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INnovations in plant VarIety Testing in Europe

Deliverable D2.2
“Shopping list”, “Do it yourself custom list”, and measurement procedure to guide Eos and PROs in the implementation of the two non RGB low-cost phenotyping tools.

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Technical References

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Project Title	INnovations in plant VarIety Testing in Europe
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Task	T 4 - Non RGB low-cost field phenotyping tools
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[1] PU = Public
PP = Restricted to other programme participants (including the Commission Services)
RE = Restricted to a group specified by the consortium (including the Commission Services)
CO = Confidential, only for members of the consortium (including the Commission Services)

Document history

V	Date	Beneficiary	Author
1	18/11/21	CRA-W	Philippe Vermeulen
2	6/12/21	CRA-W	Philippe Vermeulen
3	14/06/22 (revision)	CRA-W	Philippe Vermeulen
4			

Summary

This deliverable provides a list of protocols developed and tested during the 2 first years of this project in the framework of the task 2.4 within WP2, and to be transferred and validated by the examination offices and the post registration organisations in the framework of the task 5.1 within WP5.

The task 2.4 aimed to deliver new tools exploring beyond the human eye spectrum for specific traits of interest not accessible with regular RGB imaging devices. In particular, UAV photogrammetry was used to estimate ryegrass height by INRAE Lusignan; lidar technology was used to estimate the number of apples on trees by INRAE Montpellier; near infrared spectroscopy (NIRS) was used to predict quality parameters on apples by CRA-W; VIS-NIR spectrometer, VIS-NIR multispectral and hyperspectral imaging were used to detect fusarium head blight (FHB) on wheat in laboratory by GEVES and CRA-W; and finally, Fluorescence, VIS-NIR multispectral and hyperspectral imaging were used to detect fusarium head blight (FHB) on wheat in field by respectively AGROSCOPE, GEVES and CRA-W.

For those 5 study cases, 10 protocols (one for each device) have been written using the same template. This template gives a short introduction explaining the purpose of the protocol, a list of requirements needed to apply the protocol, the different steps to follow before sample analysis, instructions to follow to perform the analysis itself, recommendations for exporting spectral data from the device and store the raw data on the files server and finally explanations on the data treatment flow applied on the acquired data in order to produce a result.

Table of content

1 Introduction

In this deliverable entitled « 'Shopping list', 'Do it yourself custom list', and measurement procedure to guide Examination Offices (EOs) and Post Registration Organisations (PROs) in the implementation of the two non-RGB low-cost phenotyping tools », you will find a list of protocols developed and tested during the 2 first years of this project in the framework of the task 2.4 dedicated to non RGB low-cost field phenotyping tools, within the WP2, Setting up mobile high-throughput phenotyping tools to measure existing and new bioindicators**.**

Figure 1 – Non RGB devices

Shopping list: non RGB devices

	Rye Grass	Apple	Wheat	Contact
Photogrammetry	Height			INRAE Lusignan, Didier Combes
Lidar		Nb apples		INRAE Montpellier, Frédéric Boudon
VIS-Near Infrared (NIR)		DM, brix	Fusa	CRA-W, Audrey Pissard CRA-W, Philippe Vermeulen
Multispectral imaging			Fusa	GEVES, Valérie Cadot
Hyperspectral imaging			Fusa	CRA-W, Damien Vincke
Fluorescence (Fluo)			Fusa	AGROSCOPE, Simon Treier

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2 Results

2.1 Shopping list

From this work in T2.4, 10 protocols related to 5 case studies have been written:

1 protocol dedicated to the height of rye grass in plot trials using photogrammetry technology on UAV (INRAE Lusignan), see 2.3.1 ;

1 protocol dedicated to the numbering of apples on trees using lidar technology on groundbased platform (INRAE Montpellier), see 2.3.2 ;

1 protocol dedicated to the quality of apples using a Visible-NIR spectrometer, Felix device (CRA-W), see 2.3.3 ;

3 protocols dedicated to fusarium head blight detection on wheat in laboratory using
- Visible – NIR spectrometer in laboratory (CRA-W), see 2.3.4.1;
- Multispectral imaging in laboratory (GEVES), see 2.3.4.2;
- Hyperspectral imaging in laboratory (CRA-W), see 2.3.4.3;

4 protocols dedicated to fusarium head blight detection on wheat in field using
- Multispectral imaging in field using a ground based support (GEVES/INRAE), see 2.3.5.1;
- Hyperspectral imaging in field using a rotating stage (CRA-W), see 2.3.5.2;
- Hyperspectral imaging in field using a translation stage (CRA-W), see 2.3.5.3;
- Fluorescence in field (AGROSCOPE), postponed to December 2022, see 2.3.5.4;

Figure 2 – Shopping list

2.2 Protocol template

Each protocol has been written using the same template organised as follows :

