



## INTRODUCTION



Yield reduction in relation to the cyst nematode number in the sugar beet roots

The current work, carried out in collaboration with SESVANDERHAVE Company, aims to assess by NIR imaging the presence of cyst nematode on sugar beet root. The damage caused by nematode on the sugar beet root is yield reduction in relation to the cyst number. The objective of the study is firstly to discriminate between cyst, root and soil support as well as in a second step, to quantify the cyst nematode presence.



Brown and white cyst nematode (*Heterodera schachtii*)

## MATERIAL AND METHODS



Plants in laboratory

For this experiment, 5 plants of sugar beet with different level of resistance, were grown in a soil support spread in plastic plates and were infested with cyst. The number of cyst nematode was previously counted by optical microscopy at SESVANDERHAVE. Then 5 images of  $\pm 5 \text{ cm}^2$  size were acquired with the hyperspectral imaging system at CRA-W, the MatrixNIR™ Chemical Imaging System (Malvern instruments Ltd, Malvern, Worcestershire, UK).



Cyst nematodes in seedling in laboratory

### Instrument parameters:

- Wavelength range: 900-1700 nm by step of 10 nm
- Average on 16 coadds/wavelength and 4 scans/image
- 1 acquisition = 1 image =  $240 * 320$  pixels = 76800 spectra
- 1 pixel =  $80\mu\text{m} * 80\mu\text{m} = 0.0064 \text{ mm}^2$
- Analysed surface =  $\pm 5 \text{ cm}^2$
- Time of acquisition = 5 min/image

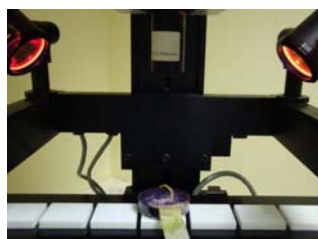


Image acquisition on sugar beet seedling with hyperspectral imaging system

### Cyst analysis:

- root system including cysts in a soil support spread in a plastic plate
- 1 cyst =  $\pm 25$  pixels =  $\pm 25$  spectra
- Analysed surface =  $\pm 5 \text{ cm}^2$  of the soil support

## CYST NEMATODE IDENTIFICATION

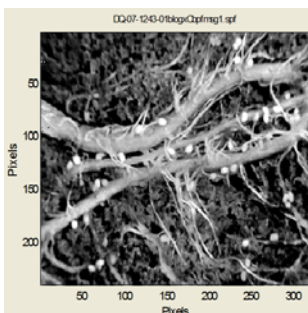
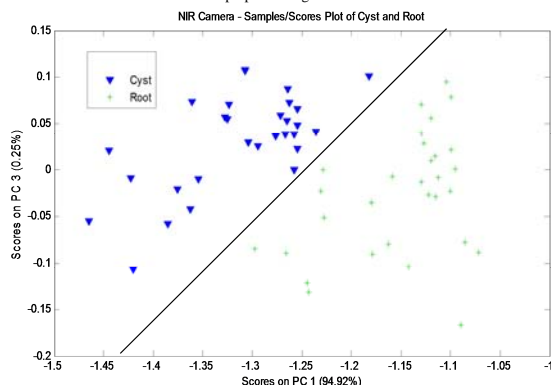


Image rebuilt at 990 nm after 1<sup>st</sup> Derivative preprocessing

The image rebuilt at 990 nm after 1<sup>st</sup> Derivative preprocessing shows clearly the different structures: soil support, root and cyst nematode.

For the identification of cysts, 30 spectra have been selected for each type of structure that can be found in a plant root (root and cyst nematode). A PCA performed on the preprocessed spectra shows that the cysts can be clearly discriminated from the root.

