

Identifying fungal leaf spots on oilseed rape: could pictures help?

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Abstract

From sowing in late summer until harvest in following summer, oilseed rape can be infected by several fungi, which foliar symptoms (leaf spots) coexist on the crop. Training an expert at their identification is quick for the typical symptoms with characteristic appearance. However, in many cases the size, colour and morphology are similar and for the atypical symptoms, there is a risk of confusion or in-decidability. Also, scouting the fields for expert training is not possible at all seasons and all diseases might not be seen in all years and all places. The aim of our study was to produce large sets of pictures annotated by several experts, from which tables illustrating the diversity of symptom appearance were chosen. These tables will enable assistance to diagnostic and expert training.

Keywords: Phenotyping, multi-pathogen, diagnostic, *Leptosphaeria maculans*, *L. biglobosa*, *Neopseudocercospora capsellae*, *Mycosphaerella brassicae*, *Alternaria brassicae*, *A. brassicicola*, *Pyrenopeziza brassicae*.

Introduction

A critical challenge in plant pathology and epidemiology is to design and implement durable crop protection strategies against pathogens. On annual crops, many plant diseases have cyclic epidemics and their dynamics are highly influenced by both temporal and spatial discontinuities, either induced by the climate (e.g. seasonality) or by human actions (e.g. sowing and harvesting) (Zadoks & Schein 1979; Bousset & Chèvre 2013). Therefore, on the one hand each disease might not be present during the whole cropping season of the host. On the other hand, a given host can simultaneously face several pathogens. Precise identification of each causal agent is needed to deploy proper crop protection strategies.

In Western Europe, oilseed rape is grown as a winter crop, sown in late summer and harvested in the following summer. During this extended period of time, several foliar pests and diseases can infect leaves and cause co-occurring symptoms on the crop. Identification of the causal agents is at stake for plant pathologists, breeders and extension service agents. Training an expert at their identification is quick and easy for typical symptoms with characteristic appearance. Training can be done in the field with real symptoms at seasons when symptom occur, or using symptom images. However, in many cases the size, colour and morphology are similar between different species, because for each of the species the symptom aspect is altered by several factors. The plant genotype, potentially with complete or partial resistance, can render the interaction less compatible, with plant defence reactions inducing e.g. darkening around lesions. Also, some of the symptom characteristics, such as darkening or yellow halo, can be convergent between two or more pathogens and bring confusion to the diagnostic. Finally, the physiological state of the plant tissues can also have an impact on the

symptoms. So in many cases the size, colour and morphology of the symptoms can be similar although the plant is infected by different fungal species and there is a risk of confusion or indecidability between several symptom-causing pathogens.

In this study we aimed at sampling the diversity of symptom appearance for foliar diseases on oilseed rape during the cropping season, with multiexpert annotation. We gathered a community of plant pathologists more or less trained to the diagnostic of foliar diseases of oilseed rape in order to build a database of consensual or problematic symptoms. Standardized pictures of a range of symptoms were taken, independently annotated and classified in different categories depending on experts' answers. This enabled us to build a validated set of "typical" and "atypical" symptom pictures for six foliar fungal pathogen species. Finally, this work enabled us to list the phenotypic traits that help distinguishing each of these species. From this diversity, illustrations are provided in addition to sets of distinctive criteria as material to help diagnostic or train experts.

Materials and methods

Experimental fields, collection of leaf symptoms and handling of leaves

Symptomatic leaves were collected from winter oilseed rape fields in cropping seasons 2018-2019 to 2020-2021 on plants ranging from cotyledon to pod formation growth stage (Table 1). To encompass the varietal diversity of winter oilseed rape, we sampled variety testing plots, plant breeding nursery, both located on the INRAE UE La Motte experimental station in Le Rheu (48.1°N, 1.5°W), in Brittany and scouted farmers' fields within 40 km around. The climate of Brittany is oceanic and most of the oilseed rape foliar fungal diseases are observed each year. To expand the range of soil and cropping conditions, samples at Grignon (48.9°N, 1.9°E) from INRAe BIOGER and TERRES INOVIA, in Ile-de-France were added. Leaves sampled were either typical i.e. with causal agent identified at first sight and atypical i.e. identification not straightforward. While scouting the fields, leaves were harvested altogether in bags. In the laboratory, leaves were arranged in buckets with petioles dipped in water so that they would keep fresh overnight. Leaves were further processed either the same or the following day by imaging the leaf lesions.

Table 1: Numbers of leaf segments sampled depending on the year and the growth stage

	All leaf fragments
Season - Month	
2018-2019 Mar.	306
2019-2020 Oct.	114
2019-2020 Nov.	46
2019-2020 Feb.	169
2019-2020 Mar.	239
2020-2021 Nov.	63
Growth stage	
Cotyledon	67
Leaf rosette	156
Stem elongation	178
Flowering	456
Pod formation	80
Total	937