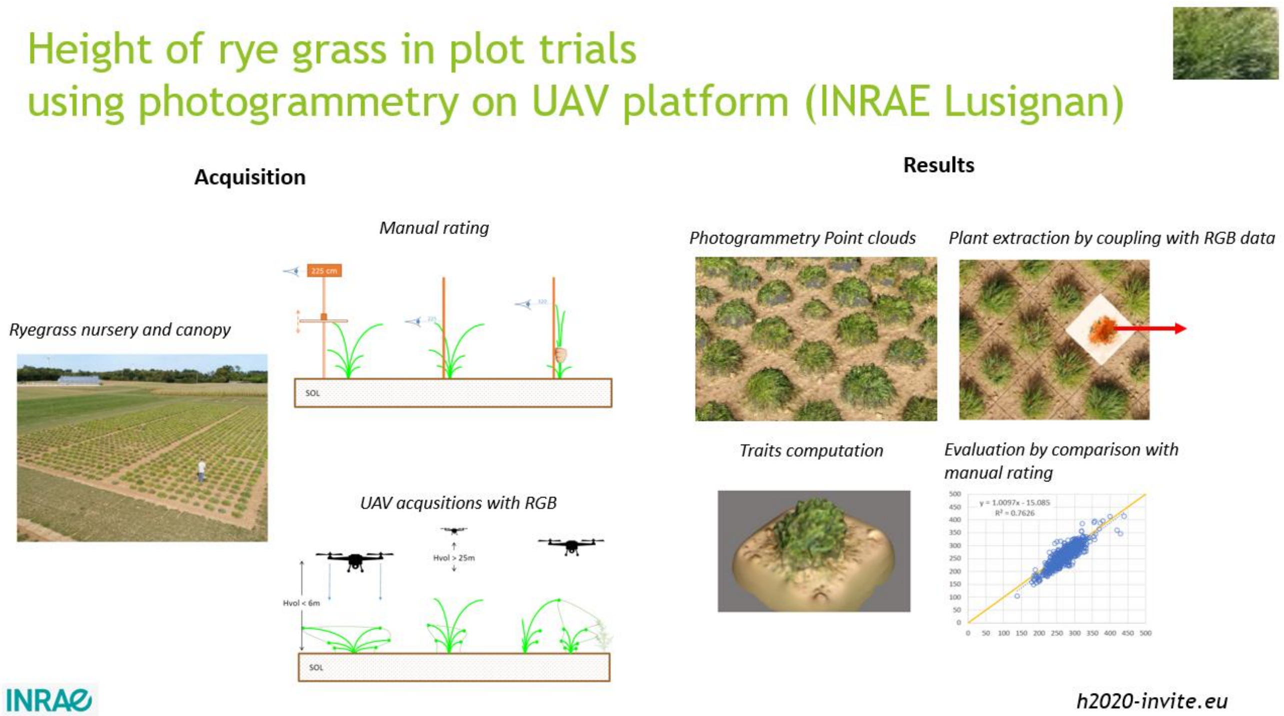
- A short introduction explaining the purpose of the protocol
- A list of requirements needed to apply the protocol
- The different steps to follow before sample analysis
- Instructions to follow to perform the analysis itself
- Recommendations for exporting spectral data from the device and store the raw data on the files server
- Explanations on the data treatment flow applied on the acquired data in order to produce a result

2.3 Protocols

2.3.1 Protocol dedicated to the height of rye grass in plot trials using photogrammetry technology on UAV (INRAE Lusignan)

The following figure summarizes the protocol from the data acquisition using unmanned aerial vehicle (UAV) to measure plant height by digital photogrammetry, up to the data treatment delivering height of rye grass by plot. The full protocol can be found in the following document joint to this deliverable: Protocol-drone-ray grass-vf.pdf.

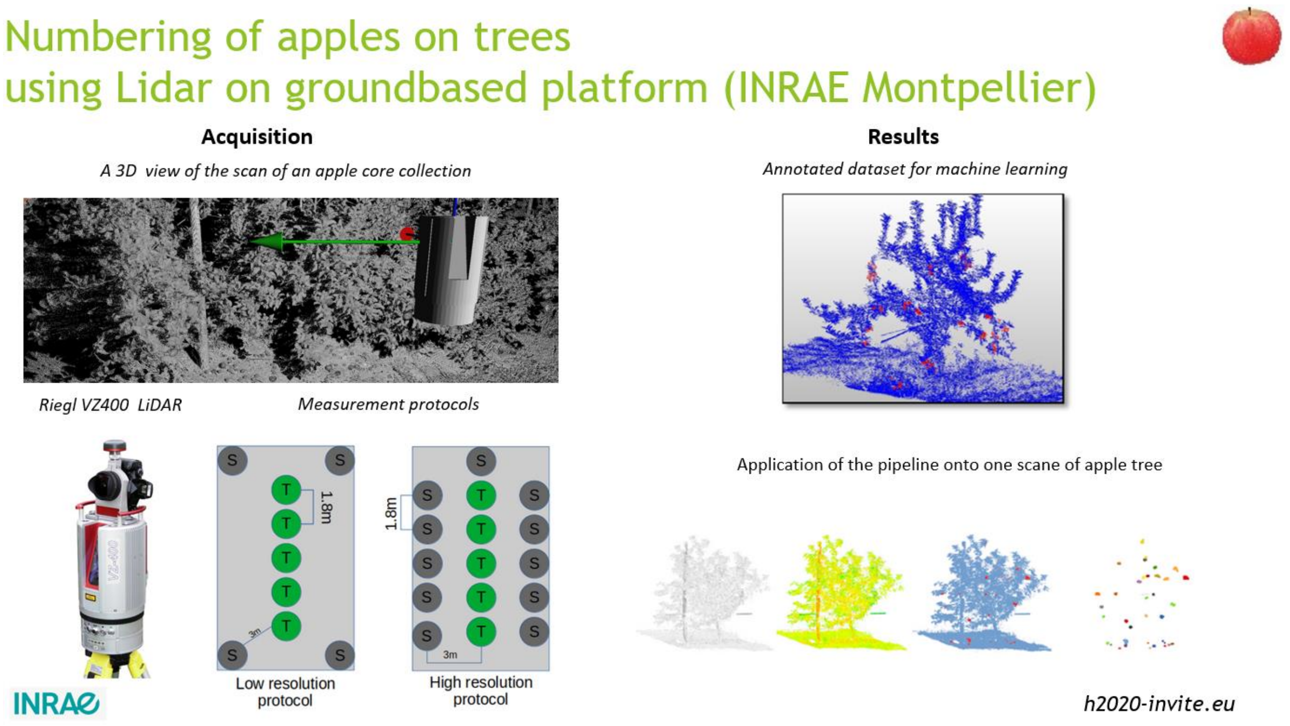
Figure 3 – Protocol to assess the rye grass height using photogrammetry on UAV



