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Application note

Thermography versus chlorophyll fluorescence imaging for detection and quantification of apple scab

Étienne Belin [a], David Rousseau [b,*], Tristan Boureau [c], Valérie Caffier [d]

[a] Laboratoire d'Ingénierie des Systèmes Automatisés (LISA), Université d'Angers, 62 avenue Notre Dame du Lac, 49000 Angers, France
[b] Université de Lyon, CREATIS; CNRS UMR 5220; INSERM U630; Université Lyon 1, INSA-Lyon, 69621 Villeurbanne, France
[c] Université d'Angers, Institut de Recherche en Horticulture et Semences (INRA, Agrocampus-Ouest, Université d'Angers), SFR 149 QUASAV, F-49071 Beaucouzé, France
[d] INRA, Institut de Recherche en Horticulture et Semences (INRA, Agrocampus-Ouest, Université d'Angers), SFR 149 QUASAV, F-49071 Beaucouzé, France

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ABSTRACT

Fluorescence imaging has recently been shown to be useful for the detection of apple scab, and thermal imaging for both detection and quantification of apple scab. We undertake a comparison of these two techniques and demonstrate the advantages of thermal imaging compared to fluorescence imaging to detect and quantify the presence of apple scab at the surface of leaves. We demonstrate, in practical environmental conditions of growth chambers, the advantages of thermal imaging compared to fluorescence imaging in terms of detection in the framework of a Neyman–Pearson strategy with the Bhattacharyya distance and ROC curves and in terms of quantification by establishing a linear relationship between percentage of leaf diseased area estimated visually and percentage of leaf area estimated by imaging segmentation. This opens perspectives for quantitative aspect of pathogenicity in the study of apple scab and constitutes a general framework for the comparison of nonconventional optical imaging applied to plant pathology.

1. Introduction

Nonconventional optical imaging, gazing outside the human visible spectrum, are powerful vision tools to study diseases in plant sciences (Sankaran et al., 2010). They offer noninvasive solutions to monitor and quantify the development of the pathogens on the surface of plant leaves with higher sensitivity than the human eye. Thermal imaging, chlorophyll fluorescence, near infrared imaging, hyperspectral imaging are among the most popular imaging technologies which have been shown to be useful in plant pathology. Thermal imaging and chlorophyll fluorescence are of particular interest since they can provide non-visible functional information on the physiology of the plant (Chaerle and Straeten, 2001; Papageorgiou and Govindjee, 2004) and allow early detection of plant pathogens (Chaerle et al., 2004) and quantification of plant disease (Oerke et al., 2006). Nature offers a great variety of pathogen strategies to infect plants and a great variety of plant responses to pathogens, which implies a wide range of host–pathogen interactions to analyze (Agrios, 2005). This results in a variety of imaging contrast patterns in the temporal development of pathogens (Sankaran et al., 2010; Chaerle et al., 2004, 2001). It is consequently important to analyze the specificity of each pathosystem with these imaging modalities. In this article, we evaluate the interest of thermal imaging compared to fluorescence imaging for the detection and quantification of apple scab pathogen.

Apple scab, which is caused by the fungus *Venturia inaequalis*, requires more than 10 fungicide treatments per year to be controlled and can be considered as the most serious disease for apple (Bowen et al., 2011). Quantifying the development of apple scab at the leaf scale is of major importance for studying the apple–scab interaction, as well as for analyzing the evolution of pathogenicity in *V. inaequalis* populations, and for breeding scab-resistant apple cultivars. Computer vision tools have been developed to detect or quantify the scab-diseased area of apple leaves. Thermography has been shown to be useful for early detection and quantification of apple scab (Oerke et al., 2011). These authors showed that the subcuticular growth of the pathogen induced a localized decrease in leaf temperature 1–3 days before the appearance of symptoms, and demonstrated that there was a relationship between the percentage of visibly diseased leaf area and the maximum temperature difference observed on the leaf by thermography. Fluorescence under light adapted conditions has also been used for early detection of apple scab infection (Delalieux et al., 2009). However, analysis of the quantum efficiency of Photosystem II did not allow the detection of apple scab in a well developed infection stage. Delalieux et al. (2009) recommended a simultaneous

* Corresponding author.
E-mail address: david.rousseau@univ-lyon1.fr (D. Rousseau).
URL: http://www.istia.univ-angers.fr/LISA/PHENOTIC (D. Rousseau).

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use of fluorescence imaging and hyperspectral techniques for the detection of apple scab, but they did not evaluate if these imaging techniques can be used for the quantification of scab disease. In this study, we propose to compare the potentials of thermal versus dark adapted chlorophyll fluorescence imaging, which is easier to handle than light adapted fluorescence (Papageorgiou and Govindjee, 2004) for the non-destructive detection and quantification of apple scab. We demonstrate that a higher contrast than that obtained with dark adapted fluorescence imaging can be obtained with thermal imaging that enables an earlier quantification of the disease. Our experimental setup with biological material and computer vision tools is described in material and methods section. We then present the results of comparison of the detectability and quantification of apple scab using fluorescence imaging and thermal imaging.

2. Material and methods

Grafted apple trees are inoculated in controlled conditions with 16 isolates of *V. inaequalis* of different levels of aggressiveness at a concentration of 80,000 spores/mL (Caffier et al., 2010). For each isolate, two types of apple plants belonging to the progeny of $TN10-8 \times Prima$ are used:

- type HS with 8 plants with a high level of susceptibility to scab,
- type LS with 8 plants with a low level of susceptibility to scab.