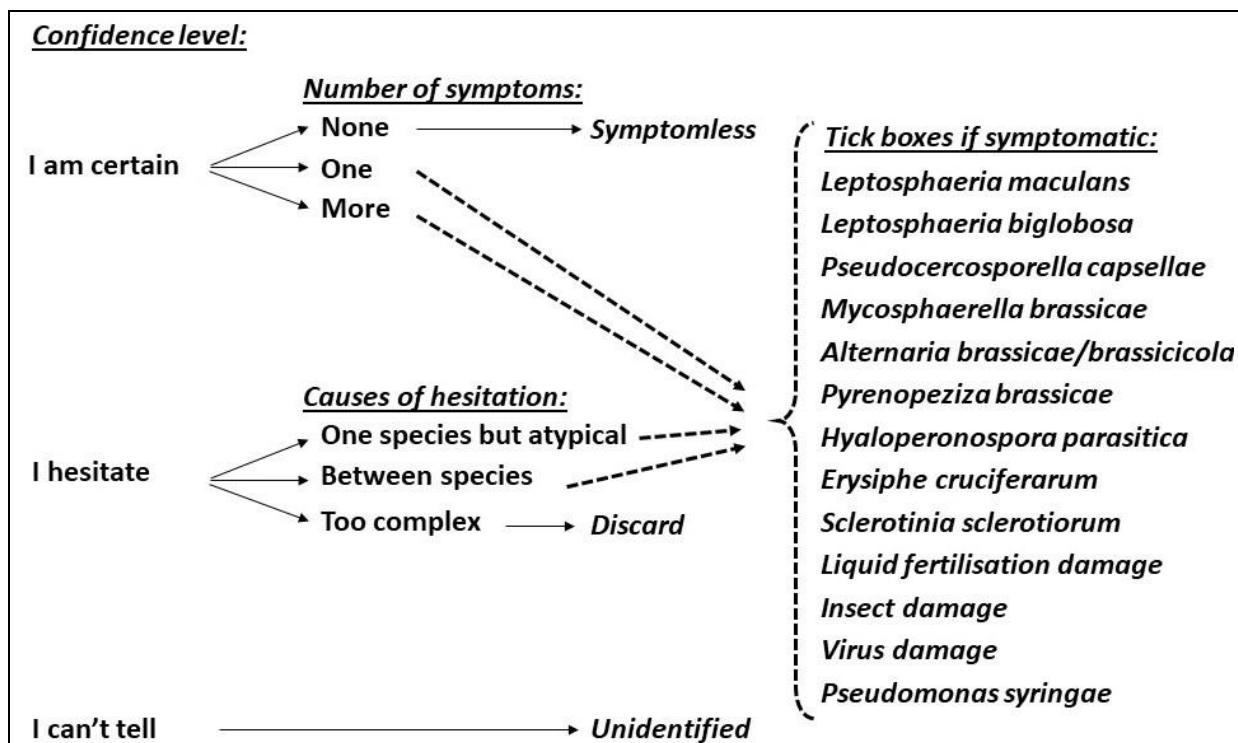
Image acquisition and pre-processing

The first step was to image the leaf lesion environment on the leaf, first taking pictures of the whole area on the recto and verso sides, then taking a third picture of the recto using a red gasket placed to circle one isolated leaf lesion. Using scissors, the second step was to cut a leaf portion containing only one leaf lesion. A pair of standardised pictures on the recto and the verso side of this fragment were taken on a blue background (PVC sheet Lastolite Colormatt electric blue), together with a label indicating sample name and a second label indicating either recto or verso, a ruler and a standardised colour test pattern with white grey and black. Two workshop led hand lights were placed on both sides of the leaf fragment within the lower 45° angle. Pictures were taken with a Nikon D5200 with an AF-S DX Micro Nikkor 40mm 1:2.8G lens, on a self-assembled stand, with a wired remote control. Aperture was set at F14 for maximal depth of field, iso 125, daylight white balance. Pictures were saved as RGB images with a resolution of 6000 x 4000 pixels. Picture pre-processing consisted in reading the barcodes to rename the files. The corresponding leaf fragments were kept frozen at -20°C.

Multi-expert annotation on a web interface

A set of 6 pathologists familiar with oilseed rape but not with each of the fungal diseases was assembled. Each of the expert had to annotate each picture on a web interface, having access to the other members' annotations only after having completed the duty. The set of 5 pictures of each symptom (general environment recto and verso, leaf lesion circled, leaf fragment recto and verso) was available with possibility to zoom. Each symptom was annotated as follows (Fig. 1). The first question was about confidence, with 3 levels ("certain"; "I hesitate" or "I don't know"). When "certain", the number of symptoms enables to set aside symptomless fragments; finally if symptomatic, tick boxes were available for 13 distinct causal agents. When "hesitating", too complex images (more than one symptom, some of which uncertain) are set aside; distinction is made between on the one hand the identification of one species but atypical symptom appearance; on the other hand hesitation between several species. When the expert "can't tell", the symptom is classified unidentified. For the symptomatic leaf fragments, tick boxes enable "and" and "or" selection of 9 fungal diseases, liquid fertilisation, insect, virus and bacterial damage.

Figure 1: Decision key for the annotation by experts on the web interface depending on expert's confidence level, numbers of symptoms seen, causes of hesitation and causal species.



Set of criteria and picture selection for the figures

Following annotation of the whole dataset, the experts devised sets of criteria distinctive for each of the fungal species frequently encountered on oilseed rape in France. They selected subsets of pictures on the one hand with typical symptoms (respecting most of the distinctive criteria); on the other hand with atypical symptoms (variants not respecting all criteria).

Results

We produced a database of 937 symptoms standardized image recto-verso pairs from a diversity of varieties and sampling times across 2019, 2020 and 2021. The annotation step distinguished between contrasted situations of expertise ranging from simple consensual cases to disagreement or lack of identification confirming the difficulty of visual and photographic diagnostic.

The 6 experts agreed on sets of distinctive criteria for each of the 6 species frequently encountered on oilseed rape in France (Fig. 2; FigS1 higher quality pictures).

Figure 2: Sets of criteria distinctive for each of the six species (right panel) and corresponding illustrative recto-verso image pairs for two symptoms per species.