2.3.2 Protocol dedicated to the numbering of apples on trees using terrestrial lidar (INRAE Montpellier)

The following figure summarizes the protocol from the data acquisition using unmanned aerial vehicle (UAV) to measure traits on apples trees by laser imaging detection and ranging (lidar), up to the data treatment delivering the number of apples by tree. The full protocol can be found in the following document joint to this deliverable: Protocol-lidar-apple trees-vf.pdf. Detailed information on the global workflow of the data treatment pipeline is also available on https://github.com/jrojas9206/fruithunter.

Figure 4 – Protocol to assess the numbering of apples using lidar on groundbased platform



2.3.3 Protocol dedicated to the quality assessment of apples using a visible-NIR spectrometer, Felix F-750 device (CRA-W)

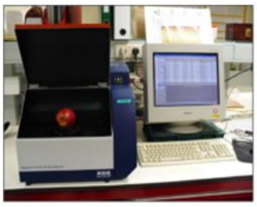
The following figure summarizes the protocol from the spectral data acquisition using laboratory and handheld device up to the data treatment delivering calibrations for 4 parameters; dry matter, brix, acidity and polyphenol. The full protocol can be found in the following document joint to this deliverable: Protocol-Felix F-750-on-apple fruit-vf.pdf.

Figure 5 – Protocol to assess the quality of apples using near infared spectroscopy

Quality of apples
using handheld VIS-NIR spectrometer Felix (CRA-W)

Acquisition

Apple measurements



Laboratory device: FOSS XDS

New generation of handheld device: FELIX F-750

Wallonie recherche CRA-W

Results

Calibration for 4 parameters

Parameter	Values (Mean; sd)	Device	Preprocess	Range (nm)	Factor	R^2Cal	R^2CV	RMSECal	RMSECV	RPDCV
DM (%)	81.5; 4.6	XDS	Der 1	729-975	8	0.85	0.84	1.4	1.4	2.6
		Felix			8	0.86	0.85	1.4	1.4	2.6
Brix (°)	10.6; 3.0	XDS	Der 1	729-975	7	0.58	0.56	1.9	2.0	1.5
		Felix			7	0.58	0.55	1.9	2.0	1.5
Acidity (g Ac mal/L)	5.0; 3.1	XDS	Der 1	729-975	12	0.61	0.57	1.9	2.0	1.6
		Felix			8	0.57	0.54	2.0	2.1	1.5
Polyphenol (µg/g)	747; 386	XDS	Der 1	729-975	12	0.55	0.47	258	279	1.4
		Felix			7	0.36	0.33	307	315	1.2

- Similar results between lab device and handheld device for the 4 parameters
- DM predicted at 80 % means DM between 77,2 and 82,8 % in 95 % of cases;
  3 groups of DM
- Brix predicted at 10 ° means brix between 6 and 14 ° in 95% of cases;
  2 groups of brix
- Acidity predicted at 5 g Ac mal/L means acidity between 1 and 9 g Ac mal/L in 95% of cases;
  2 groups of acidity
- Polyphenol predicted at 700 µg/g means polyphenol between 100 and 1300 µg/g in 95% of cases;
  2 groups of polyphenol

