Hyperspectral phenotyping as a tool for asymptomatic flax disease detection

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Early detection of plant diseases is a key issue in agriculture and plant breeding. In the actual context of climate change and global warming, plant susceptibility to some pathogens may increase [1] leading to economic losses. Verticillium wilt of flax is a fungal disease caused by *Verticillium dahliae* fungus, whose occurrence in flax growing areas has notably increased over the last decade. Most of the time, flax Verticillium infection remains silent and specific symptoms appear late after harvesting, at the retting stage [2] when fibres get separated from other components of the stem – which is an essential step before industrial fibre extraction. When fibres are damaged by the pathogen their quality is severely reduced.

This work presents the monitoring of disease progress using an automated high-throughput phenotyping system including Visible Near InfraRed (VNIR) and Short Wave InfraRed (SWIR) hyperspectral cameras . Vegetation indices could help to distinguish Verticillium infected plants from non-infected plants at late infection stages, not earlier than visual phenotyping. Meanwhile, a simple protocol based on spectral scores obtained by Principal Component Analysis (PCA) allowed to clearly separate infected from healthy plants early in the infection. This data treatment could also help to discriminate between different levels of susceptibility of flax to Verticillium wilt disease. This project intends to evaluate the potential of hyperspectral cameras in detecting infected plants.

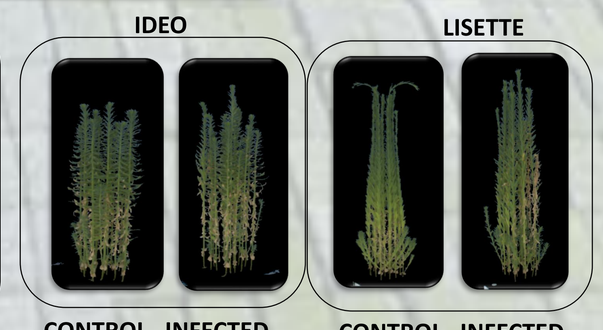
Plant High Throughput Phenotyping System

Imaging chambers:

- FluorCam
- RGB cameras
- Hyperspectral cameras

IDEO
Flax cultivar *from Linéa Semences*
less susceptible to Verticillium infection

LISETTE
Flax cultivar *from Van de Bilt Zaden en Vlas*
more susceptible to Verticillium infection



Verticillium symptoms are rather similar to normal plant ageing, rendering infection detection delicate.

→ **Late detection of fungal infection by RGB imaging**

Image data analysis

Hyperspectral specifications
VNIR: 450-900 nm
SWIR: 900-1700 nm

- MASK - Background vs plant - Removing of background pixels
- Determination of mean spectrum per plant (3180 spectra)
- For each variety:
  - Determination of the mean spectrum per stage of infection / day of analysis
  - Library of mean spectra
  - PCA analysis and extraction of the second PC score
  - Second PC score vs stage of infection / day of analysis

→ **Earlier detection of fungal infection by PCA analysis of VNIR & SWIR spectra**

Results

Fig.1: Disease scale of Verticillium infected flax
Disease scores represent the degree of infection. 0: No symptoms to 7: 100% of stem affected.

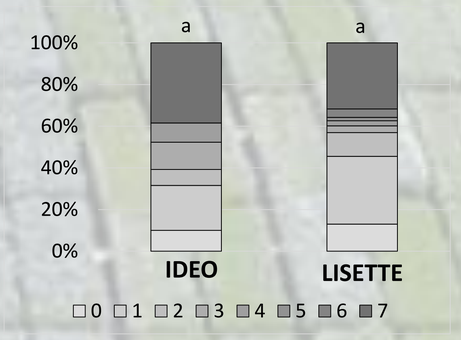


Fig.2: Disease scores of Verticillium infected flax plants 75 days post infection
Plants are scored according to the disease scale (n=122 plants). Results are percentage of plants for each score.

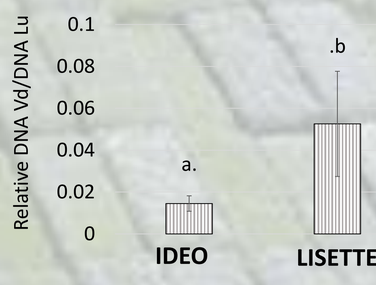


Fig.3: *in planta* Verticillium DNA quantification
Relative amount of fungus (Vd) DNA assessed by quantitative PCR and normalized by flax (Lu) DNA 75-days after infection. Results are mean values of 8x16 plants.

Need for **an imaging tool to detect**:

1. **Early** infection stages
2. **Different amount** of fungus in plants

Fig.1-3
75 days post infection in controlled conditions, visual estimation of the infection degree can not be linked to the actual amount of fungus present in the plant.