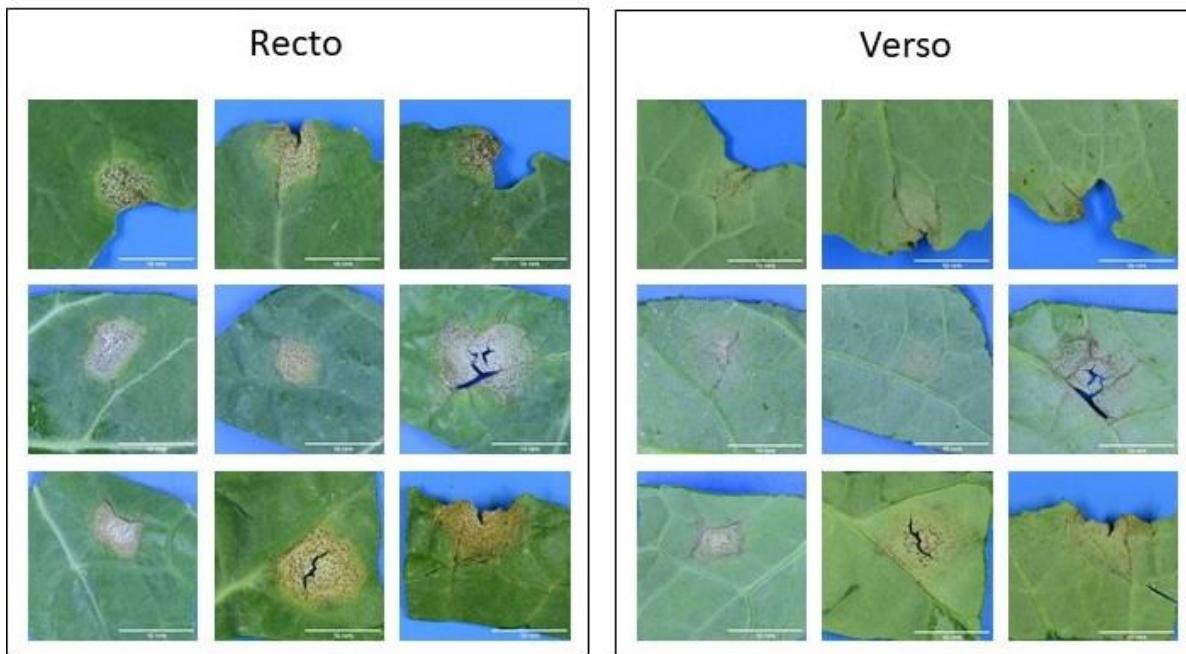


Distinctive criteria	
<p>Phoma stem canker, <i>Leptosphaeria maculans</i>: Light grey leaf spot, often in a not fully regular shape. Black pycnidia visible to the naked eye, well distinct from one another. Diffuse border, generally without margin. Often the symptom stops at a leaf vein, which is black. Sometimes the leaf spot tears over: tissue collapse render them thinner. Verso of the symptom is green lighter than the leaf, and can bear some dots/crossing pink spores (pycnidia)</p>	
<p>Phoma stem canker, <i>Leptosphaeria biglobosa</i>: Symptom seldom regular, spreading in all directions, generally stopped by leaf veins. Intense grey color, often with a darker dot in the middle (sometimes small tear). No or very few pycnidia visible to the naked eye. Often the symptom is surrounded by a yellow halo. Verso of the symptom of intense grey color. Exist a larger and more regular form, with a black dot at the center. Sometimes dry necrosis of lighter grey. Exist a smaller form, black with a yellow halo.</p>	
<p>Black spot, <i>Alternaria brassicicola</i>, <i>A. brassicace</i>: Symptom of very regular shape, most often not stopping at leaf veins. Dark grey to blackish color. No pycnidia, and in most cases darker/lighter concentric circles are visible. There often is a yellow halo around the symptom. Verso of the symptom is dark grey to black.</p>	
<p>Ring spot, <i>Mycosphaerella brassicicola</i>: Symptom fully circular, most often not stopping at leaf veins. Dark grey color due to the many pycnidia, difficult to separate with the naked eye, smaller and more contiguous than these of <i>L. maculans</i>, sometimes arranged in circles. Often border slightly undulating and light green or yellow margin (not the same yellow as around <i>Alternaria</i> and <i>L. biglobosa</i>). Variable in color on the verso, but always slightly colored (beige) compared to <i>L. maculans</i>.</p>	
<p>White leaf spot, <i>Neopseudocercosporaella capsellae</i>: Symptom of irregular shape, round or angular that sometimes stops at leaf margins. Whitish color, spot often with dark brown / black margin. Without visible pycnidia, but often marbling. On the verso, the presence of marbling/dark veins is distinctive. Exist a more circular and white form.</p>	
<p>Light leaf spot, <i>Pyrenopeziza brassicae</i>: <u>Early stages</u> of symptoms: Light grey discolored area, white sugar-like acervuli often forming a circular pattern around the discolored area. Either on the recto or the verso of the leaf. <u>Late stages</u>: dry beige necrosis of corky aspect and anarchic shape, with an intense yellow coloration (not the same yellow as the halo surrounding <i>Alternaria</i> or <i>L. biglobosa</i> symptoms). The old acervuli turn black and can be further around the crumpled symptom. In severe forms, the leaf can be crinkled and symptoms cracked.</p>	

For each of these 6 species, the 6 experts selected subsets of pictures on the one hand with typical symptoms (respecting most of the distinctive criteria); on the other hand with atypical symptoms (variants not respecting all criteria). Figures were produced for phoma stem canker *Leptosphaeria maculans* (Fig. 3 & Fig. S2 higher quality pictures) and *Leptosphaeria biglobosa* (Fig. 4 & Fig. S3 higher quality pictures); for white leaf spot *Neopseudocercosporaella capsellae* (Fig. 5 & Fig. S4 higher quality pictures); for ring spot *Mycosphaerella brassicace* (Fig. 6 & Fig. S5 higher quality pictures); for black spot *Alternaria brassicicola* and *A. brassicicola* (Fig. 7 & Fig. S6 higher quality pictures) and light leaf spot *Pyrenopeziza brassicae* (Fig. 8 & Fig. S7 higher quality pictures).