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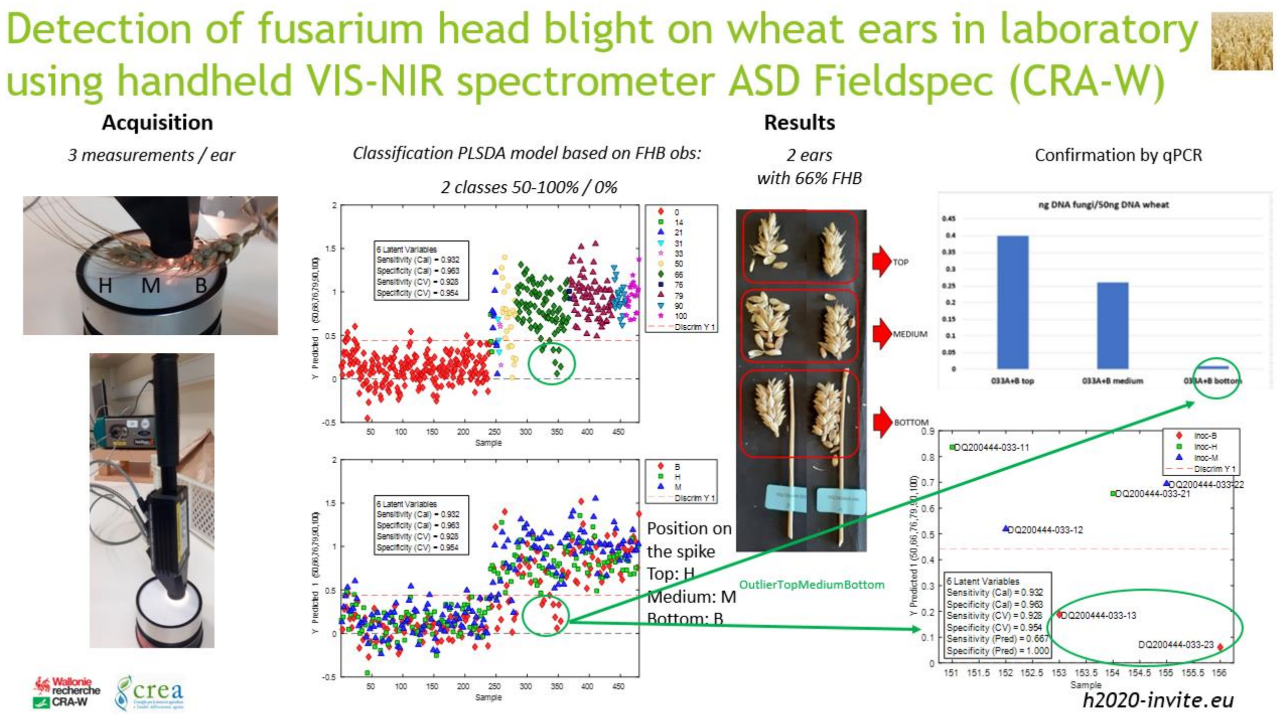
2.3.4 Protocols dedicated to the detection of fusarium head blight (FHB) on wheat in laboratory (CRA-W, GEVES/INRAE)

2.3.4.1 Protocol dedicated to FHB detection on wheat using Visible – NIR spectrometer (CRA-W)

The following figure summarizes the protocol from the spectral data acquisition in laboratory, using an handheld ASD fieldspec spectrometer, up to the data treatment delivering a classification, as infected by FHB or not, for the point measurement on the ear. The full protocol can be found in the following document joint to this deliverable: Protocol-Fieldspec4-Lab-FHB-on-wheat-ears-vf.pdf. Those predicted results can be confirmed by reference method such as the qPCR or dPCR method developed by CREA:

Morcia C, Tumino G, Gasparo G, Ceresoli C, Fattorini C, Ghizzoni R, Carnevali P, Terzi V. (2020). Moving from qPCR to chip digital PCR assays for tracking of some Fusarium species causing fusarium head blight in cereals. Microorganisms, 8(9), 1307.

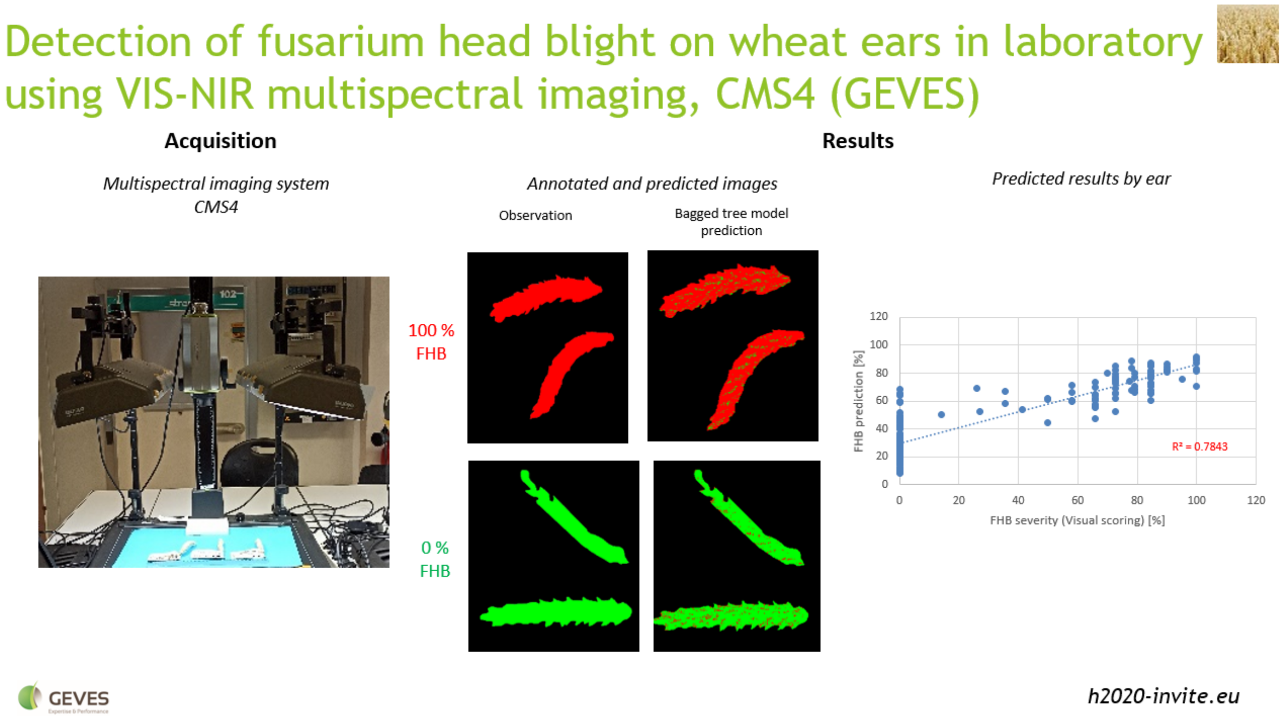
Figure 6 – Protocol to detect FHB on wheat using near infared spectroscopy in laboratory