The percentage of diseased leaf area (i.e. the area that is covered by spores of the fungus) is assessed visually by an expert using a seven step quantized scale (1: 0–1%, 2: 1–5%, 3: 5–10%, 4: 10–25%, 5: 25–50%, 6: 50–75%, 7: 75–100%) adapted from (Croxall et al., 1952). This notation, operated on the second younger fully developed leaf at inoculation time, is performed between 8 and 21 days after inoculation in usual ambient environmental conditions (20 Celsius and 60% hygrometry). Twenty one days after inoculation, seven leaves that are representative of these seven steps are chosen. It is quite difficult to visualize scab on pictures with diseased leaves. As pictures of apple scabbed leaves generally do not provide a clear visualization of the symptoms, we use adhesive tapes as the ground truth. The adhesive tape is applied on each leaf to stick the spores of the fungus out of the apple leaf. The adhesive tape is then pasted on a paper sheet in order to obtain a print of each leaf with sporulating and non-sporulating areas.

Thermal images are acquired with a FLIR SC 5000 cooled infrared camera with a spectral range between 2 and 5 μm, a pixel resolution of 320 by 240, and a 12-bit dynamic. Fluorescence images are performed with a PSI Open FluorCam FC 800-O. The system sensor is a CCD camera with a pixel resolution of 512 by 512 and a 12-bit dynamic. The system includes four LED panels divided to two pairs. One pair provides an orange actinic light with a wavelength of around 618 nm, with an intensity that can vary from 200 to 400 μmol/m^2/s. The other pair provides a saturating pulse in blue wavelength, typically 455 nm, with an intensity of up to 3000 μmol/m^2/s. Both pairs of LED panel produce a 5 cm by 5 cm area, larger than the typical size of leaf considered in this study, at the center of the setup which receive a uniform lighting (the spatial standard variation of the lighting in this area is 0.07% of the dynamic of the CCD sensor). Fluorescence chlorophyll imaging is used in a dark adapted mode (Papageorgiou and Govindjee, 2004) to produce two maps with the initial fluorescence state F_0 and the fluorescent quantum efficiency $Q_{ymax} = \frac{F_m - F_0}{F_m}$ where F_m is the maximum fluorescence state. For all image acquisitions, the observed leaf is maintained horizontally on a white sheet of paper with nylon strings.

3. Detecting apple scab

We compare the detectability of apple scab at the surface of apple tree leaves using a multimodal image stack with four components for each of the seven leaves representative of the development of apple scab. The four components are the visible scan image of apple scab print, the initial fluorescence state image F_0, the fluorescent quantum efficiency image Q_{ymax} and the thermal image. All the components of the stack are registered and interpolated. Results are shown in Fig. 1. Fig. 1A shows the print which is considered, after thresholding (0 for healthy tissue, 1 for tissue with sporulation), as the ground truth. The initial fluorescent state F_0 in Fig. 1B and the thermal imaging in Fig. 1D appear close to the prints. Thermal imaging shows lower temperatures (around 0.3 K) and initial fluorescence state F_0 shows lower values with spatial patterns located for both imaging modalities specifically where print reveals apple scab. The fluorescent quantum efficiency Q_{ymax} of Fig. 1C is not showing such a spatial similarity with the ground truth.

We also propose to assess the visual observation in Fig. 1 using a quantitative procedure. To this purpose, manual segmentation of the numerical ground truth obtained from the print of Fig. 1A is performed by an expert to define a binary mask for the infected area *I* in Fig. 2B and a binary mask for the non-infected area *NI* in Fig. 2C.

For each component of the stack, a contrast can be computed with the Bhattacharrya distance (Kailath, 1967) between two probability density functions defined as

$$BD = -\ln\left[\sum \sqrt{h_I h_{NI}}\right], \quad (1)$$

where h_I and h_{NI} are the normalized histograms of probability density functions for the infected and non-infected areas. The Bhattacharrya is known to be particularly useful to give a contrast scalar directly connected to detection performance (Goudail et al., 2004) in noisy images. Table 1 presents the Bhattacharrya distance between the infected and non-infected areas in each of the imaging modalities.

We observe in Table 1 that the Bhattacharrya distance between infected and noninfected areas is relatively small at all the stages of the development of the pathogen in the fluorescent quantum efficiency image Q_{ymax} but longer in the initial state fluorescence image F_0 and thermal imaging. The highest contrasts measured by the Bhattacharrya distance are systematically obtained at all levels of scab development with thermal imaging. Furthermore, a limitation of the F_0 fluorescence imaging is that it is sensitive to non-uniform lightning while thermal imaging is a passive imaging technique. This demonstrates that apple scab is better detected by thermal imaging than by fluorescence imaging.

To further investigate the comparison of thermal imaging and chlorophyll fluorescence imaging for apple scab detection, we now study a detection criterion on a population of infected and noninfected leaves. As visible in Fig. 1B and D, apple scab seen with thermal and F_0 fluorescence imaging produce a large variety of spatial patterns with spots (B1, D1, B2, D2, B6, D6), or large homogeneous areas (B5, D5, B7, D7) but also elongated areas following the vascular net of the leaves (B3, D3, B4, D4). For these reasons, a detection threshold based on a spatial signature is difficult to define. Also, acquisition conditions such as room temperature for thermal imaging or dark adapted conditions for F_0 fluorescence imaging, although fixed for each acquisition, may vary from one acquisition to another. As a result, a criterion based on an absolute temperature or on an absolute level of initial state fluorescence is not appropriate. Hence, adapted from the method used by (Oerke et al., 2011) with the maximum temperature difference (MTD), we propose to use a simple radiometric criterion with a threshold