Figure 3: Phoma stem canker *Leptosphaeria maculans*

3a. Typical symptoms: examples respecting most of the distinctive criteria for the species



3b. Atypical symptoms: examples of variations respecting only some of the distinctive criteria

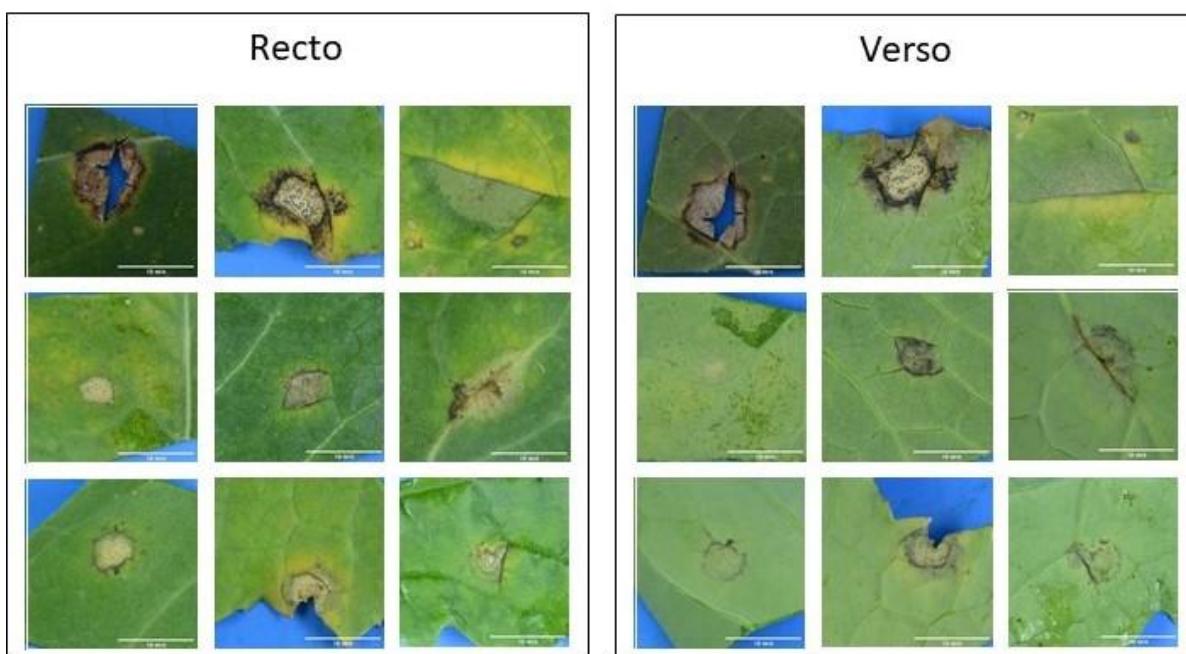
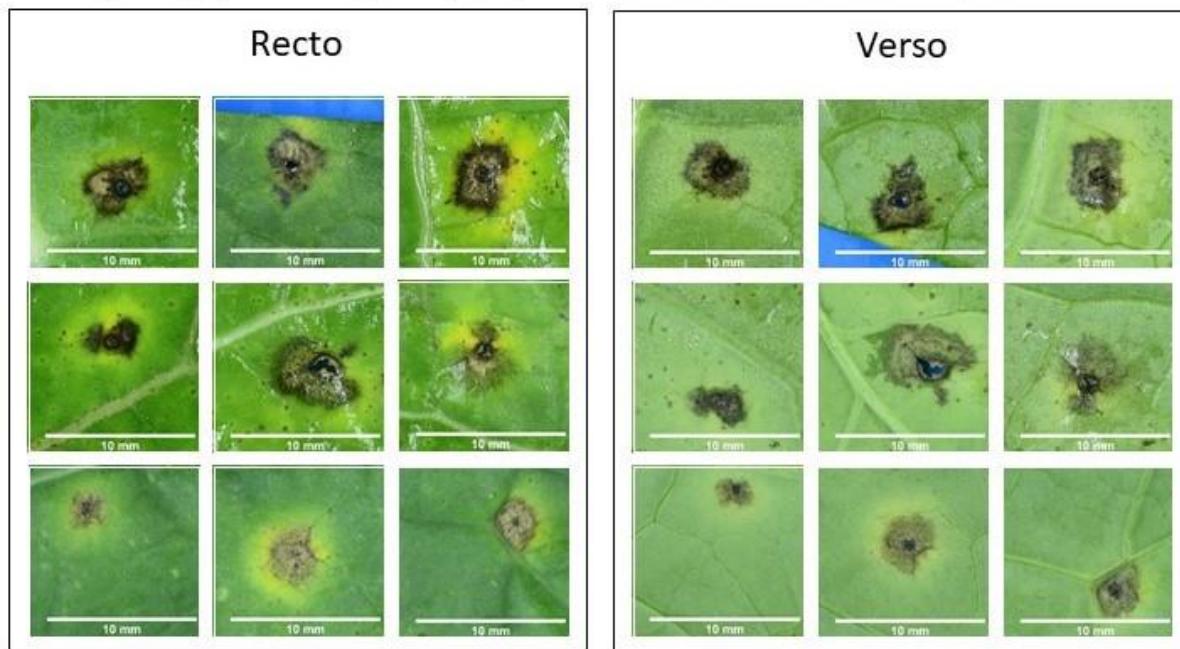


Figure 4: Phoma stem canker *Leptosphaeria biglobosa*

4a. Typical symptoms: exemples respecting most of the distinctive criteria for the species



4b. Atypical symptoms: examples of variations respecting only some of the distinctive criteria