2.3.4.2 Protocol dedicated to FHB detection on wheat using multispectral imaging, CMS-4 (GEVES/INRAE)

The following figure summarizes the protocol from the spectral image acquisition in laboratory, using the multispectral imaging system CMS-4, up to the images treatment delivering an assessment of the % FHB infection by ear. The full protocol can be found in the following document joint to this deliverable: Protocol-multispectral-camera-Lab-Field-FHB-on-wheat-ears-vf.pdf.

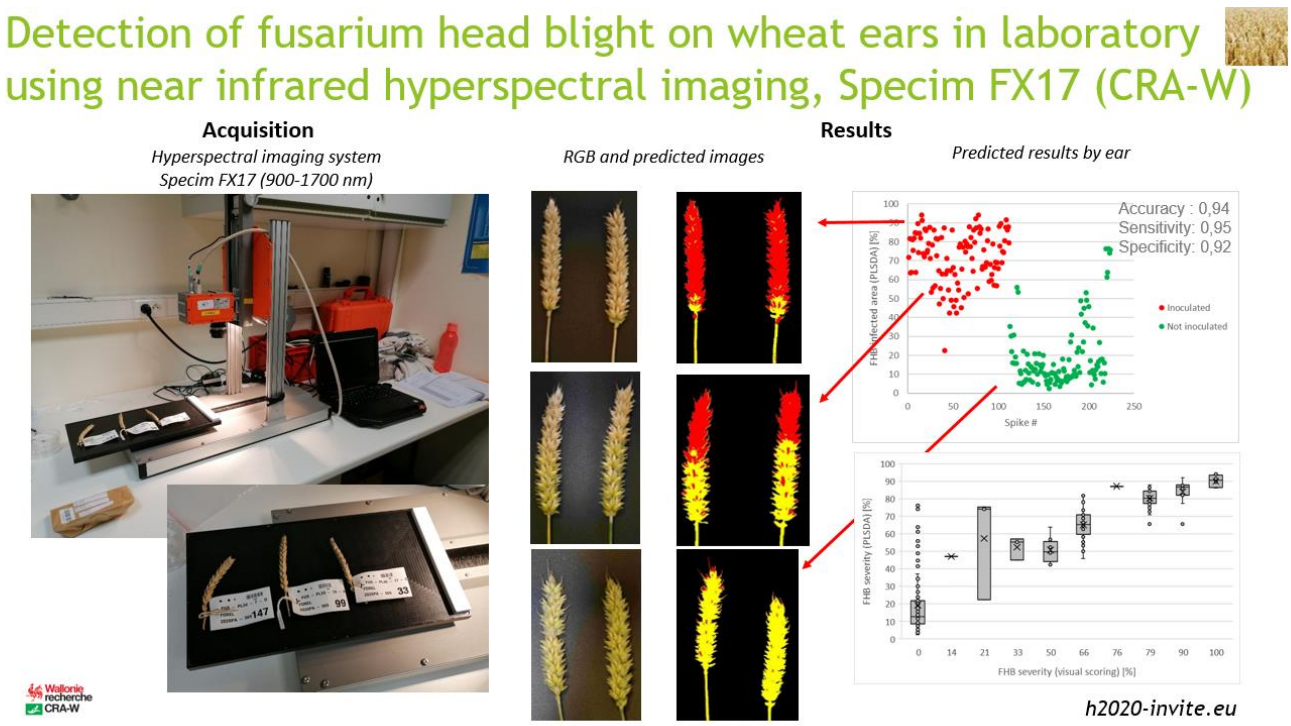
Figure 7 – Protocol to detect FHB on wheat using VIS-NIR multispectral imaging in laboratory



2.3.4.3 Protocol dedicated to FHB detection on wheat using near infrared hyperspectral imaging (CRA-W)

The following figure summarizes the protocol from the spectral image acquisition in laboratory, using the hyperspectral imaging system Specim FX17, up to the images treatment delivering an assessment of the % FHB infection by ear. The full protocol can be found in the following document joint to this deliverable: Protocol-FX17-Lab-FHB-on-wheat-ears-vf.pdf.