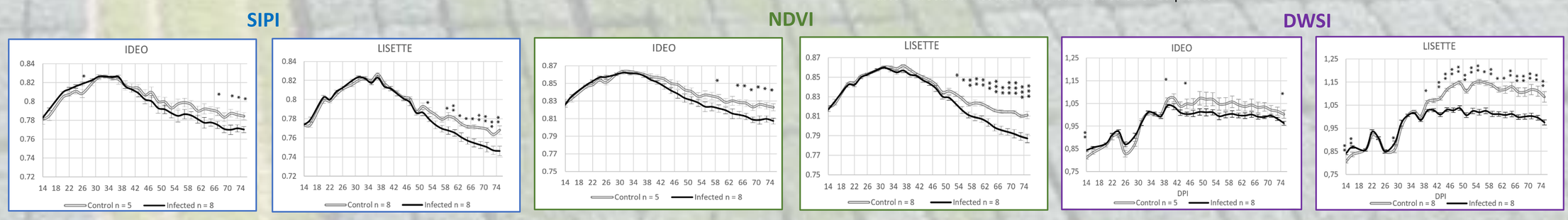


Fig.4: Evolution of Vegetation Indices over the course of disease progression. Vegetation indices are calculated as follows: Structure Insensitive Pigment Index (SIPI) $(R_{790}-R_{450}) / (R_{790}+R_{650})$, Normalized Difference Vegetation Index (NDVI) $(R_{800}-R_{670})/(R_{800}+R_{670})$, Disease Water Stress Index (DWSI) $(R_{802}-R_{547}) / (R_{1657}+R_{682})$.

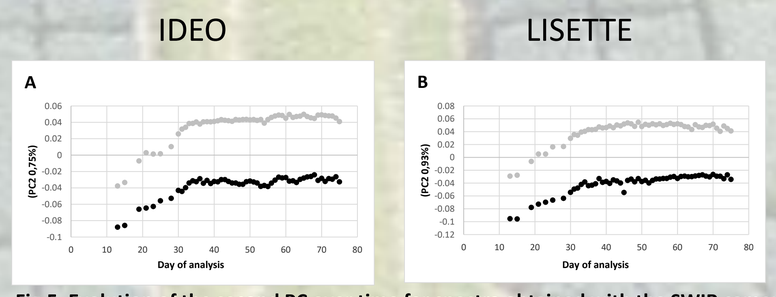


Fig.5: Evolution of the second PC over time for spectra obtained with the SWIR camera

PCA on SWIR spectra

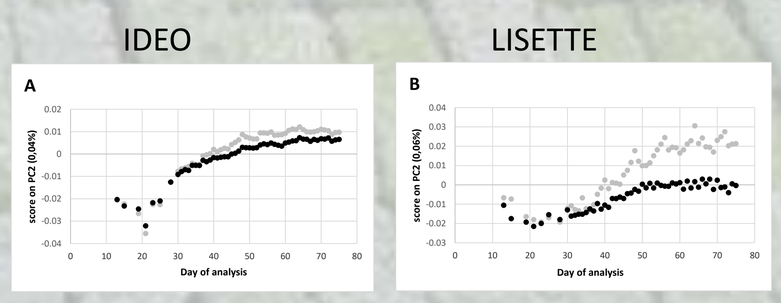


Fig.6: Evolution of the second PC over time for spectra obtained with the VNIR camera

PCA on VNIR spectra

Fig.4
Vegetation indices SIPI, NDVI and DWSI are usually relevant to estimate global plant health. Interestingly, they show **differences between healthy and diseased plants**, but this detection occurs quite late during plant growth.

Fig.5-6
PCA analysis of SWIR spectra could separate both conditions at **very early stages of the infection**. It is not the case with VNIR spectra, whereas this analysis provides another level of information since each PCA curve displays a **specific pattern depending on the pathogen susceptibility**. An approach combining both data treatment could greatly help breeders screening varieties for Verticillium resistance.

Perspectives

Usual vegetation indices showed differences between healthy and ill plants not earlier than the onset of visible symptoms. Moreover, the visual symptoms do not always correlate with the disease impact on the final plant product (fibers for example). The use of the PCA approach using spectra from VNIR and SWIR cameras allowed an earlier detection and could separate flax varieties according to their susceptibility level to Verticillium wilt. This study lays the foundation for the development of a screening tool to help breeders identifying flax varieties according to their susceptibility to *Verticillium dahliae.*

References: [1]Arvalis report, 2019, [2]Nuez et al., 2022

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