Application Note

PLANT PATHOGEN MONITORING WITH THE NIGHTSHADE USING IMAGING OF PROMPT AND DELAYED FLUORESCENCE

Abstract

Plant pathogens cause important yield losses in food crops, which makes the study of plant diseases an area of research of high priority. In this application note, imaging of the prompt fluorescence of a transgenic pathogen is combined with imaging of the delayed fluorescence of chlorophyll to follow the infection and asses its effects using the NightSHADE In Vivo Imaging System. Results show a good correlation between both imaging methods and suggest that the combination of both imaging methods can be a useful tool for the study of plant diseases.

Introduction

Plant pathogens are the cause of many diseases. The impact of those diseases varies depending on the specific pathogen and plant, but crop yield loss is one of the most important ones: pathogens and pests cause global yield losses from 8 to 41% in food crops, depending on crop and country [1] It is expected that the frequency and severity of plant disease outbreaks will increase as a result of climate change [2]. This makes the study of plant pathogens an area of research of high priority.

This application note provides 2 examples of methods useful for studying plant pathogens: Firstly, infection monitoring using fluorescence-labelled pathogenic fungi that enable visualisation of infected areas through prompt fluorescence imaging.; and secondly, the assessment of plant health based on the delayed fluorescence of chlorophyll, which is an indicator of the state of the photosynthetic system [3].

Materials and Methods

- NightSHADE evo In Vivo Plant Imaging System (Berthold Technologies).
- Excitation filter 475/20.
- Emission filter 520/10.
- indiGO™ image analysis software (Berthold Technologies).

The pathogen fungus to be used in the experiment was transfected with the fluorophore ZSgreen, and wheat was infected with this transgenic fungus.

For imaging, two leaves, one with a high level of infection and one with a low level of infection were

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fixed on a clipboard and transferred to the imaging chamber of the NightSHADE.

For imaging of delayed fluorescence, leaves were illuminated for 30 s with the LED panels, light was turned off, and the image was immediately acquired in Luminescence mode without filters, with 20 s exposure time and 4x4 pixel binning.

For imaging of prompt fluorescence of ZSgreen, the filters above were used, and imaging was performed in Fluorescence mode with a 2 s exposure time and 90% lamp energy.

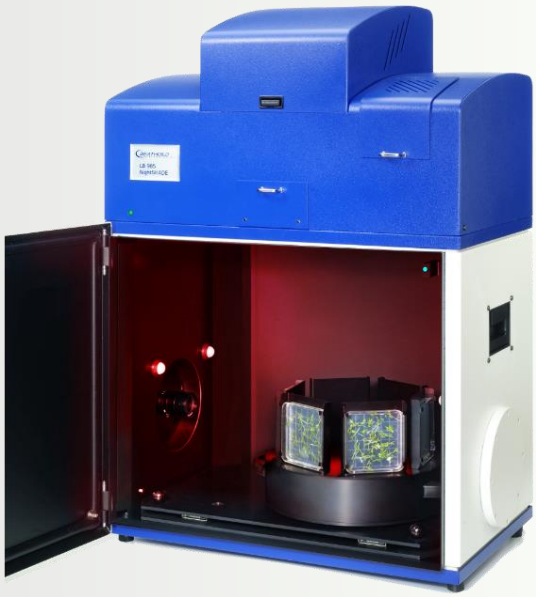
A picture in Photo mode was also acquired and is used in the figures to show the position of the fluorescence in the leaves.

The intensity of fluorescence was quantified using the indiGO™ image analysis software and expressed in counts per second (cps).

The Berthold Technologies NightSHADE evo LB 985N In Vivo Plant Imaging System

The NightSHADE evo LB 985N In vivo Plant Imaging System is a modular, easy to use optical imaging system dedicated to in vivo analysis of plants. Equipped with an absolutely light-tight cabinet and a cooled CCD camera it enables sensitive luminescence and fluorescence monitoring in tissues, seedlings, and whole plants.

The camera can be attached either to the ceiling or the side walls of the dark room – the sample chamber – to facilitate imaging from above and from the side. The latter position of the camera enables processing of multiple seedlings in parallel while growing plants vertically oriented to enable observation of the complete plant. Furthermore, key environmental conditions like temperature or humidity as well as daylight can be simulated to provide a controlled growth environment.



Results

In the image showing the prompt fluorescence from the transfected fungus (Fig. 1, left), the left leaf shows high levels of prompt fluorescence close to the tip, with lower levels of fluorescence around the right edge and in the central part of the leaf. The right leaf shows no fluorescence coming from the fungus, which indicates low or no infection. In the image showing the delayed fluorescence of chlorophyll (Fig. 1, right), the left leaf shows no delayed fluorescence near the tip and around the left edge, and reduced levels of delayed fluorescence in the central part of the leaf, but also areas of very low ZSgreen fluorescence (below 300 cps), indicating low but detectable infection. These areas show a significant reduction in delayed fluorescence (highlighted by red ovals). The right leaf displayed high levels of delayed fluorescence on the entire surface, except for the tip. Horizontal lines with high (left image) or low (right image) fluorescence are artifacts caused by the sample holder.