Figure 8 – Protocol to detect FHB on wheat using NIR hyperspectral imaging in laboratory

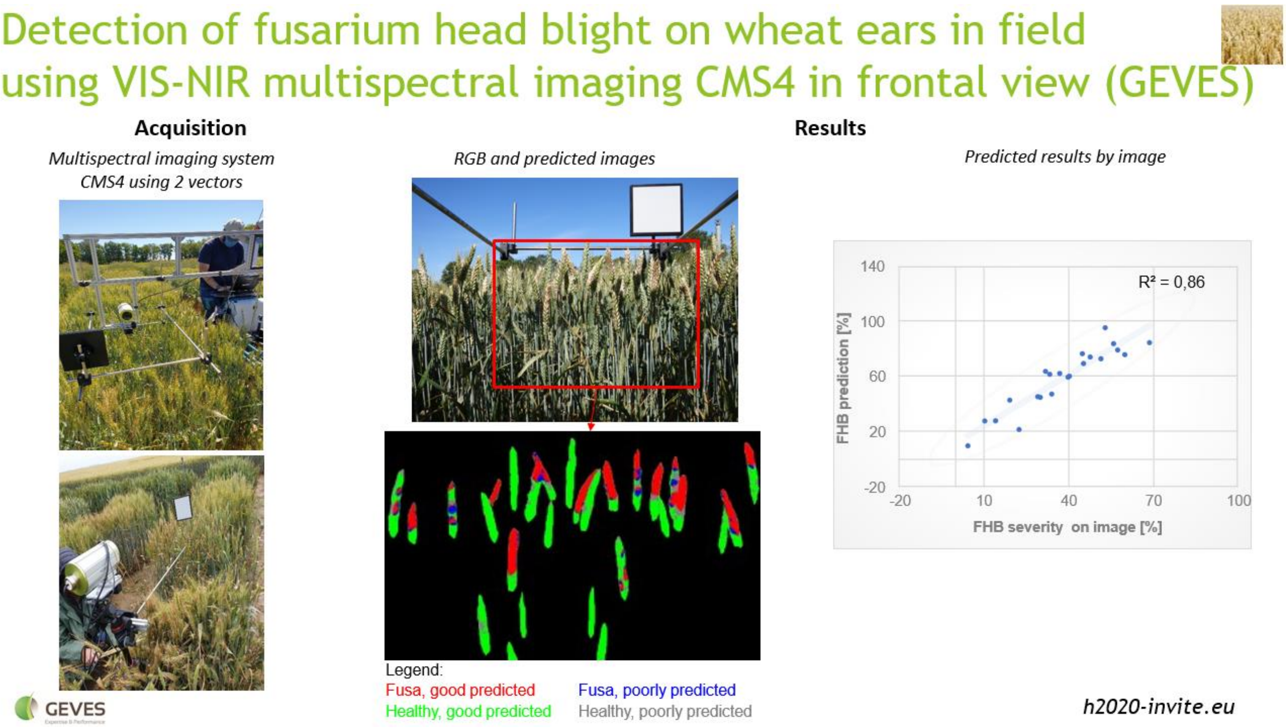


2.3.5 Protocols dedicated to the detection of fusarium head blight (FHB) on wheat in field (CRA-W, GEVES, AGROSCOPE)

2.3.5.1 Protocol dedicated to FHB detection on wheat using VIS-NIR multispectral imaging and a ground based support with frontal view (GEVES)

The following figure summarizes the protocol from the spectral image acquisition in field up to the images treatment delivering an assessment of the % FHB infection by ear. The full protocol can be found in the following document joint to this deliverable: Protocol-multispectral-camera-Lab-Field-FHB-on-wheat-ears-vf.pdf.

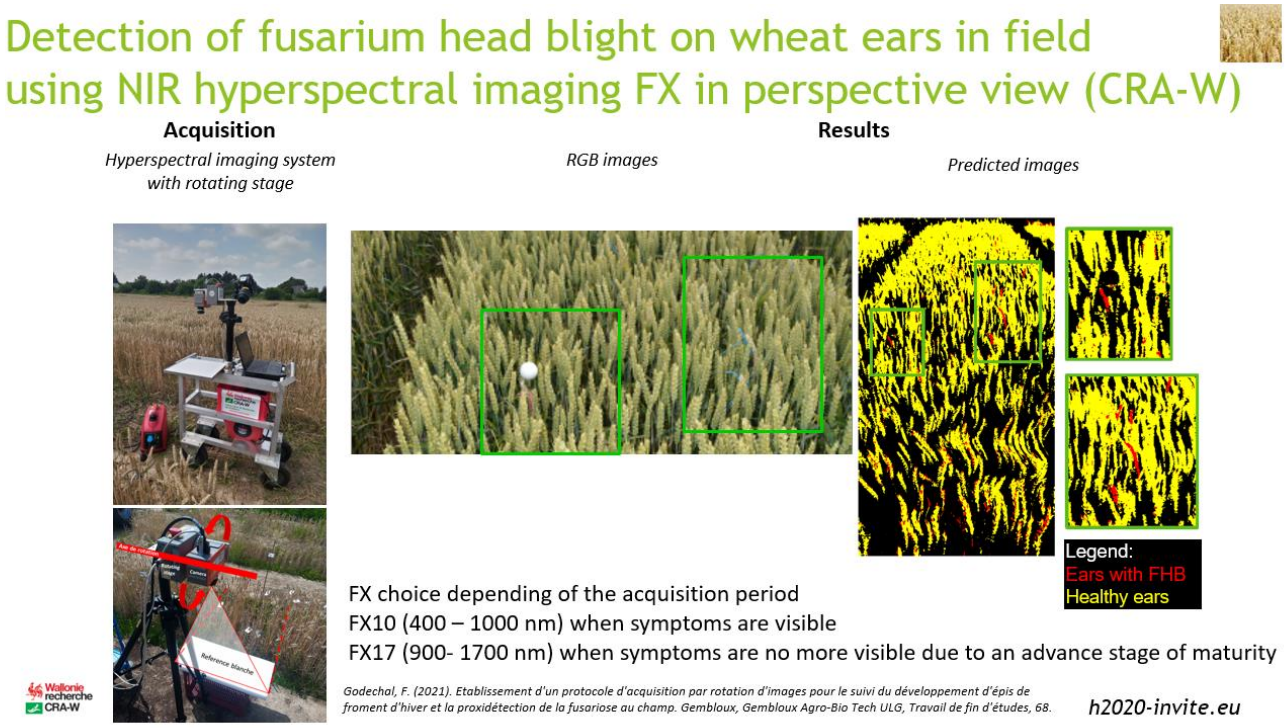
Figure 9 – Protocol to detect FHB on wheat using VIS-NIR multispectral imaging in field