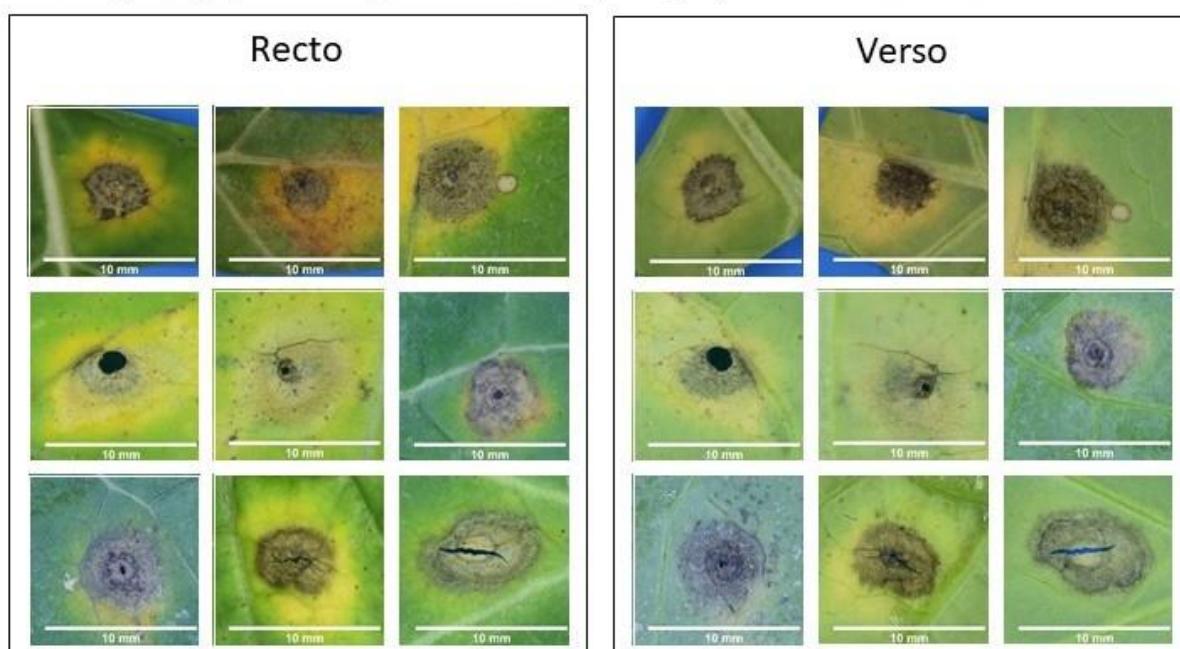
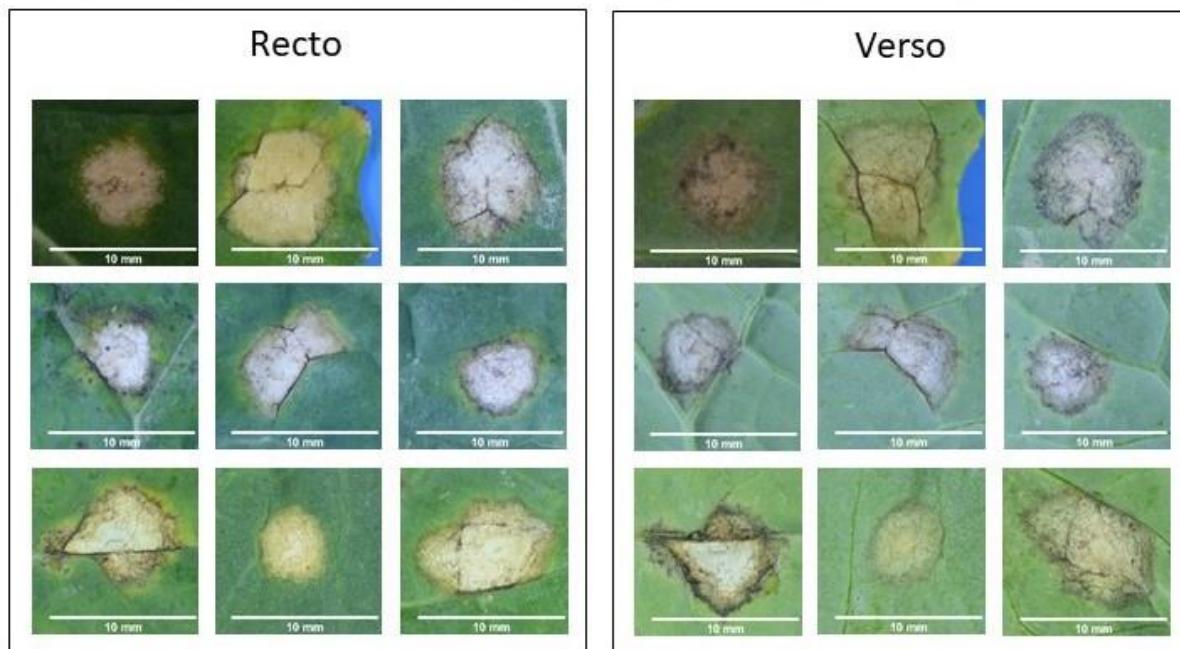


Figure 5: White leaf spot *Neopseudocercospora capsellae*

5a. Typical symptoms: exemples respecting most of the distinctive criteria for the species



5b. Atypical symptoms: examples of variations respecting only some of the distinctive criteria

