurements. Furthermore, the effect of other biotic stresses for apple plants like scab or powdery mildew on the reflectance characteristics should be investigated.

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