2.3.5.2 Protocol dedicated to FHB detection on wheat using near infrared hyperspectral imaging and a rotating stage with perspective view (CRA-W)

The following figure summarizes the protocol from the spectral image acquisition in field, using the hyperspectral imaging system Specim FX with rotating stage, up to the images treatment delivering an assessment of the FHB infection in a plot. The full protocol can be found in the following document joint to this deliverable: Protocol-FX-rotating-stage-Field-FHB-on-wheat-ears-vf.pdf. Additional information can be found in the end of study work of François Godechal: Godechal, F. (2021). *Etablissement d'un protocole d'acquisition par rotation d'images pour le suivi du développement d'épis de froment d'hiver et la proxidétection de la fusariose au champ.* Gembloux, Gembloux Agro-Bio Tech ULG, Travail de fin d'études, 68.

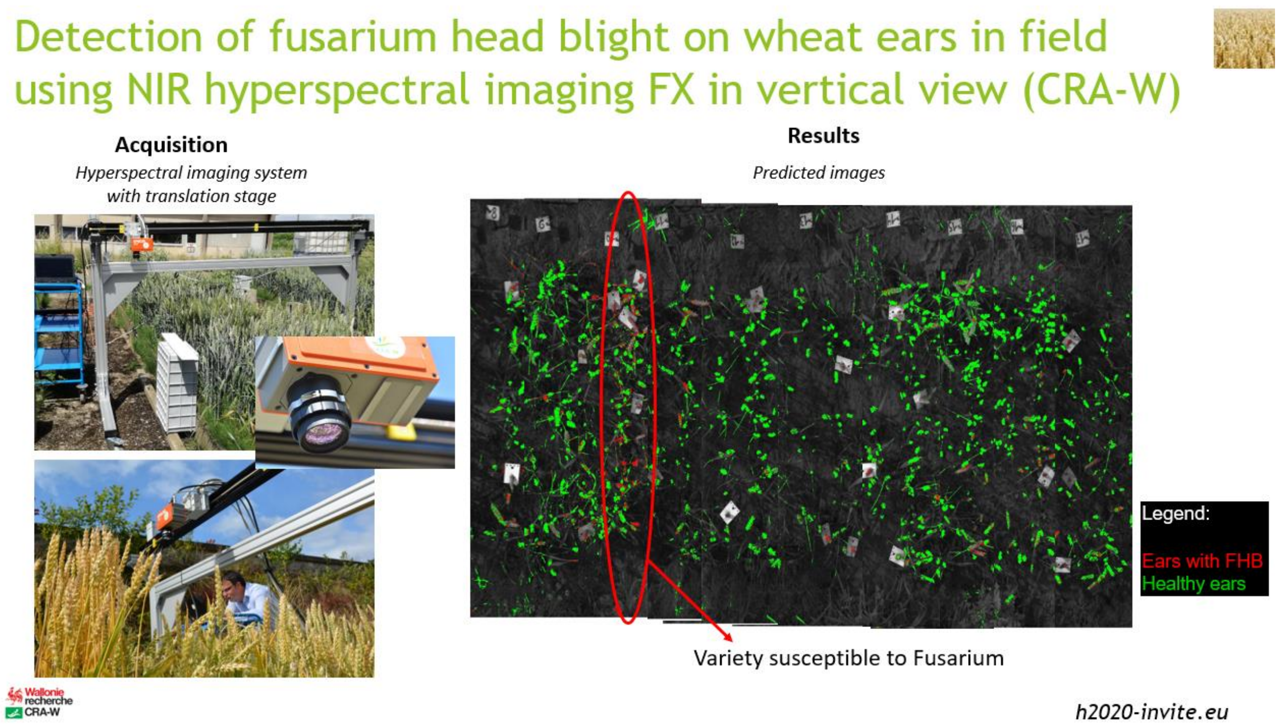
Figure 10 – Protocol to detect FHB on wheat using NIR hyperspectral imaging with rotating stage in field



2.3.5.3 Protocol dedicated to FHB detection on wheat using near infrared hyperspectral imaging and a translation stage with vertical view (CRA-W)

The following figure summarizes the protocol from the spectral image acquisition in field, using the hyperspectral imaging system Specim FX with translation stage, up to the images treatment delivering an assessment of the FHB infection in a plot. The full protocol can be found in the following document joint to this deliverable: Protocol-FX-translation-stage-Field-FHB-on-wheat-ears-vf.pdf.