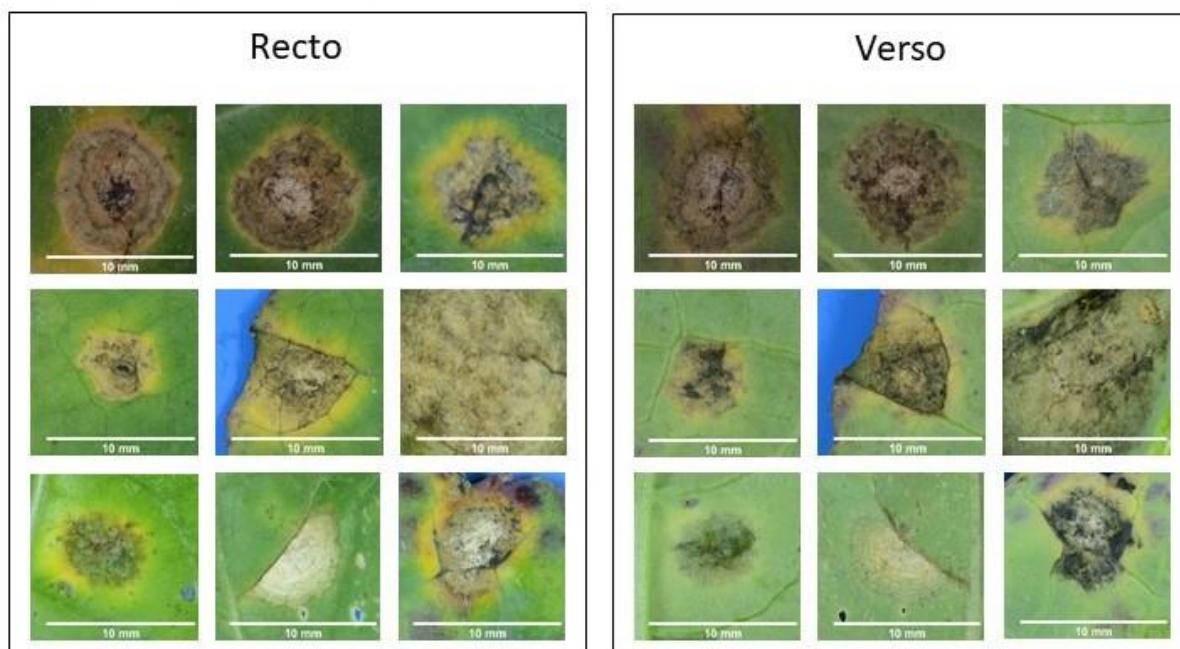
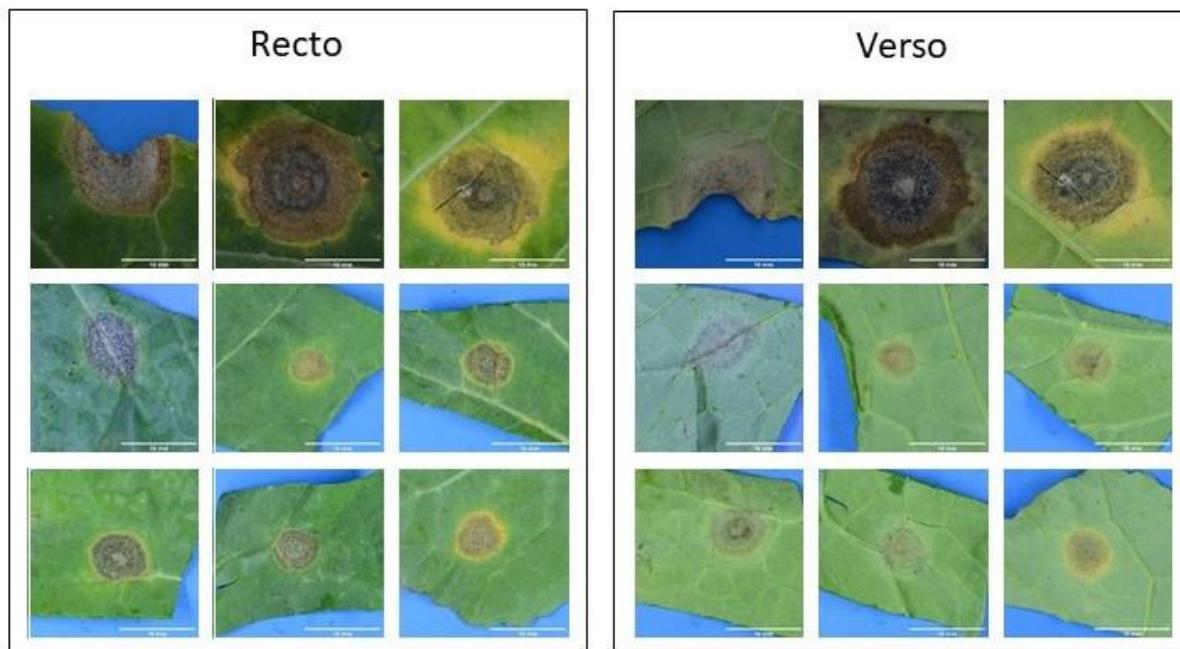


Figure 6: Ring spot *Mycosphaerella brassicae*

6a. Typical symptoms: examples respecting most of the distinctive criteria for the species



6b. Atypical symptoms: examples of variations respecting only some of the distinctive criteria