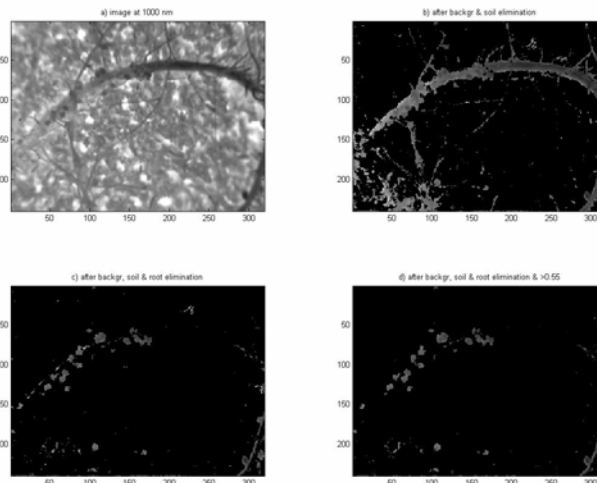


PCA on preprocessed NIR spectra acquired with the NIR camera instrument on cysts and roots

## CYST NEMATODE QUANTIFICATION

In order to assess the number of cysts in the roots of a plant, 5 samples have been analysed. Three spectral libraries have been built by selecting pixels in the 5 images at 1000 nm: 26724 pixels corresponding to soil support, 4829 pixels corresponding to root and 1558 pixels corresponding to cyst nematodes. Those libraries were used for the building of discrimination equations in order to identify cysts from root and soil support. SVM, Support Vector Machines, was used as classification method for the construction of these models. Two equations have been built: "soil support vs. root + cyst" and "root vs. cyst".

Then, these equations have been applied successively to all the pixels in the 5 images in order to build a mask, by isolating the cysts, and then assess the number of cysts by surface unit. The figure here below shows the 4 steps (a to d) to detect the cysts. Figure a shows the image at 1000 nm without preprocessing. Figure b shows the pixels detected as roots and cysts after background elimination and "soil support vs. root + cysts" equation application. Pixels corresponding to background and soil support are displayed in black color. Figure c shows the pixels detected as cysts after "root vs. cysts" equation application on the not black pixels of Figure b. The pixels classified as root are displayed in black as well. The rest includes pixels detected as cysts and miss classified pixels from the previous equations. Figure d shows the pixels detected as cysts after removing of pixels with color intensity upper than 0.55. Those pixels correspond to a light color close to blank color. All the pixels not classified as cysts are displayed in black color.

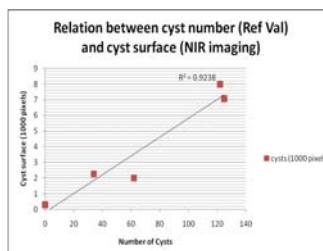


(a) image at 1000 nm; (b) pixels detected as roots and cysts after background elimination and "soil support vs. root + cysts" equation application; (c) pixels detected as cysts after "root vs. cysts" equation application on the not black pixels of Figure b; (d) pixels detected as cysts after removing of pixels with color intensity > 0.55

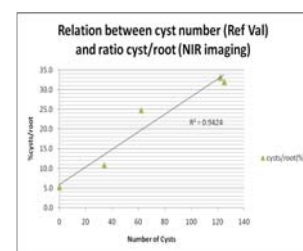
A correlation of 0.92 has been calculated between the number of cysts counted under microscope and the number of pixels recognized as cyst nematode by the NIR imaging. It has to be noted that cysts are counted on the total surface of the glass plate while NIR imaging analyses a part of this surface. Moreover; the cyst number could be depending on the root development. To reduce the surface effect as well as the sampling bias, the ratio between the cyst pixels number and the root pixels number was calculated. The correlation obtained between the number of cysts counted under microscope and this ratio is 0.94.

The table here beside shows, for each sample, the number of cysts counted under optical microscope by a VDH expert and the number of pixels of each structure type recognized by NIR hyperspectral imaging spectroscopy data treatment.

Samples	Optical microscope Cysts (number)	NIR hyperspectral imaging				Cysts/Root (%)
		Background (pixels)	Soil support (pixels)	Roots (pixels)	Cysts (pixels)	
DQ-07-1243-01	125	85	47505	22156	7074	32.0
DQ-07-1243-02	122	197	44429	24176	7998	33.1
DQ-07-1243-03	62	121	66587	8087	2005	24.8
DQ-07-1243-04	34	112	53632	20801	2255	10.8
DQ-07-1243-05	0	367	70790	5359	284	5.3



Relation cyst number/cyst surface (1000 pixels)



Relation cyst number/ratio cyst-root (%)

## CONCLUSION

This study showed the potential of the NIR imaging to discriminate the cysts from the root and the soil support in a sugar beet root as well as to quantify the number of cysts. Additional samples will be analysed to confirm those results.