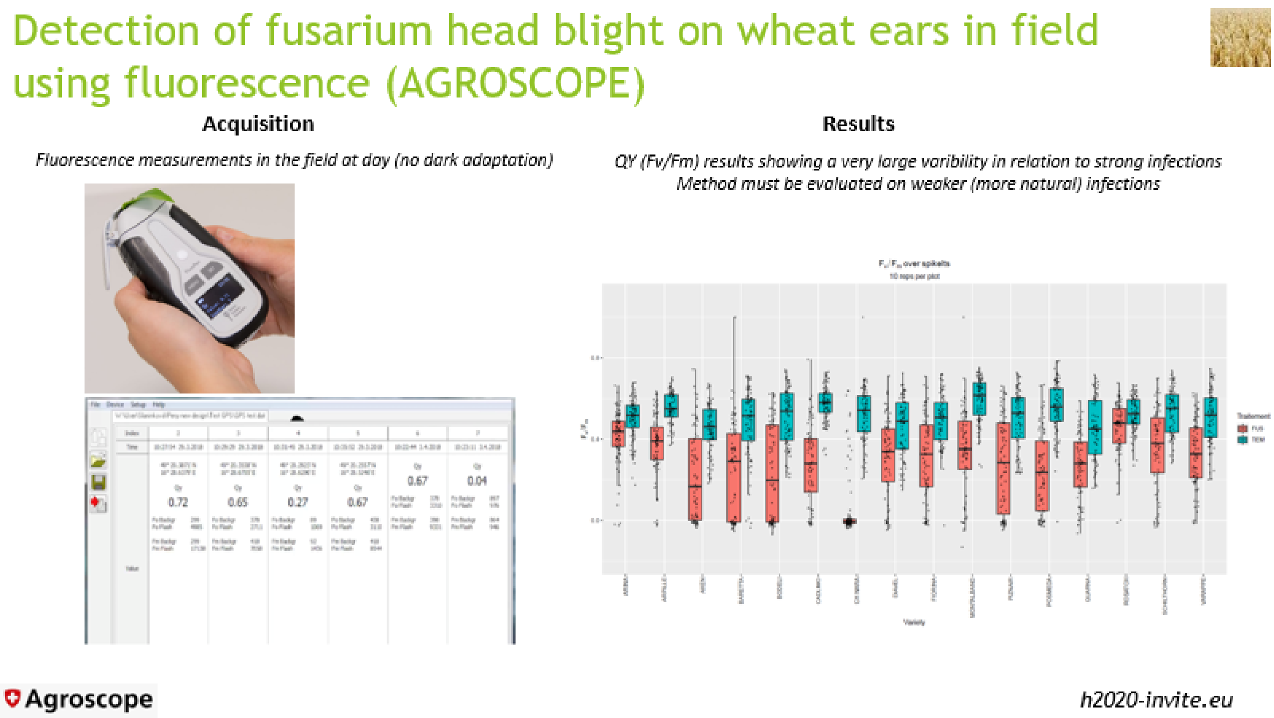
Figure 11 – Protocol to detect FHB on wheat using NIR hyperspectral imaging with translation stage in field



2.3.5.4 Protocol dedicated to FHB detection on wheat using fluorescence (AGROSCOPE)

The following figure summarizes the protocol from the fluorescence data acquisition in field. One additional year was added to improve the protocol because we found out that there was room to improve the configuration and operation of the devices and this may improve the accuracy of the measurements. It is planned to be delivered it by end of 2022.

Figure 12 – Protocol to detect FHB on wheat using fluorescence in field



3 Conclusions

Within the task 2.4, new non RGB tools have been explored for specific traits of interest not accessible with regular RGB imaging devices. In particular, UAV photogrammetry was used to estimate ryegrass height by INRAE Lusignan; lidar technology was used to estimate the number of apples on trees by INRAE Montpellier; near infrared spectroscopy (NIRS) was used to predict quality parameters on apples by CRA-W; VIS-NIR spectrometer, VIS-NIR multispectral and hyperspectral imaging were used to detect fusarium head blight (FHB) on wheat in laboratory by GEVES and CRA-W; and finally, Fluorescence, VIS-NIR multispectral and hyperspectral imaging were used to detect fusarium head blight (FHB) on wheat in field by respectively AGROSCOPE, GEVES and CRA-W.

All the protocols are available for the partners from the examination offices or post registration organisations in order to be tested/validated/transferred under other environmental conditions. That will be done in the framework of the WP5 activities.

4 Annexes

All the protocols developed in the framework of this work are available under doc or pdf file.