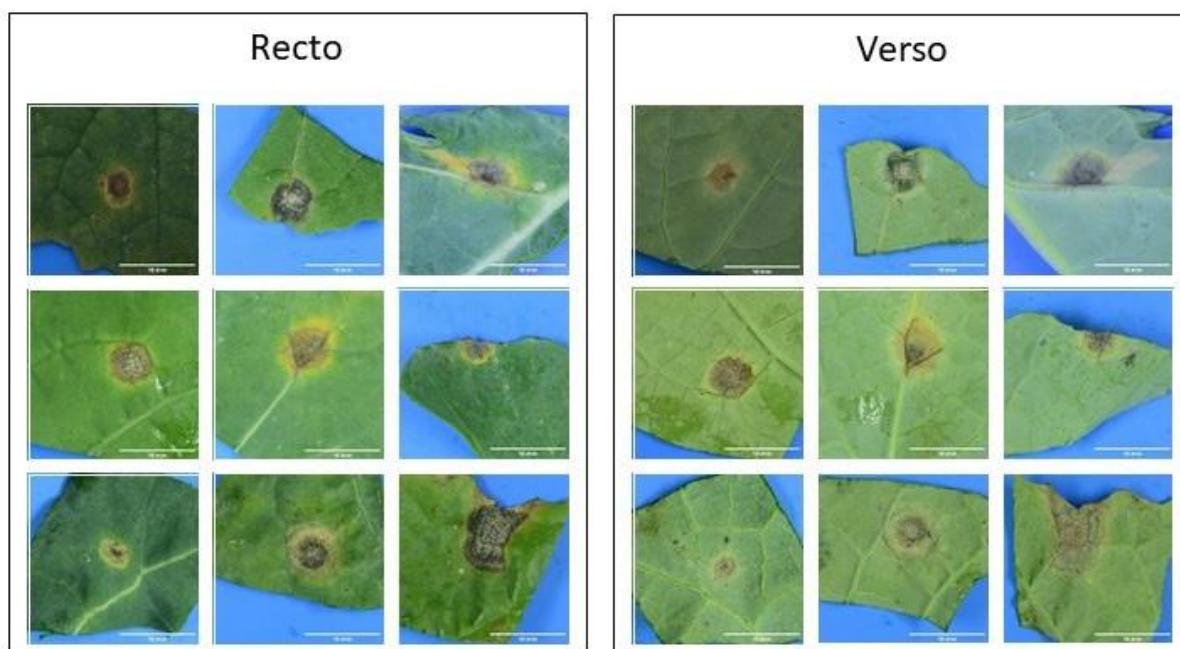
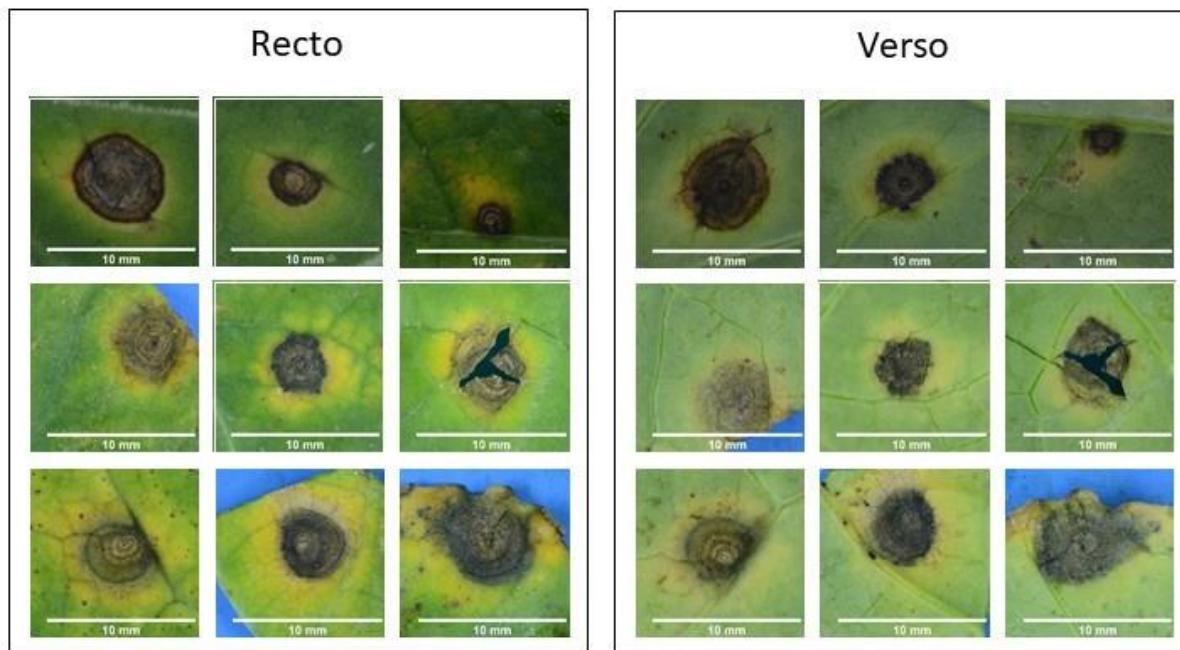


Figure 7: Black spot *Alternaria brassicae* and *A. brassicicola*

7a. Typical symptoms: examples respecting most of the distinctive criteria for the species



7b. Atypical symptoms: examples of variations respecting only some of the distinctive criteria