Overall, results show a good correlation between the position of the prompt fluorescence of the fungus and the reduction of delayed fluorescence of chlorophyll.

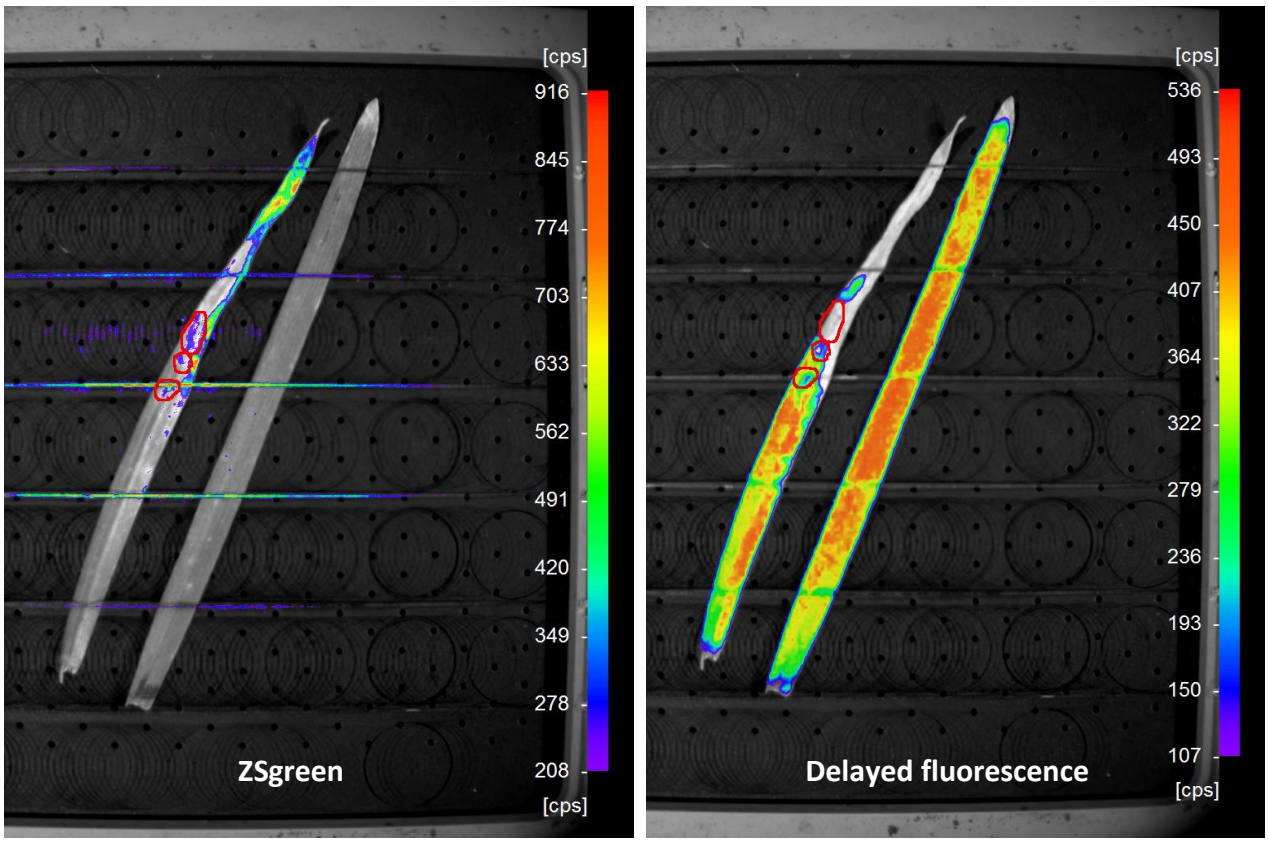


Figure 1. *Prompt fluorescence of ZSgreen (left) and delayed fluorescence of chlorophyll (right). Red ovals mark areas with low levels of ZSgreen fluorescence and reduced delayed fluorescence.*

Conclusions

Imaging the prompt fluorescence of fungal pathogens expressing ZSgreen with the NightSHADE permits localisation of the pathogen in the leaves of infected wheat and an assessment of the extent of infection. Furthermore, imaging the delayed fluorescence of chlorophyll with the NightSHADE shows a significant reduction in the health of the plant´s photosynthetic system in areas infected by the fungus.

The fluorescence spectra of ZSgreen partially overlaps with those of chlorophyll. While the results obtained in this application note were good, labelling the pathogen with other fluorophores without spectral overlap with chlorophyll might improve the performance of the assay.

As a conclusion, the combination of imaging of the prompt fluorescence of transgenic pathogens with imaging of the delayed fluorescence of chlorophyll using the NightSHADE In Vivo Plant Imaging System can be a useful method to study plant diseases.

References

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