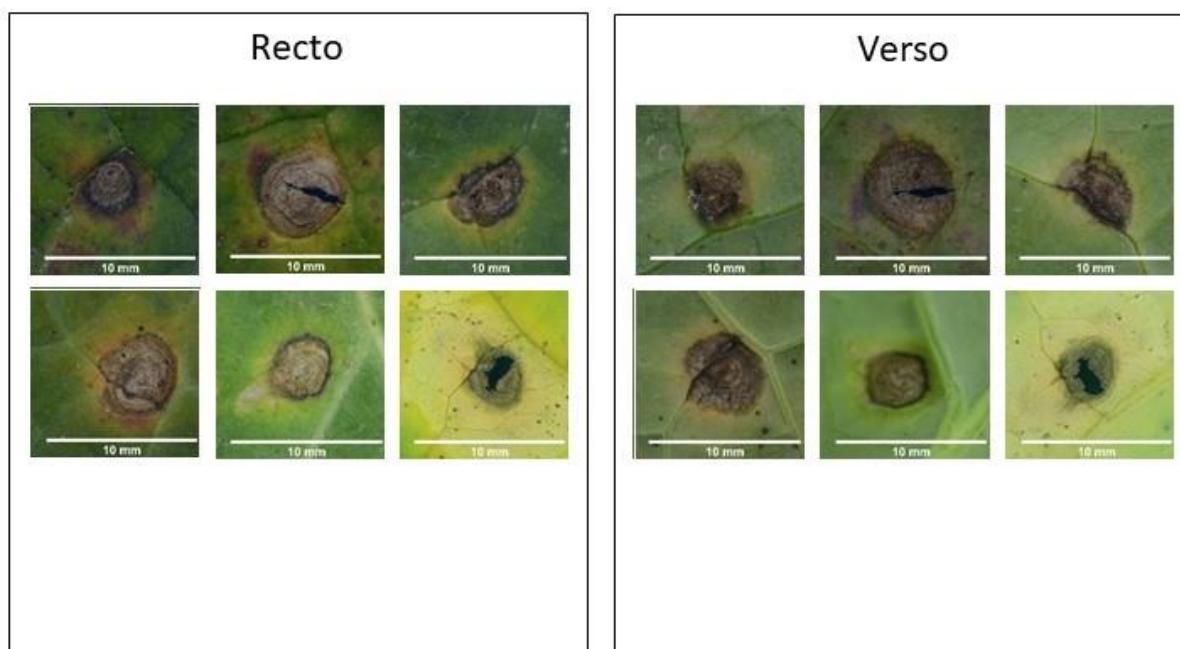
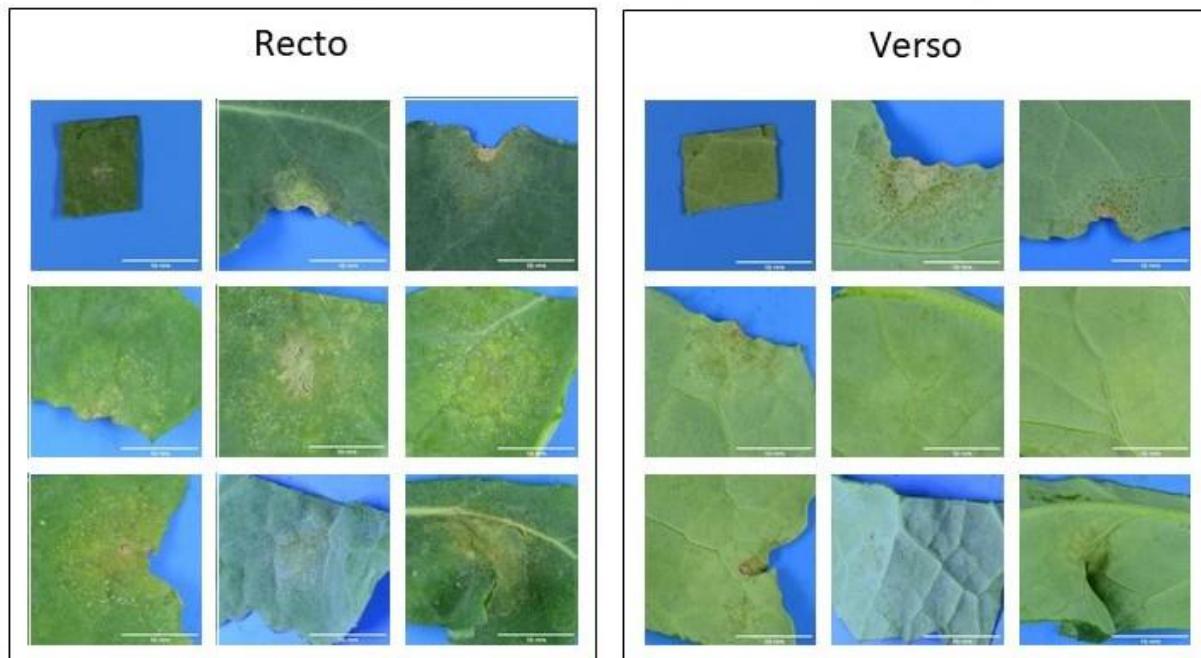
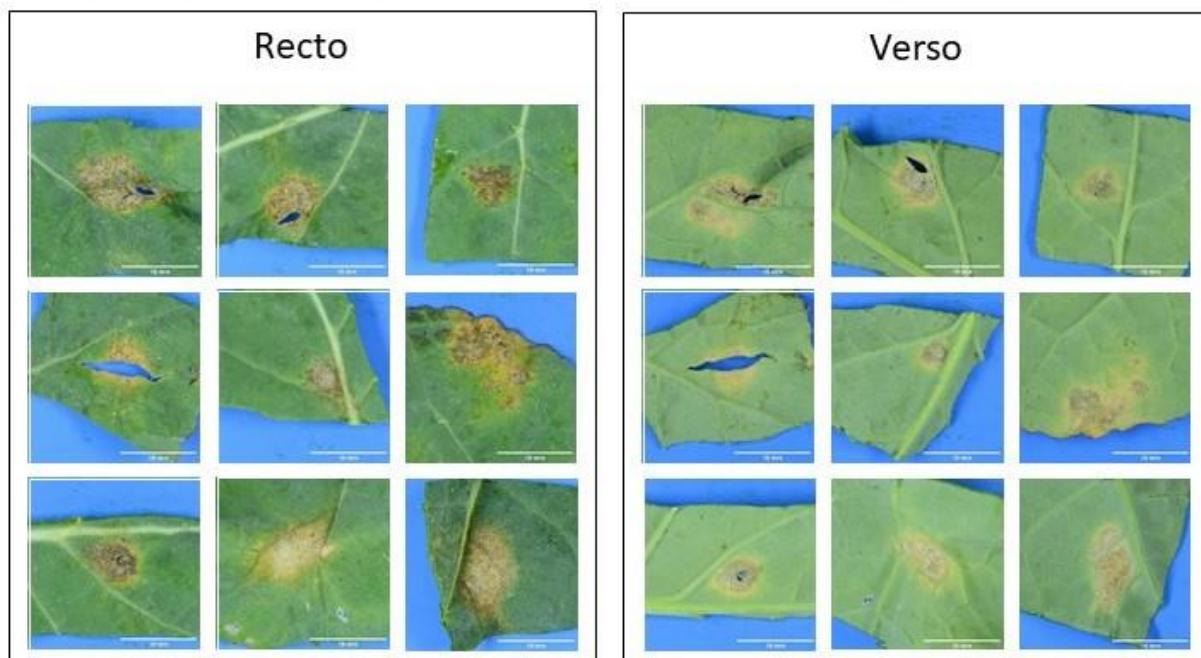


Figure 8: Light leaf spot *Pyrenopeziza brassicae*

8a. Typical symptoms: examples respecting most of the distinctive criteria for the species



8b. Atypical symptoms: examples of variations respecting only some of the distinctive criteria



Discussion

We confirmed that along the growth of oilseed rape several fungal diseases co-occur on leaves. This might not be the case in all regions, but the oceanic climate of Brittany allowed observing all the major diseases.

The experts were not able to identify distinctive criteria that would be respected by all the symptoms of a given species and not frequently or occasionally shared with other species. However, combining several criteria for selection or exclusion allows to produce guidelines for typical symptoms. For the atypical symptoms (variations respecting only some of the distinctive criteria, the availability of standardized pictures will be of great help for the reader.

In addition to the current illustrative figures, the availability of our picture database will enable developing computer applications to train experts. The annotated images can be selectively presented to candidates, rated and performance evaluated before going to the field.

As the leaf fragments corresponding to leaf fragments in the image database were kept frozen, the prospect is open to confirm expert visual diagnostic by DNA molecular characterisation.

Acknowledgements

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Supplementary Materials

In Supplementary figures Fig. S1 to Fig. S7, the contents of Figure 2 to Figure 8 are provided with higher quality pictures.